ASSOCIATIVE INTERACTIONS BETWEEN SEPTO-DENTATE AND PERFORANT PATH AFFERENTS TO THE RAT DENTATE GYRUS

By

GILBERT BRADLEY ROBINSON, B.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

ASSOCIATIVE INTERACTIONS IN THE RAT DENTATE GYRUS .

DOCTOR OF PHILOSOPHY (1984)
(Psychology)

McMASTER UNIVERSITY
Hamilton, Ontario.

TITLE: Associative Interactions Between Septo-dentate and Perforant Path Afferents to the Rat Dentate Gyrus

AUTHOR: Gilbert Bradley Robinson, B.Sc. (Dalhousie University)

SUPERVISOR: Professor R.J. Racine

NUMBER OF PAGES: (xii), 150

ABSTRACT

Cooperative interactions between the septal (septo-dentate; SD) and perforant path (PP) inputs to the dentate gyrus granule cells (GCs) of the rat were examined to determine the effect on long-term potentiation (LTP). Tetanization of SD afferents produced LTP of the SD-GC response but not of the PP-GC response, and PP tetanization produced LTP of the PP-GC response but had no effect on SD-GC responses. Despite this specificity, concurrent SD and PP tetanization produced significantly greater LTP of the PP-GC population spike than was produced following PP-only trains. In addition to increasing its magnitude, concurrent trains also increased the duration of LTP. Concurrent SD and PP tetanization had no additional effect on SD-GC responses.

The heterosynaptic cooperativity effect depended on both the temporal interval between application of the SD and PP trains and on their order of activation. When the SD trains were applied less than 100 ms prior to the PP trains there was a large additional increment in LTP magnitude. Intertrain intervals between 100 and 1000 ms produced significantly smaller increments. Beyond 1000 ms, no interaction effects were observed. If the PP trains preceded the SD trains there was little or no evidence of a cooperativity effect. The PP-SD sequence actually appeared to inhibit the appearance of a cooperativity effect when the trains were subsequently applied concurrently.

Paired-pulse tests, designed to examine the effect of the SD trains on recurrent inhibition, raised the possibility that the SD trains may reduce the strength of the GC's recurrent inhibitory circuits and thereby increase the postsynaptic effect of each PP train.

These results increase the attractiveness of the LTP phenomenon as a candidate neural mechanism for learning and memory. The possible role of the heterosynaptic interactions in classical conditioning were discussed.

ACKNOWLEDGEMENTS

Many of the ideas that form the backbone of this thesis were developed during discussions with Dr. R.J. Racine, in which my vaguely stated ideas were shaped into answerable questions. Perhaps more than anything, Dr. Racine has shown the value of phrasing a question so that it is both interesting and answerable. I thank him for his patience, constant encouragement, and for allowing me to "pick" his brain these last four years.

I would also like to thank Dr. H.P. Weingarten for reading earlier drafts of this thesis, and for always asking, "do you really believe that this has anything to do with learning or memory?"

Appreciation is also due to my wife, and best friend, Sandra, for her support these last few years, and to my parents, for the lifetime of opportunities they have provided.

Finally, I would like to thank the Natural Sciences and Engineering Research Council of Canada for the postgraduate scholarships that provided the financial support necessary to complete this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

ACh	acetylcholine
AMP	cyclic 3',5' adenosine monophosphate
Ca^{2+}	calcium
CS	conditioned stimulus
EEG	electroencephalograph
EPSP	excitatory ppstsynaptic potential
GABA	gamma-aminobutyric acid
GC	granule cell
im	intramuscular
IP	intraperitoneal
K^{+}	potassium
LTP	long-term potentiation
PP	perforant path
SD	septo-dentate
SEM	standard error of the mean
STP	short-term potentiation
UCS	unconditioned stimulus
VOR	vestibulo-ocular reflex

CHAPTER I. INTRODUCTION

Neuroscientists have long been interested in the physiological mechanisms of learning and memory. Demonstrations of causal relationships between neural events and learning, however, have been relatively rare. This is especially true for the study of mammalian information storage and retrieval mechanisms where numerous difficulties are encountered. A determination of the complete neural circuit mediating a particular behaviour, for example, is generally not possible in mammalian systems. Also, learning must be inferred from changes in the animal's behaviour. These changes may be affected by variables not directly related to the learned behaviour (e.g. motivation, arousal) and therefore difficult to control experimentally.

One approach to the above problems has been the utilization of model systems or model phenomena (Andersen, 1983). The modelling approach is built upon the philosophy that to understand neural mechanisms it is neither necessary to study complicated forms of behaviour nor to utilize the mammalian nervous system (or at least not the entire mammalian nervous system). In the model systems approach, for example, the variables controlling task performance are usually known and the neural circuit is at least partially mapped. This allows experimental control of all relevant inputs to the system. Invertebrate preparations provide some of the more popular model systems (for reviews of various invertebrate model systems see Krasne, 1976).

One of the most successful examples of a model systems approach is the work of Kandel and his associates on the invertebrate preparation, Aplysia (Kandel, 1976; Kandel, 1979; Kandel and Schwartz, 1982). The Aplysia has a simple nervous system consisting of a few ganglia, each composed of relatively few cells. The cells are easily identified from preparation to preparation and the connections between many of the cells have been mapped. For certain types of behaviour the complete neural circuit is known, and this has allowed investigators to examine learning-induced circuit changes.

In particular, the mechanisms underlying relatively simple forms of learning, such as habituation and sensitization, have been the subject of intense investigation. Habituation is due to a decrease in synaptic efficacy at the sensory-motor synapse. This decrease is the result of reduced levels of transmitter release by the sensory neurons (Castellucci and Kandel, 1974). The reduction in transmitter release, in turn, is believed to be due to either an increase in the uptake of intracellular calcium (Ca^{2+}; upon which release depends) by terminal mitochondria or to a decrease in the permeability of the terminal membrane to Ca^{2+} (Kandel, 1976; Kandel, 1979).

The mechanism of sensitization is better understood. Neurons contacting the sensory terminals release serotonin which increases cyclic 3',5' adenosine monophosphate (cyclic-AMP) activity in the sensory terminals. Cyclic-AMP phosphorylates a membrane protein resulting in the closing of potassium (K^{+}) channels. As a consequence, the duration of action potentials in the sensory terminals is increased, so that during subsequent action potentials there is a greater influx of

Ca^{2+} and therefore increased transmitter release (Kandel and Schwartz, 1982).

Although a great deal has been gained from the utilization of a model systems approach, it does suffer from two serious limitations. First, it is questionable whether habituation and sensitization are representative of higher, associative forms of learning. Yet, it is often the associative mechanisms that are of greatest interest to the neuroscientist. Second, the neural mechanisms responsible for invertebrate learned behaviour may be quite different from those operating in mammalian systems.

Work now being done on classical and operant conditioning, in a variety of invertebrate preparations, is aimed at this first criticism. Carew, Walters and Kandel (1981) have demonstrated simple associative learning of the defensive withdrawal reflex in Aplysia, and Carew, Hawkins and Kandel (1983) recently demonstrated differential classical conditioning of this defensive behaviour. The mechanism of differential classical conditioning may be similar to sensitization; both are presynaptic and involve an increase in spike duration as a result of increased Ca^{2+} influx (Hawkins, Abrams, Carew and Kandel, 1983; Walters and Byrne, 1983).

Sahley, Rudy and Gelperin (1981) have shown that the terrestrial slug, Limax maximus, is also capable of higher associative forms of learning such as higher-order conditioning, blocking, and an unconditioned stimulus (US) pre-exposure effect. The neurons involved in the feeding motor program of Limax are known, and aversive training

has been shown to suppress the feeding motor response (Chang and Gelperin, 1980). Davis and Gillette (1978) have also demonstrated classical conditioning of a complex feeding behaviour in the mollusc, Pleurobranchaea californica. In this system, the command neurons for feeding, the paracerebral neurons, elicit a burst of hyperpolarizing potentials when food stimuli, previously paired with shock, are applied to the oral veil. These inhibitory potentials eliminated feeding behaviour. Feeding behaviour could be reinstated if depolarizing current was injected into the paracerebral neurons.

Recent work with vertebrate systems is aimed at the second criticism, that invertebrate and vertebrate learning mechanisms may be fundamentally different. Preparations such as the hippocampal slice (Skrede and Westgaard, 1971) are being used to examine the biochemical and cellular mechanisms of changes in cortical synaptic efficacy, while relatively simple behaviours, such as the rabbit's nictitating membrane reflex (Gormezano, 1972; Thompson, Patterson and Berger, 1978) are now providing important insights into the mechanisms of behavioural and neural plasticity. A third vertebrate model system is that mediating the vestibulo-ocular reflex (VOR). The VOR system mediates compensatory eye movements that are used to stabilize retinal images during head rotations. The anatomy and physiology of the neural circuitry controlling this reflex are partially understood, and models based on this circuitry have been suggested (Davies and Melvill Jones, 1976; Galiana and Outerbridge, 1984). Although much is now known of this system, investigators have not yet determined the site at which the change in neural plasticity occurs. Nevertheless, these preparations

are providing valuable insight into vertebrate mechanisms of neural and behavioural plasticity. It is not yet possible, however, to apply the same level of analysis as is used in many of the invertebrate preparations.

The second modelling approach utilizes model phenomena. Model phenomena are neural events with characteristics that mimic those present in the phenomenon of interest. Examination of these characteristics, and their mechanisms, may provide information regarding the neural mechanisms of complex behaviours that may not otherwise be approachable. For learning and memory, the basic characteristics should be neural changes that are large and relatively permanent, but which are nevertheless capable of decay. Kindling, for example, refers to a progressive and long-lasting increase in the magnitude of both the cellular and behavioural response to epileptogenic electrical stimulation of certain forebrain sites. Although it does not appear to produce tissue damage, the effects are large and permanent. The magnitude and permanence of these neural changes made kindling an attractive model for learning and memory mechanisms (Goddard, McIntyre and Leech, 1969). More recent work, however, indicates that kindling might still be based upon degenerative changes and is, perhaps, more appropriately viewed as an epilepsy model (Kairiss, Racine and Smith, 1984; Racine, 1978; Racine and Burnham, 1984; Racine, Milgram and Hafner, 1983). Nevertheless, kindling does result in large and long-lasting increases in synaptic efficacy, as do other model phenomena utilized to study learning and memory. Within certain forebrain sites, for example, classical conditioning procedures produce a long-lasting

increase in the frequency of cell firing (Berger, Alger and Thompson, 1976; Brons and Woody, 1980).

This thesis will examine the characteristics of a model phenomenon that is currently one of the best candidate neural mechanisms for learning and memory (Teyler and Discenna, 1984). The phenomenon of interest is long-term potentiation (LTP), a long-lasting increase in the strength of synaptic drive between central nervous system neurons. LTP occurs following the activation of a cell's input pathways with high-frequency trains of electrical pulses. This phenomonon has many of the characteristics one would hope to find in a neural system that encodes and stores information (Goddard, 1980; McNaughton, 1983; Teyler and Discenna, 1984). These characteristics will be discussed in detail in a later section of the Introduction.

In particular, this thesis examines the effect, on LTP, of coactivation of inputs to the dentate gyrus. The system of interest was the perforant path (PP) and medial septal inputs (septo-dentate; SD) to the dentate gyrus granule cells (GCs) of the hippocampal formation. High-frequency activation of the PP and SD inputs, both separately and at various temporal delays, was used to induce long-term changes in GC excitability (LTP). The remainder of this Introduction will provide a basic description of the LTP phenomenon, describe the anatomy and physiology of the hippocampus and septum and evidence for their involvement in learning and memory, and review features which make LTP an attractive neural candidate for learning and memory.

I. A. THE LONG-TERM POTENTIATION PHENOMENON

1. LTP: Basic Description

Theories on the neural mechanisms of learning and memory have generally specified relatively permanent increases in synaptic efficacy (Ajmone-Marsan and Matthies, 1982; Fillenz, 1972; Gardner-Medwin, 1969; Hebb, 1949). In addition, many of these theories have suggested the need for some type of associative interaction. Hebb (1949), for example, postulated that learning occurred whenever the postsynaptic cell discharged at the same time it was activated by one of its afferents. Also, an input too weak to discharge the postsynaptic cell by itself, could acquire the necessary strength if it activated the cell concurrently with a strong input. This acquisition of neural strength through association was thought to be a mechanism whereby whole neuronal networks or "cell assemblies" could be constructed. According to Hebb (1949), these networks were the storage sites for acquired information. Such a theory, however, required the demonstration that long-lasting changes in synaptic efficacy could occur in the central nervous system. The LTP phenomenon was one such demonstration.

Long-term potentiation is a long-lasting increase in synaptic efficacy as a result of prior high-frequency activation. It is a widespread phenomenon which is known to occur at most forebrain pathways (Racine et al., 1983). LTP, for example, has been shown to occur in the cerebellum (Racine, in preparation), the anterior neocortex (Wilson and

Racine, 1981; Wilson and Racine, 1983a), the striate cortex (Komatsu, Toyama, Maeda and Sakaguchi, 1981) and the superior cervical ganglion (Brown and McAfee, 1982). The largest and longest-lasting LTP effects, however, occur in the hippocampus (Racine et al., 1983). As a result, the hippocampus and its extrinsic and intrinsic pathways have been the structure of choice in most LTP studies.

LTP is readily observed at all excitatory hippocampal synapses (Alger and Teyler, 1976; Andersen, Sundberg, Sveen and Wigstrom, 1977; Buzsaki, 1980; Racine et al., 1983; Robinson and Racine, 1982b; Yamamoto and Chujo, 1978; Yamamoto, Matsumoto and Takagi, 1980), and recent evidence suggests LTP may also occur in hippocampal inhibitory interneurons (Buzsaki and Eidelberg, 1982). Of the excitatory pathways, the PP-GC synapse has received the most attention in LTP studies (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; Douglas, 1977; Douglas and Goddard, 1975; Levy and Steward, 1979; McNaughton, 1983; McNaughton, Douglas and Goddard, 1978; Robinson and Racine, 1982b; Robinson and Racine, 1984). Application of stimulation trains to PP fibres induces LTP that can persist for several weeks or longer (Barnes, 1979; Bliss and Gardner-Medwin, 1973; Douglas and Goddard, 1975; Racine et al., 1983; Robinson and Racine, 1984).

There are actually several different types of increased synaptic efficacy exhibited in the hippocampal formation, as well as elsewhere in the mammalian forebrain (Racine and Milgram, 1983; Racine et al., 1983). On the basis of decay time constants they have been separated into 5 distinct categories (McNaughton, 1983; Racine and Milgram, 1983; Racine

et al., 1983). Facilitation is the shortest-lasting phenomenon (Creager, Dunwiddie and Lynch, 1980; Lomo, 1971b; Racine and Milgram, 1983; Steward, White and Cotman, 1977; White, Nadler and Cotman, 1979). It has a decay time constant of approximately 100 ms (McNaughton, 1982; Racine and Milgram, 1983) and is evoked by twin pulse stimulation. The first pulse serves as a conditioning pulse while the second pulse serves as the test pulse. The amplitude of the response evoked by the test pulse is usually greater (facilitated) than that evoked by the conditioning pulse. At short interpulse intervals the magnitude of the facilitation effect may be reduced by depression effects (due to transmitter depletion).

The remaining types of synaptic efficacy are all evoked by high-frequency activation of the cell's afferents. Two of these, augmentation and potentiation, are short-term components with decay time constants of approximately 5 s and 90 s, respectively (McNaughton, 1980; McNaughton, 1982; McNaughton, 1983; Racine and Milgram, 1983). The decay of these short-term components is usually superimposed on the decay of longer-term components.

The longer-term components, formerly known simply as LTP, have recently been divided into two separate components by Racine et al. (1983). They are referred to as LTP1 and LTP2 with decay time constants of approximately 1.5 hrs and 5 days, respectively. It is these long-term components which initially resulted in the consideration of LTP as a candidate neural memory mechanism (Bliss and Gardner-Medwin, 1973). It is not known to what extent these categories represent different underlying processes, but McNaughton (1982) has suggested that

LTP is not simply a longer-lasting form of potentiation (short-term), as the presence of LTP does not appear to affect the kinetics of short-term potentiation.

2. LTP: Mechanisms

The mechanism(s) of LTP is (are) not yet known, although several candidate mechanisms have been proposed (Bliss, 1979; Bliss and Dolphin, 1982). At present, these mechanisms can be divided into two categories, presynaptic and postsynaptic. LTP, for example, could be due to a presynaptic increase in transmitter release. Transmitter release depends on the action potential-induced influx of Ca^{2+} into the presynaptic terminal. If tetanization altered the Ca^{2+}-buffering properties of the cell, thereby increasing the level of free intracellular Ca^{2+}, subsequent single pulses would increase the level of transmitter release.

In support of hypotheses proposing a Ca^{2+}-dependence for LTP, Baimbridge and Miller (1981) found increased Ca^{2+} uptake in area CA1 (CA-cornu ammonis) following high-frequency activation of the Schaffer Collaterals. Turner, Baimbridge and Miller (1982), in addition, showed that LTP could be induced simply by bathing hippocampal slices in medium containing a high concentration of Ca^{2+} ions, and Dunwiddie, Madison and Lynch (1978) found that LTP was blocked in a bathing medium which contained low concentrations of Ca^{2+}. These results were confirmed by Dunwiddie and Lynch (1979). It is, of course, possible that

postsynaptic mechanisms might also be Ca^{2+}-dependent and therefore equally affected by increasing extracellular Ca^{2+} (see below).

In more direct tests of the presynaptic hypothesis, two studies have found increased levels of transmitter release following tetanization of afferent fibres. Skrede and Malthe-Sorenssen (1981) found that tetanization increased both the resting and stimulus evoked release of glutamate in area CA1. They did not, however, measure LTP so the correlation was not established. Dolphin, Errington and Bliss (1982) found that LTP of PP-GC synapses was correlated with a prolonged increase in the release of newly synthesized glutamate. It is possible, however, that release was increased as a secondary consequence of the LTP being induced (e.g. from the second stage of excitatory recurrent loops; Schwartzkroin and Wester, 1975) rather than being the primary cause of LTP.

Postsynaptic mechanisms are generally better accepted and seem better able to account for associative effects. There have been numerous demonstrations of changes in the morphology of postsynaptic spines following high-frequency activation of their afferents (Desmond and Levy, 1983; Lee, Schottler, Oliver and Lynch, 1980; Van Harreveld and Fifkova, 1975). Tetanization of either the medial or lateral components of the PP, for example, increases the width of the spine head and spine stalk in the middle and distal thirds of the dentate molecular layer (Fifkova and Andersen, 1981). A reduced spine resistance would allow more of the synaptic current to reach the central portions of the cell. If all other factors remained constant, this would tend to increase the amplitude of the synaptic response.

A postsynaptic role for Ca^{2+} ions in LTP, in contrast to the above mentioned presynaptic hypotheses, has been proposed by Baudry and Lynch (1980). According to their theory, tetanization results in an influx of Ca^{2+} ions into the postsynaptic membrane which increases the number of postsynaptic glutamate receptors. Briefly, the elevation of intracellular Ca^{2+} is believed to activate a membrane associated proteinase which splits the peptide bonds in the membrane proteins. This leads to membrane destabilization and the uncovering of additional glutamate receptors (Lynch, Halpain and Baudry, 1983). LTP is correlated with increased glutamate binding to hippocampal membranes (Baudry, Oliver, Creager, Wieraszko and Lynch, 1980; Lynch, Halpain and Baudry, 1982) and is reduced by certain postsynaptic glutamate antagonists which should leave presynaptic mechanisms intact (Krug, Brodemann and Ott, 1982). Furthermore, LTP of the intracellular EPSP recorded in area CA1 of the hippocampal slice is blocked by intracellular injection of the Ca^{2+} buffering agent EGTA. EGTA could block the Ca^{2+} activated proteinase controlling postsynaptic receptors and thereby block the development of LTP (Lynch, Larson, Kelso, Barrionuevo and Schottler, 1983).

Although intuitively attractive, two recent findings have provided evidence against the glutamate-receptor hypothesis. First, leupeptin, an inhibitor of Ca^{2+}-sensitive proteinases, does not appear to block LTP in the chronic rat preparation (Wilson, personal communication). Second, Sastry and Goh (1984) have recently reported that synaptic depression, not LTP, results in increased glutamate binding in the hippocampus.

I. B. ANATOMY

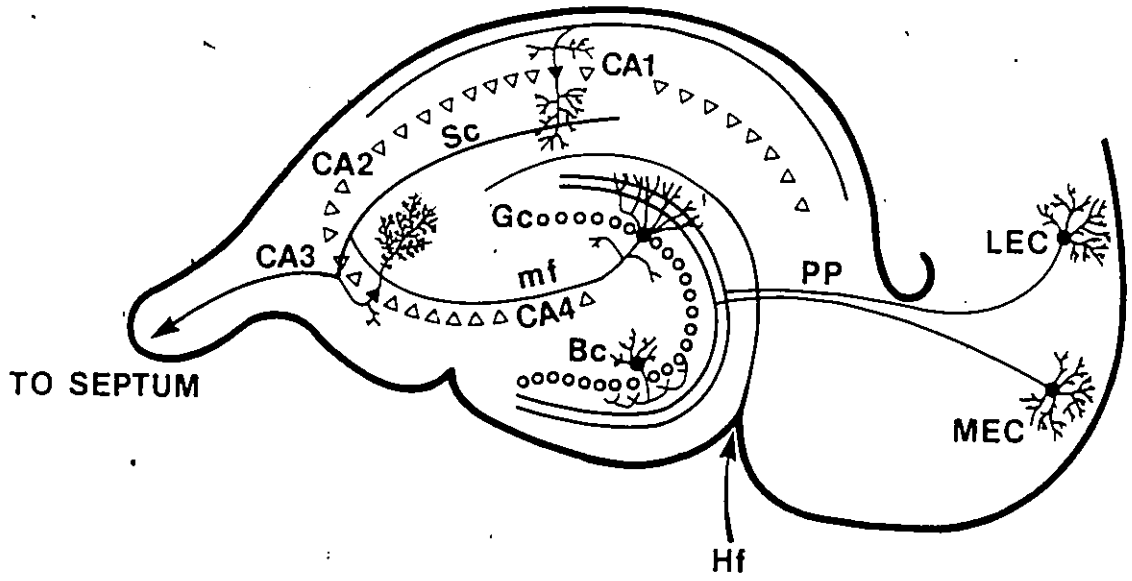
1. The Hippocampal Formation

The hippocampal formation is one of the most prominent structures in the mammalian forebrain. It consists of both the hippocampal gyrus, or Ammons Horn, and the area dentata, or dentate gyrus. The structure has a shape which has been likened to that of a banana (Gray, 1982) or sausage (O'Keefe and Nadel, 1978) and lies just beneath the neocortex.

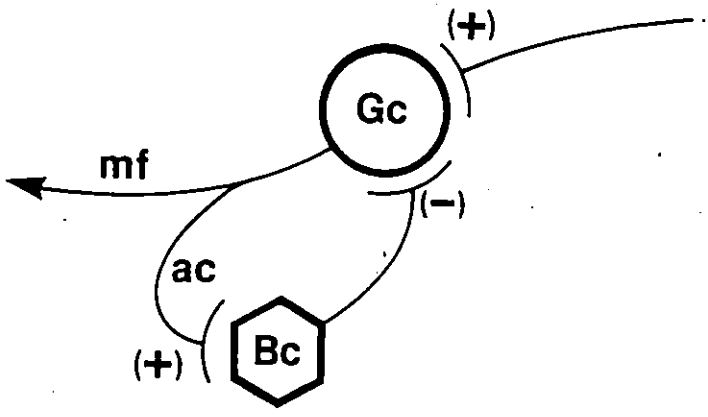
Figure 1A is a cross-sectional representation of the hippocampal formation and adjacent entorhinal cortex, showing the major cell types of Ammon's Horn and the dentate gyrus. The hippocampal and dentate gyri are interlocked and are made up of a series of lamallae, from the temporal to the septal pole (Andersen, Bliss and Skrede, 1971b). Each lamella is nearly identical in terms of neural organization and internal circuitry (Andersen, 1975). On the basis of cytoarchitecture and afferent and efferent connections these lamella have been divided into a number of subdivisions and layers. The major internal subdivisions of the hippocampal gyrus are areas CA1, CA2, CA3 and CA4. Each of these areas consist of five layers. The outermost layer is the alveus, followed by stratum oriens, stratum pyramidale (pyramidal cell layer), stratum radiatum and finally stratum moleculare which lies immediately above the hippocampal fissure.

Figure 1: A. Schematic diagram of hippocampal formation and adjacent entorhinal cortex (lateral-LEC; medial-MEC). Bc, basket cell; CA, cornu ammonis; Gc, granule cell; Hf, hippocampal formation; mf, mossy fibre; PP, perforant path; Sc, Schaffer collateral. B. Dentate gyrus circuit diagram indicating excitatory (+) and inhibitory (-) connections that affect granule cell firing. ac, axon collateral.

A



B



Stratum pyramidal contains the cell bodies of the main cell type of the hippocampus, the pyramidal cells. The pyramidal cells have both basal and apical dendrites which extend into stratum oriens and stratum moleculare, respectively. The pyramidal cell axons project through the alveus towards the subiculum and lateral septal nucleus.

The GCs are the main cell type of the dentate gyrus. The apical dendrites of the GCs extend into stratum moleculare ending immediately below the hippocampal fissure. The unmyelinated mossy fibre axons of the GCs form the output from the dentate gyrus. Thus, the dentate gyrus consists of a molecular layer, a GC layer and a polymorph layer. The dentate gyrus actually consists of two blades that are separated by area CA4.

Another major cell type within the hippocampal formation is the basket cell. These cells are located below the cell body layers of the dentate and hippocampal gyri. They receive input from granule and pyramidal cell axon collaterals and project back to the cell bodies of granule and pyramidal cells, respectively (see Figure 1B). Their function is to inhibit cell discharge. Intracellular and extracellular recording techniques have verified that the inhibitory synapses are located on the cell bodies of the principle cell types and that their activation results in an inhibitory postsynaptic potential (hyperpolarization) in the principle cells (Andersen, Eccles and Loyning, 1964a,b; Andersen, Holmqvist and Voorhoeve, 1966b; Lomo, 1971a).

The main input to the dentate is via the PP. The PP fibres originate from the entorhinal cortex to make synaptic contact with the

apical dendrites of the GCs (Nafstad, 1967; Steward, 1976). These contacts are made in well defined layers of the molecular zone. On the basis of degeneration studies, the PP input has been divided into a medial and lateral component. The medial PP (Hjorth-Simonsen and Jeune, 1972) originates from cells in the dorsomedial entorhinal cortex and synapses within the middle third of the GC molecular layer. The lateral PP originates from the ventrolateral entorhinal cortex (Hjorth-Simonsen, 1972) to synapse within the distal thirds of the GC dendritic tree. These findings have been confirmed using electrophysiological criteria (Lomo, 1971a; McNaughton, 1980; McNaughton and Barnes, 1977). Both the medial and lateral PP make synaptic contact en passage so that activation of any one fibre excites a whole strip of GCs, within a given lamella.

The output from the GCs is via the mossy fibres. These fibres course thru the hilus to make synaptic contact with the pyramidal cells of area CA3. The main branch of the CA3 axon, in turn, projects out of the hippocampus, via the fimbria, to the lateral septum (Andersen, Bland and Dudar, 1973; Raisman, 1966; Raisman, Cowan and Powell, 1965). Collateral branches of the CA3 axons, the Schaffer collaterals, project to the basal dendrites of CA1 pyramidal cells. The CA1 axons travel through the alveus to the subiculum (Andersen, Blackstad and Lomo, 1966a; Andersen et al., 1973) and lateral septal nucleus. The PP, mossy fibres, Schaffer collaterals and axons of the CA1 cells all run in nearly the same plane and transversely to the longitudinal axis of the hippocampus (Andersen, et al., 1971b; Andersen et al., 1966a; Lomo, 1971a). The sequential flow of information, from GCs to CA3 pyramidal

cells to CA1 pyramidal cells, following PP activation, forms the basic trisynaptic excitatory pathway of the hippocampal formation (Andersen, 1975; Andersen et al., 1971b). This thesis is concerned with the first synaptic relay (PP-GC) in this circuitry.

In addition to PP terminals, the GCs also receive associational (Gottlieb and Cowan, 1973; Zimmer, 1971) and commissural projections (Blackstad, 1956; Deadwyler, West, Cotman and Lynch, 1975; Raisman et al., 1965) to the inner third of the molecular layer. These projections arise from cells in the ipsilateral and contralateral hilus, respectively (Hjorth-Simonsen and Laurberg, 1977; Laurberg, 1979; Swanson, Wyss and Cowan, 1978). There are also serotonergic fibres, from the median raphe nucleus (Azmitia and Segal, 1978; Conrad, Leonard and Pfaff, 1974; Kohler and Steinbusch, 1982; Moore and Halaris, 1975), noradrenergic fibres from the locus coeruleus (Koda and Bloom, 1977; Loy, Koziell, Lindsey and Moore, 1980; Segal and Landis, 1974; Ungerstedt, 1971), an input from the reticular formation (Winson, 1981), various neuropeptide containing fibres (Roberts, Woodhams, Polak and Crow, 1984) and a possible dopaminergic projection (Ishikawa, Ott and McGaugh, 1982; Storm-Mathisen, 1977b, 1978). These projections are relatively diffuse and project to much of the hippocampal formation. Terminal density does, however, appear to be greatest in the hilus of the dentate gyrus. The serotonergic (Assaf and Miller, 1978; Segal, 1980; Winson, 1980) noradrenergic (Abraham and Goddard, 1982; Assaf, Mason and Miller, 1979; Segal, 1982) and reticular terminals (Winson, 1981) all influence GC activity. There are also cholinergic fibres from the medial septum that will be further discussed in the next section.

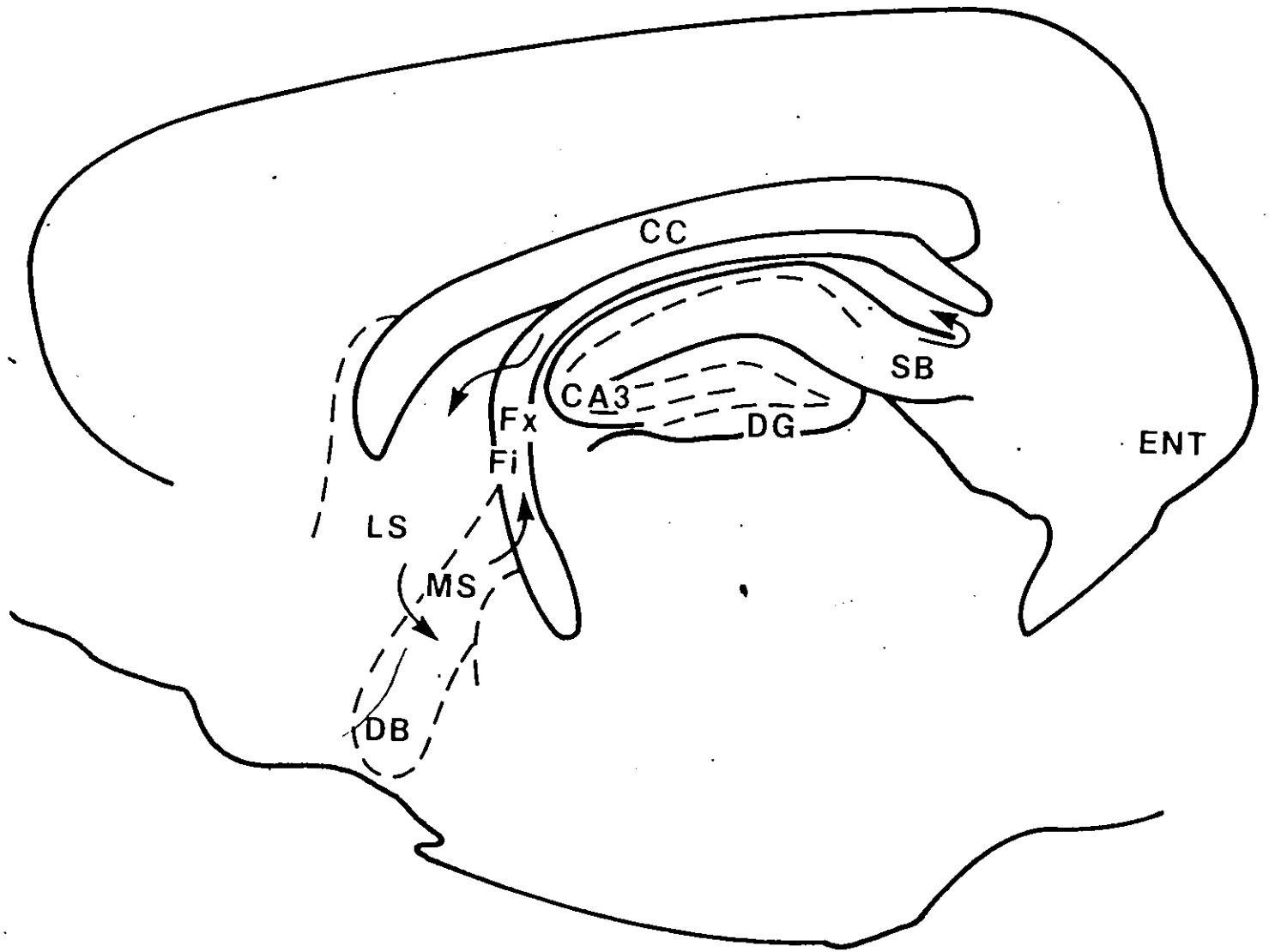
2. The Septum

Figure 2 illustrates the anatomical relationship between the hippocampal formation and the septal nuclei. The septum is situated between the anterior lateral ventricles, beneath the callosal fibres, and above the anterior commissure (Swanson and Cowan, 1976). Its organization is not as clear as that within the hippocampus. There is, for example, no sharp demarcation between the septal nuclei such as is found between the hippocampal and dentate gyri.

The septum is composed of four basic subdivisions, the lateral and medial septum and the ventral and posterior divisions. The lateral and medial nuclei are the most distinct, and have their major connections with the hippocampal formation. In terms of cytoarchitecture the medial septum is mainly composed of large cells, whereas the lateral septum mainly consists of medium sized cells (Swanson, 1978; Swanson and Cowan, 1976). The medial septum also contains numerous fibres of passage. These arise from various brain stem nuclei such as the locus coeruleus, median raphe and reticular formation.

The hippocampus projects to the lateral septum via the fimbria and the precommissural fornix. These fibres arise from areas CA1, CA3 and the subiculum. The projection from CA1 and the subiculum is believed to be unilateral (Meibach and Siegel, 1977). In contrast, the septal input from CA3 pyramidal cells is bilateral (Swanson, 1978). Both inputs are believed to be topographically organized, with the

Figure 2: Schematic diagram of hippocampus and septum illustrating the reciprocal connections between the two structures. CA, cornu ammonis; CC, corpus callosum; DB, nucleus of the diagonal band; DG, dentate gyrus; ENT, entorhinal cortex; Fi, fimbria; Fx, fornix; LS, lateral septum; MS, medial septum; SB, subiculum.


CC
SB
CA3
DG
Fx
Fi
ENT
LS
MS
DB

ventral hippocampus innervating the most dorsal areas of the lateral septum and the dorsal hippocampus the more medial areas.

The lateral septum, in turn, projects to the medial septum and the nucleus of the diagonal band (Swanson, 1978). These latter two structures give rise to efferents that project to the hippocampus via the fimbria and dorsal fornix (Crutcher, Madison and Davis, 1981; Lynch, Rose and Gall, 1978; Mosko, Lynch and Cotman, 1973; Raisman et al., 1965; Stanfield and Cowan, 1982; Swanson, 1978; Swanson and Cowan, 1976). There is some evidence that this projection is also topographically organized, with the more medial aspects of the medial septum projecting to the rostral hippocampus and more lateral regions projecting to successively more caudal and ventral portions of the hippocampal formation (Segal and Landis, 1974).

These anatomical findings have been confirmed using electrophysiological techniques. Electrical activation of the fimbria results in orthodromic activation of the lateral septum and antidromic activation of the medial septum (McLennen and Miller, 1974). Thus, there is a loop from hippocampus to lateral septum to medial septum and finally back to the hippocampus (see Figure 2).

The density of septal terminals varies within the hippocampal formation. The densest projection is to the dentate hilus and stratum oriens of area CA3 (Crutcher et al., 1981; Mosko et al., 1973). In the dentate gyrus, septal terminals appear throughout the hilus with the majority of terminals concentrated in a small zone immediately above and below the GC layer (Lewis and Shute, 1967; Lewis, Shute and Silver, 1967; Stanfield and Cowan, 1982; Mosko et al., 1973). There is a modest

input to the dentate molecular layer and stratum radiatum of area CA3. The projection to stratum oriens and stratum lacunosum of area CA1 is very sparse (Crutcher et al., 1981; Mosko et al., 1973).

Mosko et al. (1973) first suggested that the dense hilar projection may indicate a direct projection from the medial septum to the basket cell interneurons. This proposal was investigated by Lynch et al. (1978) using radioactively labeled adenosine, a nucleotide that is able to cross synaptic junctions. Following injections into the medial septum they found label in one cell, whose morphology and location was consistent with it being a basket cell. Thus, it appears that at least part of the septal input to the dentate gyrus may make synaptic contact with basket cell interneurons. These findings are consistent with the proposed disinhibitory action of the septal terminals (Valentino and Dingledine, 1981).

In addition to the above differences, there are indications that the PP and SD afferents utilize different transmitters. The former pathway appears to utilize glutamate as its transmitter (Crunelli, Forda and Kelly, 1983; Monaghan, Holets, Toy and Cotman, 1983; Nadler, Cotman and Lynch, 1974; Nadler, Vaca, White, Lynch and Cotman, 1976; Storm-Mathisen, 1977a,b; Storm-Mathisen, 1978; Storm-Mathisen and Fonum, 1971; Wheal and Miller, 1980; White, Nadler, Hamberger Cotman and Cummins, 1977). In contrast, the SD afferents appear to be cholinergic. Evidence that the septal to hippocampal projection is cholinergic has come from both assay and mapping studies using various cholinergic metabolites (Fonnum, 1970; Lewis and Shute, 1967; Lewis et al., 1967; Shelton, Nadler and Cotman, 1979; Storm-Mathisen, 1970), stimulation

induced increases in hippocampal acetylcholine (ACh) release (Dudar, 1975; Szerb, Hadhazy and Dudar, 1977), the effects of septal lesions on hippocampal ACh levels (Potempska, Gradkowska and Oderfeld-Nowak, 1975; Szerb et al., 1977) and the presence of both hippocampal muscarinic and nicotinic receptor proteins (Ben-Barak and Dudai, 1979; Segal, Dudai and Amsterdam, 1978; Yamamura, Kuhar and Snyder, 1974; Yamamura and Snyder, 1974a,b).

I. C. PHYSIOLOGY

The geometry of the hippocampus, particularly that of the dentate gyrus, is ideal for the recording of extracellular field potentials. Activation of the PP results in the influx of current into the dendrites of a large population of GCs, thereby producing a non-uniformly charged membrane. As a result, intracellular current (positive ions) flows toward the more negative regions of the cell body (see Figure 3A and B). To complete the current path there will be a corresponding flow of outward current, from soma to dendrite, in the extracellular space (Andersen et al., 1966b; Andersen et al., 1971b; Lomo, 1971a).

An extracellular electrode placed in the molecular layer, in the region of the active sink (area of current influx), will record a negative potential. This negativity reflects the summed activity of the dendritic current sinks. The magnitude of this negativity is a measure of the excitatory postsynaptic potential (EPSP). A second electrode in the somatic layer would simultaneously record this event (EPSP) as a

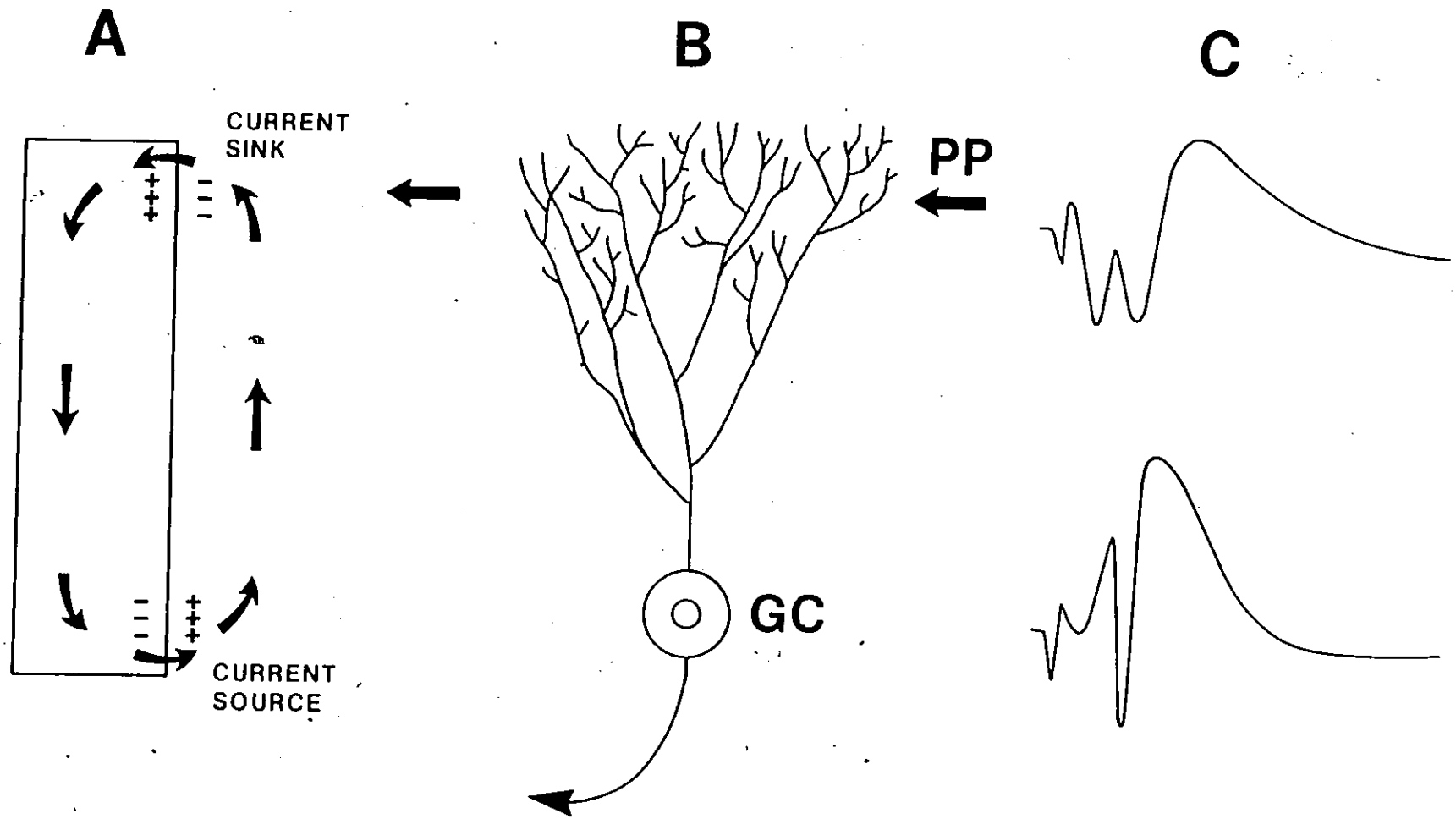

A
B
C
CURRENT SINK
CURRENT SOURCE
PP
GC

Figure 3: Schematic diagram showing an (A) idealized cell, (B) granule cell, and (C) typical field potentials recorded in the molecular layer and in the granule cell layer. Upward deflections are positive in this figure and in all subsequent figures.

positive potential as the cell somata act as current sources for the dendritic sinks (Lomo, 1970, 1971a).

The magnitude of the negative extracellular EPSP will gradually diminish as the electrode is moved from the active sink towards the cell body layer. There will be a polarity reversal at the cell body layer where the GC somata act as current sources for the dendritic sinks. All recordings for this thesis were made in the hilus of the fascia dentata, immediately below the GC bodies, and hence EPSPs were recorded as a positive potential (see Figure 3C).

As the intensity of electrical pulses applied to the PP is increased, there will be a corresponding increase in the magnitude of the population EPSP. Eventually, when some threshold number of PP afferents are activated, a negative potential will appear that is superimposed on the positive EPSP waveform (if recorded near the somatic layer). This negativity reflects the near synchronous discharge of a large population of GCs (Andersen et al., 1971a). It has been calculated that roughly 400 PP fibres (approximately 5% of the GC synapses) must be activated to discharge a GC (McNaughton, Barnes and Andersen, 1981). The magnitude of the population spike will also continue to increase as the intensity of PP stimulation is further raised.

In addition to these two major components of the field response there is, with optimal electrode placement, a fibre response recorded. Following PP activation, the onset latency of the fibre response, EPSP

and population spike are approximately 1.6, 2.0 and 4.5 ms, respectively (Douglas and Goddard, 1975; McNaugton and Barnes, 1977).

Intracellular recordings have verified that these extracellular field potentials reflect events occuring at the single cell level (Andersen et al., 1971a; Dudek, Deadwyler, Cotman and Lynch, 1976; Lomo, 1971a). The experiments reported in this thesis utilized extracellular recording techniques, and these secondary measures were used to infer processes occuring at the single-cell level. The validity of these measures is important, as it would be impossible to record from a single GC for the long periods of time necessary to study LTP. In addition, one could not be sure of what effect the damage caused by cell penetration during intracellular recording would have on subsequent LTP measures. It has, for example, been shown that there is less LTP of the intracellular EPSP than of the extracellular EPSP (Deadwyler, Dudek, Cotman and Lynch, 1975) and this has been attributed to electrode induced damage (Wigstrom, McNaughton and Barnes, 1982).

Hippocampal field potentials following septal activation have not been clearly characterized and there is, in fact, some controversy about whether they can be recorded. Andersen, Bruland and Kaada (1961a,b) recorded field responses in both area CA1 and the dentate gyrus following septal activation. Hippocampal field responses were also reported by DeFrance, Stanley, Marchand and Chronister (1978) and Wheal and Miller (1980). On the other hand, Alvarez-Leefmans and Gardner-Medwin (1975), Fantie and Goddard (1982) and Krnjevic and Ropert (1981) failed to detect field responses in the hippocampus or dentate as a result of septal stimulation. More recently, however, Ropert and

Krnjevic (1983) have reported that septal activation evokes "minimal field responses" in the hippocampus, and Goddard (personal communication) has recorded septal to dentate field responses.

Whatever response is produced, it may be partly cholinergically mediated. Stanley, DeFrance and Marchand (1980) were able to reduce the septal to CA1 response amplitude by over 40% with iontophoretic application of the cholinergic antagonist, atropine. Incomplete blockade of the response may have been due to the contribution of non-cholingergic fibres, to an insufficient blockade of muscarinic receptors or to a nicotinic component of the response.

I. D. LEARNING AND MEMORY

One of the major reasons for investigating the septo-dentate system is that, in addition to its demonstrated ability to undergo plastic changes at the synaptic level, both the hippocampus and septum are thought to be involved in learning and memory. The interest in these two areas is apparent from the large number of books recently written in attempts to relate septo-hippocampal physiology to behaviour (Ciba Foundation Symposium 58, 1978; DeFrance, 1976; Gray, 1982; Isaacson, 1974; Isaacson and Pribram, 1975a,b; O'Keefe and Nadel, 1978; Seifert, 1983; Swanson, Teyler and Thompson, 1982). The dentate gyrus is part of the septo-hippocampal system and, in addition, not only receives the majority of septal fibres but is the first synaptic relay station for cortical input.

A role for the hippocampus in learning and memory was first suggested from studies on humans. Lesions of the hippocampus and related structures, in attempts to control temporal lobe epilepsy, were shown to impair the acquisition and/or retention of learned events in humans (Penfield and Milner, 1958; Scoville and Milner, 1957). In addition, electrical stimulation in the area of the hippocampus was shown to evoke specific and detailed recall of previously experienced events (Penfield and Perot, 1963).

Similar lesions in animals were later shown to impair their ability on a variety of learning and memory tasks (see Douglas, 1967). The wealth of animal experiments which followed have provided the basis for at least two major theories on the role of the septohippocampal system in behaviour. O'Keefe and Nadel (1978) proposed that the hippocampus was involved in cognitive or spatial mapping of the environment. As the animal explored its environment, the objects or places it encountered and the relationship between these places, were thought to be represented in a spatial map. Olton (Olton, 1983; Olton, Becker and Handelmann, 1980; Olton, Walker and Wolf, 1983), on the other hand, has argued that the hippocampus is necessary to perform tasks that require working memory. Working memory is a form of memory that allows the animal to perform correctly from trial to trial by allowing it to use information acquired on previous trials. Consider, for example, a radial arm maze (usually eight arms radiating out from a central platform like the spokes on a bicycle wheel) with food placed at the end of each arm. The animal's task is to get all the food, which requires going down each arm no more than once. Working memory allows the animal

to recall which arms were visited on previous trials. According to Olton, the information stored in this memory may be spatial or non-spatial.

There is still a great deal of controversy concerning which theory is best able to account for the experimental findings. Although it is not the purpose of this thesis to discuss the relative merit of these two theories, it should be mentioned that specific experiments, designed in an attempt to determine whether the animal is using a spatial strategy or relying on an intact working memory system, have been unable to solve this problem (Morris, 1983; Olton et al., 1980). Rather, it appears that the animal utilizes a spatial strategy on some tasks and working memory on others, and what strategy is used may depend on the specific task requirements as well as the information available. Whatever the theory, performance on a radial arm maze and other spatial tasks depends on an intact septo-hippocampal system (Buzsaki, Bors, Nagy and Eidelberg, 1982; Crutcher, Kesner and Novak, 1983; Ellen and Delouche, 1968; Morris, 1983; O'Keefe, 1983a,b; O'Keefe and Black, 1978; O'Keefe, Nadel, Keightley and Kill, 1975; Olton, 1978; Olton et al., 1983).

There have also been a number of demonstrations of changes in hippocampal cellular activity related to behaviour or learning (Buzsaki, Grastyan, Czopf, Kellenyi and Prohaska, 1981; Deadwyler, West and Robinson 1981a; Haubenreiser, Hansen and Haschke, 1982; Jaffard and Jeantet, 1981; Leung, 1980; O'Keefe and Black, 1978; Ott, Ruthrich, Reymann, Lindinau and Matthies, 1982; Ruthrich, Matthies and Ott, 1982; Segal, 1977; Vanderwolf, 1969; Winson and Abzug, 1978). In some

instances, the changes in cellular activity appear to be differentially controlled by the entorhinal cortex and the medial septum (Deadwyler, West and Robinson, 1981b).

Perhaps one of the strongest correlations between neural activity and behaviour is that between hippocampal theta activity and motor behaviour (Leung, 1980; Vanderwolf, 1969). Theta rhythm, in the rat, is a rhythmical sinusoidal wave pattern of 6-10 Hz that appears in the hippocampal electroencephalograph (EEG) during certain behaviours. Theta rhythm appears during such behaviours as locomotion, or postural change, but is not present during motionless behaviour, or eating and drinking (Vanderwolf, 1969). The exact nature of the relationship between theta and learning and memory is not known, although several possibilities, such as an involvement in attention or arousal, have been suggested (see Swanson et al., 1982). It is difficult, however, to separate motor-related theta (a component of most learning situations) from theta that might be related to learning and memory per se (Vanderwolf and Leung, 1983; Vanderwolf and Ossenkopp, 1982). Whatever the relationship, it is fairly well established that theta is generated by the rhythmical discharge of cells within the hippocampus and that this rhythmicity is probably induced by the cells of the medial septum (Monmaur and Thomson, 1983; O'Keefe and Nadel, 1978).

I. E. LTP AND LEARNING AND MEMORY

1. Associative Nature of LTP

A neural phenomenon such as LTP should have several characteristics that make it an attractive neural candidate for associative learning and memory mechanisms. It was previously noted that LTP involved extremely large and long-lasting increases in response magnitude. These are just two of the characteristics that have made LTP an attractive candidate mechanism. There are, however, several additional features of LTP which have increased it's attractiveness as a candidate mechanism.

It has been shown that LTP of the EPSP, recorded either in area CA1 or the dentate gyrus, is specific to the activated synapses (Andersen, Sundberg, Sveen, Swann and Wigstrom, 1980; Andersen et al., 1977; Levy and Steward, 1979; McNaughton and Barnes, 1977; McNaughton and Miller, 1979; Yamamoto and Sawada, 1981). Thus, it is not likely that the increased response strength is caused by non-specific changes in the postsynaptic cell. If a cell is capable of selective alteration of its synaptic connections, the information storage capacity of the system would be correspondingly increased.

It should be noted, however, that heterosynaptic LTP effects have been reported for the mossy fibre input to the CA3 pyramidal cells (Misgeld, Sarvey and Klee, 1979; Yamamoto and Chujo, 1978). It is not

known to what extent overlapping fibres were being activated in these studies. Heterosynaptic LTP has not been previously reported in either the dentate gyrus (McNaughton and Barnes, 1977) or area CA1 (Andersen et al., 1980) of the hippocampus.

It has also been demonstrated that LTP of one pathway may produce depression in the cell's responsiveness to other inputs (Abraham and Goddard, 1983; Berger and Levy, 1983; Levy and Steward, 1979). The significance of these heterosynaptic depression effects is not known but several proposals have been made. Heterosynaptic depression may, for example, result in a relative increase in the strength of the signal carried by the potentiated synapses (Swanson et al., 1982).

Another attractive feature of LTP is that repeated tetanization over the course of several days will increase the duration of LTP (Barnes, 1979). This is similar to learning and memory, where rehearsal improves both performance and the length of time that memory is retained.

One of the most attractive features of LTP, however, is its associative nature. If LTP is to model a phenomenon like classical conditioning, then activation of converging pathways should influence the magnitude of LTP. These converging inputs may represent information carried by those pathways responding to the unconditioned stimulus (UCS) and the conditioned stimulus (CS). McNaughton et al. (1978) demonstrated this associative property of LTP at the PP-GC synapses. Low intensity, high-frequency activation of PP afferents produced short-term potentiation (STP), lasting less than 5 minutes, but did not induce LTP. At an intensity sufficient to evoke a population spike,

however, both STP and LTP were induced. Further increases in the strength of PP tetanization and thus, the number of coactive PP fibres, resulted in further increases in the magnitude of LTP. LTP, therefore, is dependent on the activation of some critical number of PP afferents, below which LTP is never observed and above which the magnitude of LTP increases as a function of the number of coactive afferents.

Furthermore, although tetanization of the medial component of the PP will not produce LTP in the lateral component (specificity property), it will increase the level of LTP in a concurrently tetanized lateral component (McNaughton et al., 1978). In these studies only the population EPSP was measured so the effect of these manipulations on the population spike is not known. In addition, these effects broke down when delays were interposed between the two trains. As behavioural conditioning is normally demonstrated with CS-UCS delays of a few hundred milliseconds or more, this phenomenon has limited utility as a model for associative memory mechanisms.

Levy and Steward (1979), utilizing the ipsilateral and crossed entorhinal-dentate projections, have provided an additional demonstration of the cooperative nature of LTP. Ordinarily, the crossed projection, which arises from collaterals of the ipsilateral system (Goldowitz, White, Steward, Cotman and Lynch, 1975; Wilson, Levy and Steward, 1979), is too sparse to produce LTP. If, however, a lesion is placed in the ipsilateral system, the crossed projection will sprout and fill the vacated synaptic sites. The system, with its increased innervation, is now capable of LTP (Wilson et al., 1979). It is also possible to produce LTP in the crossed projection if it is concurrently

tetanized with the ipsilateral projection (Levy and Steward, 1979). These findings provide additional support for the cooperative nature of LTP.

The crossed and ipsilateral entorhinal-to-dentate projections, unlike the medial and lateral PP, will show cooperative interactions even when delays are interposed between the two trains. Both the order and temporal interval between activation of the crossed and ipsilateral PP systems are important. LTP of the crossed projection is induced only if it is activated within a 20 ms period prior to activation of the ipsilateral system. If the crossed projection is activated after ipsilateral activation, or more than 200 ms prior to ipsilateral activation, the magnitude of the crossed PP-GC response is depressed or depotentiated (Levy and Steward, 1983). Depotentiation of previously activated synapses provides a possible mechanism (in addition to passive decay) for the reduction of LTP levels.

Although these are interesting results, there are a number of problems associated with examining interactions between such anatomically similar pathways. First, when examining the two components of the PP it is possible that McNaughton et al. (1978) were actually activating some additional subset of the same pathway. Second, the two components of the PP have a similar site of origin and probably use the same transmitters. Third, as far as associative mechanisms are concerned, the pathways probably convey similar information to the hippocampus as they originate from a common cortical structure. Finally, the allowable temporal delays, even in the crossed projection of the PP, are rather restricted.

Associative interactions have, however, been demonstrated between less similar pathways, for example, between the PP and commissural projection to the dentate gyrus (Buzsaki and Czeh, 1981; Buzsaki and Eidelberg, 1981; Douglas, Goddard and Riives, 1982; Douglas, McNaughton and Goddard, 1983; Goddard and Riives, 1981; McNaugton et al., 1978). In a paired-pulse paradigm the commissural input was shown to suppress subsequent PP-GC population spikes (Buzsaki and Czeh, 1981; Buzsaki and Eidelberg, 1981; Buzsaki and Eidelberg, 1982; Douglas et al, 1982; Douglas et al, 1983). One mechanism proposed for this inhibitory effect was a direct excitation of the hilar inhibitory basket cell interneurons by the commissural input, a feed-forward inhibition (Buzsaki and Czeh, 1981; Douglas et al., 1983).

Given the inhibitory nature of this interaction, the commissural-PP system was utilized to test Hebb's (1949) hypothesis that increases in synaptic strength occurred whenever cell discharge was concurrent with afferent activation. If LTP is a component of learning, and if learning depends on cellular discharge concurrent with afferent activation, commissural stimulation may be expected to block LTP by inhibiting GC discharge. This hypothesis was tested by Douglas et al. (1982) and McNaughton et al. (1978). Both single pulses and high-frequency commissural trains were capable of blocking GC discharge but only the trains were found to reduce LTP levels. These findings did not appear to support Hebb's (1949) hypothesis. They did, however, provide further evidence for the associative nature of LTP.

Again, there was a critical window for these interaction effects. The commissural trains had to occur within a 50 ms period

prior to the PP trains and not more than 1 ms after the PP trains. Although an associative memory mechanism could perhaps be based upon such a negative interaction effect, the brief duration of the window also limits its suitability.

There is also evidence for heterosynaptic interactions between pathways more distinct, both anatomically and neurochemically, than the different components of the PP or commissural system. Laroch and Bloch (1982), for example, examined the effects of intermittant (6 s on and 3 s off for 90 s) posttrain reticular tetanization on the duration of PP-GC LTP, the rate at which LTP was established, and the asymptotic magnitude of LTP. Trains applied to the mesencephalic reticular formation were shown to increase all the above measures of PP-GC LTP. The duration of LTP, for example, was extended from 1 day to several days by posttrial reticular trains. This increase may, however, have simply been due to the greater magnitude of LTP induced by the paired trains. A second, and more likely, explanation is that the difference was due to the short duration (one-day) of the LTP effect induced by the PP-only trains. LTP in control animals generally lasts for at least one week (Barnes, 1979; Douglas and Goddard, 1975; Racine et al., 1983; Robinson and Racine, 1984).

Evidence for the involvement of specific neurochemical systems in LTP is still controversial. Depletion of central noradrenaline, for example, has been found to either decrease (Robinson and Racine, 1984) or have no effect on LTP of the PP-GC population spike (Bliss, Goddard and Riives, 1983). LTP of the PP-GC population EPSP was either increased (Robinson and Racine, 1984) or decreased (Bliss et al., 1983).

These differences are most likely due to the effect depletion has on the evoked response (i.e. a masking effect) rather than a direct effect on LTP. If masking effects are taken into account, noradrenaline depletion has no effect on LTP (Robinson and Racine, 1984). Masking effects, when examined, have been reported for several drug-LTP studies (Dolphin, 1983; Sastry, Chirwa, Goh, Maretic and Pandanoboina, 1984; Sastry, Goh and Pandanoboina, 1984).

Concurrent tetanization of the PP and locus coeruleus, with short-duration trains, also does not affect LTP (Abraham and Goddard, 1982). Harley, Lacaille and Milway (1982), however, found that long-lasting (10-60s) locus coeruleus trains will increase the amplitude of subsequently evoked PP-GC population spikes for up to 30 minutes. These effects are mimicked by iontophoretically applied noradrenaline (Neuman and Harley, 1983).

2. LTP: Relationship to Learning and Memory

Despite having many of the characteristics one would hope to find in a neural system which encodes and stores information, it has become clear that LTP-like phenomena must be shown to occur during learning if it is to have any utility as a model phenomenon. Also, it should not occur simply as a consequence of learning but, should somehow be related to the learned behaviour itself. For example, the incremental changes in behavioural performance, which occur across acquisition sessions, should be matched by some form of incremental change in LTP (Thompson et al., 1978). Such changes could, however, be

distributed throughout the central nervous system and therefore be difficult to detect.

As previously mentioned, one proposed role for the hippocampus is to form a spatial map of the environment (O'Keefe and Nadel, 1978). Barnes and her collegues have utilized a number of approaches in an attempt to relate LTP to spatial learning in rats. First, Barnes (1979) has shown that an animal's retention of a spatial learning task was correlated with the persistence of LTP. Second, Sharp, McNaughton and Barnes (1983) attempted to induce LTP in rats by exposing them to complex spatial environments. Rats transferred from an impoverished to a spatially enriched environment exhibited a gradual increase in the amplitude of both the PP-GC population EPSP and spike (an LTP-like effect). With continued exposure, however, the amplitude decreased to that level observed in the impoverished environment. LTP could be induced again if the animal was transferred to a second complex environment. Third, animals were trained to asymptotic performance on a circular maze platform, given LTP stimulation, and then retested on either the same maze or a different maze. LTP was found to have no effect on task retention but did impair the animals ability to learn a new spatial task (Barnes and McNaughton, 1983). Together, these results raise the possibility that the hippocampus may be necessary for the acquisition of a spatial map and that a process similar to LTP may occur during the formation of the spatial map.

Berger et al. (1976) have utilized a model systems approach to investigate the relationship between LTP and learning. They found that CS-UCS (tone-corneal air puff) pairings, which condition the nictitating

membrane response, increased the level of hippocampal unit activity above spontaneous pre-CS levels. This increase was specific to the paired paradigm. Presentation of the tone by itself resulted in no behavioural response and no increase in unit activity. UCS-only presentations resulted in a reflexive nictitating membrane response, as would be expected, but only a small non-significant increase in unit activity.

The increased neural activity was therefore specific to those animals which formed an association between the CS and UCS. In addition, there was no evidence of behavioural learning until unit activity had increased significantly above spontaneous levels. Furthermore, variables such as the CS-UCS interval, which influence conditioned behaviour, also influenced hippocampal unit activity, and the direction of this influence could be predicted from the known behavioural effects (Hoehler and Thompson, 1980). The spatio-temporal characteristics of the increased unit activity, however, were correlated with the form of the behavioural response, suggesting a role in performance rather than association (Morris, 1983).

The medial septum was shown to provide a different type of information to the hippocampus. Unit activity of the medial septum increased to the onset of both the tone CS and air-puff UCS (Berger and Thompson, 1977), but failed to increase further with extended training. It was also evoked by unpaired as well as paired CS and UCS presentations. The increased unit activity recorded in the medial septum was thought to reflect an increase in arousal. This suggestion received some support from a subsequent study demonstrating that

spontaneous hippocampal EEG activity, in the range of theta, was predictive of faster learning of the nictitating membrane response than was EEG at frequencies greater than theta (Berry and Thompson, 1978). Furthermore, medial septal lesions, which impair hippocampal theta, also impaired conditioning of the nictitating membrane response (Berry and Thompson, 1979).

Given the large increases in unit activity that occur with classical conditioning of the nictitating membrane response, it was hypothesized that this increase may also appear in the PP-GC field potential. In a test of this hypothesis, it was shown that the amplitude of the PP-GC population spike increased in animals that received CS-UCS presentations that resulted in behavioural conditioning (Berger, Laham and Thompson, 1980; Thompson, Berger, Berry, Hoehler, Kettner and Weisz, 1980). The population spike amplitude did not change in animals who had received unpaired or random CS-UCS presentations. Also, there was no evidence of behavioural conditioning until the amplitude of the PP-GC population spike was near asymptotic levels.

There were a number of similarities between the "potentiated" response to the CS in these learning studies, and potentiation seen in LTP studies. Both required a small number of stimulations, had a similar time course for development, a similar magnitude of change, were very long-lasting and were produced by a specific range of stimuli (see Swanson et al., 1982). Clark, Berger and Thompson (1978) have also shown that entorhinal unit activity increases above spontaneous levels as the CS-UCS association is formed.

More recent evidence suggests that the critical circuits for the acquisition and retention of standard delay conditioning of the rabbit's nictitating membrane may be located in cerebellar nuclei (McCormick, Lavond, Clark, Kettner, Rising and Thompson, 1981). It is interesting that cerebellar nuclei also exhibit LTP (Racine, et al., 1984).

The increase in hippocampal unit activity that occurs during conditioning is, however, similar to that recorded from cerebellar nuclei, suggesting the two structures receive similar information. It is possible that the two structures utilize the information for different purposes. Rabbits with hippocampal lesions, for example, can still learn a standard delay conditioning task (Berthier and Moore, 1980; Orr and Berger, 1981; Solomon, 1977; Solomon and Moore, 1975) but are unable to learn in situations such as latent inhibition, blocking, discrimination reversal and trace conditioning (Berthier and Moore, 1980; Orr and Berger, 1981; Solomon, 1977, 1979, 1980; Solomon and Moore, 1975; Weisz, Solomon and Thompson, 1980). Given these results, Solomon (1979; 1980) has suggested that the hippocampus may be involved in temporal mapping, rather than the actual association of the CS and UCS.

According to the temporal mapping view, the hippocampus encodes the relationships between stimuli and "tunes out" irrelevant stimuli. Therefore, hippocampal animals may not be expected to show conditioned behaviour when there is a delay between the CS offset and the UCS onset, such as occurs in trace conditioning. With a delay the animal may ignore the second stimulus. Temporal coding may be important for attentional processes, helping the organism attend to important

stimuli and to ignore those of no survival value (see Solomon, 1979, 1980).

O'Keefe, Nadel and Wilner (1979) have taken exception to this addition to their theory, arguing that the animal can condition to background spatial cues, and that a temporal mapping function is not necessary. Yet, Berthier and Moore (1980) found that animals with hippocampal lesions had no difficulty learning a differential conditioning task when the CS^+ and CS^- differed in terms of their spatial location. A strictly spatial mapping view would have predicted an acquisition deficit on this task. A temporal mapping view, however, would predict normal differential conditioning as the CS- would be a relevant stimulus predicting the absence of reinforcement (the hippocampus only tuning out irrelevant stimuli) (Solomon, 1979). As the cognitive mapping theory has difficulty in accounting for conditioning of the rabbit nictitating membrane response, as well as other classical conditioning tasks, Hoehler and Thompson (1979) have suggested that these tasks have a working memory component rather than a spatial component.

Together, the nictitating membrane and LTP data suggest that classical conditioning and PP trains induce similar changes in the amplitude of the PP-GC population spike. Furthermore, both stimulation (electrical)-induced and learning-induced LTP may share a common mechanism. Thompson, Mamounas, Lynch and Baudry (1983) have shown that classical conditioning of the nictitating membrane response increases the number of hippocampal glutamate receptors. The number of glutamate

receptors does not change in animals that receive random CS-UCS pairings. Similarly, high-frequency activation increases the number of postsynaptic glutamate receptors (Lynch et al., 1982). Although these results are encouraging, it is difficult to imagine why such a limited experience would produce such large changes in the hippocampal cell population.

I. F. PRESENT EXPERIMENTS

I have been interested in heterosynaptic interaction effects between neural systems more distinct, both anatomically and neurochemically, than the components of the PP. The septal (septo-dentate, SD) and entorhinal inputs (perforant path, PP) to the dentate gyrus satisfy these criteria. As noted above, both the SD-GC and the PP-GC pathways exhibit LTP. It was not, however, known if these two inputs showed cooperativity effects in the production of LTP. Yet, the anatomical and neurochemical differences between these two sites make the SD and PP an attractive system to investigate the mechanisms underlying associative interaction effects.

Interaction effects between these two pathways have not been previously described for LTP phenomena. There was evidence, however, that the septum exerts a powerful influence on transmission from the PP to the dentate gyrus GCs. These effects were short-lasting and involved the application of single pulses to the two inputs. Alvarez-Leefmans and Gardner-Medwin (1976), for example, showed in the rabbit that paired stimuli delivered to the medial septum and the PP

increased the magnitude of the PP-GC population spike but had no effect on the population EPSP. Facilitation of the population spike occured if the septal pulse was delivered up to 600 ms before the PP pulse. There was, however, depression of the spike at SD-PP interpulse intervals from 2 to 10 ms. The facilitation, but not the depression, is present in the rat dentate gyrus (Fantie and Goddard, 1982). Obviously, a neural phenomenon of such limited duration has little utility as an associative model for long-term memory.

I have previously shown that high-frequency coactivation of SD and PP afferents to the dentate gyrus increases the level of LTP of the PP-GC population spike above that produced by PP-only trains (Robinson and Racine, 1982a, b). Preliminary evidence from this lab, and others (Fantie, 1982), suggests that coactivation may also increase the level of LTP of the synaptic component (EPSP) of the PP-GC field potential. This thesis is directed first towards a description of the cooperativity phenomenon and the parameters which affect SD and PP interactions (e.g. effects of SD train intensity and temporal order), and second towards the elucidation of mechanisms.

CHAPTER II. GENERAL METHODS

II.A. SURGERY

Male Long-Evans Hooded rats (310-550 g) were anesthetized with sodium pentobarbitol (65 mg/ml) or urethane (250 mg/ml - Chapter V, Experiment 2). The depth of anesthesia was sufficient to eliminate reflexive movement to a tail pinch.

With the upper incisor bar at 5 mm above the interaural line, the skull was exposed and bone flaps, approximately 2mm by 2mm, were removed to allow for bilateral electrode implantation. The coordinates, relative to bregma, were 3.5 mm posterior and 2.2 mm lateral for the dentate recording electrodes and 7.9 mm posterior and 4.4 mm lateral for the PP stimulating electrodes. The SD electrode was lowered, on the midline, approximately 2 mm anterior to bregma. In addition, ground electrodes were placed in the skull posterior to lambda and anterior to bregma. The exposed cortex was kept moist by the application of warm mineral oil.

II. B. STIMULATION AND RECORDING

While monitoring evoked potentials, the stimulating electrodes (two 245 µm diameter, teflon-coated stainless steel wires, twisted together and separated at the tips by 0.5 mm) and recording electrodes (single strand, 245 µm diameter, teflon-coated stainless steel) were lowered to the PP and the hilus of the fascia dentata, respectively. When a positive field potential was located, indicating the recording

electrode was in the cell body layer, small adjustments were made in the position of the recording and stimulating electrodes in order to maximize the response amplitude.

The SD electrode, used to activate the medial septal input, (same construction as that for the PP) was then lowered while monitoring responses in the dentate gyrus. When a SD-GC response was obtained, the SD and PP stimulation pulses were arranged to produce paired-pulse facilitation effects. Typically this involved applying the SD pulse 2 ms before the PP pulse while monitoring the PP-GC response. The SD electrode was then lowered to that level of the medial septum which produced the maximum facilitation of the PP-GC population spike. The amplitude of the SD-GC field response corresponding to a maximum facilitation of the PP-GC population spike was highly variable, ranging from 0.10 to 2.0 or 3.0 mV.

In initial experiments, all electrode locations were verified by standard histological procedures. Utilizing the electophysiological criteria mentioned above, however, always produced accurate electrode placement. Thus, in subsequent experiments electrode locations were rarely verified by histological procedures.

Granule cell field responses were amplified by a Grass P15 preamplifier (1 Hz and 3KHz cut-off frequencies). Subsequent amplification was provided by an additional Grass preamplifier (Model 7P5B). Data was collected on-line by an LSI-11 computer. Stimulation during testing consisted of both monophasic single test pulses (0.1 Hz or 0.05 Hz) and brief high-frequency trains, delivered by a Grass S88

stimulator. A photoelectric stimulus isolation unit (Grass model PSIU6) was used to deliver constant current pulses.

High-frequency PP stimuli consisted of 3 trains, 1-5 s apart, each composed of 20 pulses at a frequency of 400 Hz. The intensity, determined individually for each animal, was sufficient to consistently evoke a small population spike in response to a single PP volley. A minimum of 4 sets of trains were applied separately to ensure the level of LTP had reached its maximum level (saturated) for that intensity. Test responses were recorded for at least 5 min before applying the next set of trains. The final 2 sets of PP trains were each followed by at least a 15 min test period to confirm that the LTP effects had reached saturation. When further increases in LTP were not apparent, the trains were applied to both the SD and PP fibres. SD trains also consisted of 20 pulses at 400 Hz.

During the train run the control hemisphere received constant intensity PP test pulses to control for any effects common to both hemispheres. A constant level of anesthesia was maintained by IP infusion of sodium pentobarbitol (Harvard Apparatus, Model 901). The flow rate was 0.10-0.15 ml/hr.

II.C. DATA ANALYSIS

The amplitude of the population EPSP's, evoked by single test pulses, was measured as the slope of the initial positivity. The slope provides a measure of the strength of the synaptic response in the population (Lomo, 1971a). EPSP amplitude measures were not used, as the

intensity of PP stimulation was consistently high enough so that a population spike was superimposed on the EPSP waveform. Thus, the true amplitude of the EPSP could not be measured. The relative change in the slope measure, however, is comparable to that of an amplitude measure provided the slope is measured prior to spike onset. The population spike, an indication of cell discharge (Anderson et al., 1971a), was measured from the tangent, joining spike onset and offset, to the spike peak height (Adamec, McNaughton, Racine and Livingston, 1981).

The EPSP and spike measures were stored on a hard disk (DSD 880) for later analysis. To determine the magnitude of LTP the absolute values were converted to a percent change relative to the mean amplitude of the pretrain baseline. LTP levels were determined from the average percent increase 13-15 min after the trains. The magnitude of LTP effects depend, to some extent, on the initial magnitude of the EPSP and spike, so some method was needed to standardize initial response amplitudes. This was done by applying test pulses at an intensity sufficient to consistently evoke a small population spike. This intensity was also used for the PP trains.

CHAPTER III. HETEROSYNAPTIC INTERACTIONS BETWEEN SEPTAL AND ENTORHINAL AFFERENTS TO THE DENTATE GYRUS: LONG-TERM POTENTIATION EFFECTS

Several lines of investigation have demonstrated that LTP is a cooperative process dependent on the coactivation of a critical number of afferents (Levy and Steward, 1979; McNaughton et al., 1978). Concurrent tetanization of both the medial and lateral components of the PP, for example, increases the level of LTP of the PP-GC population EPSP above that produced by tetanization of either component alone (McNaughton et al., 1978). Furthermore, increasing the strength of the PP trains, and thus the number of coactive fibres, increases the magnitude of LTP. This cooperativity effect occurs regardless of which component of the PP is tested (McNaughton et al., 1978).

There are, however, numerous problems associated with the examination of heterosynaptic interactions between pathways that are so closely related anatomically. One major problem is the magnitude of current spread from the stimulating electrodes. It is possible, for example, that McNaughton et al. (1978) were actually activating some additional subsets of the pathways when applying concurrent stimulation. If this were true, then the manipulation would be equivalent to increasing the intensity of the PP trains. Although still a cooperativity effect, it becomes less interesting than interactions between distinct pathways.

To alleviate this problem, the present thesis will examine heterosynaptic interaction effects between the PP and SD afferents to the dentate gyrus. Not only are these two pathways widely separated anatomically (so that current spread is not a confounding variable) but they also differ in both the location of their terminal fields within the dentate gyrus and in the chemical neurotransmitter used for synaptic transmission. In addition, the possibility that these two nuclei convey different types of information to the dentate gyrus (see Introduction) serves to increase their attractiveness for the study of associative mechanisms. The present series of experiments are concerned with the effect of concurrent tetanization of the PP and SD inputs to the GCs of the dentate gyrus.

EXPERIMENT 1. HETEROSYNAPTIC COOPERATIVITY IN ANESTHETIZED RATS: BASIC DEMONSTRATION

The purpose of Experiment 1 was to first determine if the magnitude of PP-GC LTP could be increased by concurrent tetanization of the SD and PP inputs, and second to determine if the SD response was similarly affected. It was also of interest to determine which component (population spike and/or population EPSP) of the evoked response was affected by the concurrent trains.

METHODS

High-frequency PP stimuli consisted of 3 trains, 1 s apart, each composed of 20 pulses at 400 Hz. The intensity, determined

individually for each animal, was sufficient to consistently evoke a small population spike in response to a single PP pulse. A minimum of 4 sets of PP trains were applied, at least 5 min apart, to ensure that the level of LTP had reached its maximum level (saturated) for that intensity. Test responses were recorded (0.1 Hz) during the intervals between sets. The final two sets of trains were followed by at least a 15 min test period to ensure the LTP effects had saturated. When further increases in LTP were not observed, the trains were applied to both the SD and PP afferents. SD trains also consisted of 20 pulses at 400 Hz. The amplitude and width of the pulses in each SD train were 1200 μA and 100 μs, respectively. Seventeen male Long-Evans Hooded rats were used for this study.

In 10 of these animals, the effects of separate and combined SD and PP trains on the SD-GC field response was examined. This was done to determine if the trains, either separately or concurrently, also affected the SD-GC response. In these animals, trains were initially applied to the SD input alone, and test pulses were applied to the SD as well as the PP input. Test pulses were applied alternately to the PP and SD at 5 s intervals. The test pulse frequency for each pathway was 0.1 Hz. Population responses were recorded in the dentate gyrus.

RESULTS

In agreement with previous reports (McNaughton and Barnes, 1977; McNaughton and Miller, 1979; and others), LTP was specific to the inputs which were activated by the high-frequency trains. LTP of the SD-GC

EPSP occurred following SD-tetanization but was not observed following PP tetanization (see Figure 4). SD tetanization did, however, result in a small transient heterosynaptic increase in the PP-GC population spike. Two examples of this heterosynaptic increase are shown in Figure 5. This heterosynaptic effect was not observed in all animals and, when present, rarely lasted longer than 3 min. This effect therefore represents short-term, not long-term, potentiation. The SD train-induced increment in spike amplitude was not accompanied by any measurable change in the PP-GC EPSP.

LTP of the PP-GC response was specific to the PP trains, and resulted in a significant potentiation of both the population EPSP and spike which lasted for at least 15 min (see Figure 6). PP trains had no effect on the amplitude of the SD-GC field EPSP. These results have, in part, been presented elsewhere (Robinson and Racine, 1982a,b). In a brief communication, McNaughton and Miller (1979) also showed that LTP of either the PP or SD to GC response was specific to those pathways activated at a high-frequency.

Although LTP was specific to the activated inputs, there were, nevertheless, heterosynaptic interactions between the SD and PP inputs. Concurrent tetanization of the SD and PP fibres resulted in a significant additional increment in the amplitude of the PP-GC population spike that was above that observed following PP trains alone (see Figure 6). This additional increment lasted for at least 15 min and, therefore, represents an increase in the magnitude of LTP. There was no comparable increase in the level of LTP of the SD-GC EPSP as a

Figure 4: LTP of the SD-GC field response. Continuous record of the mean percent change in the SD-GC field response following SD-only, PP-only and combined trains. Sample average field potentials for the pretrain condition and for the last 2 min following application of the separate and combined SD and PP trains are also shown. Trains were applied at times indicated by the astericks (*).

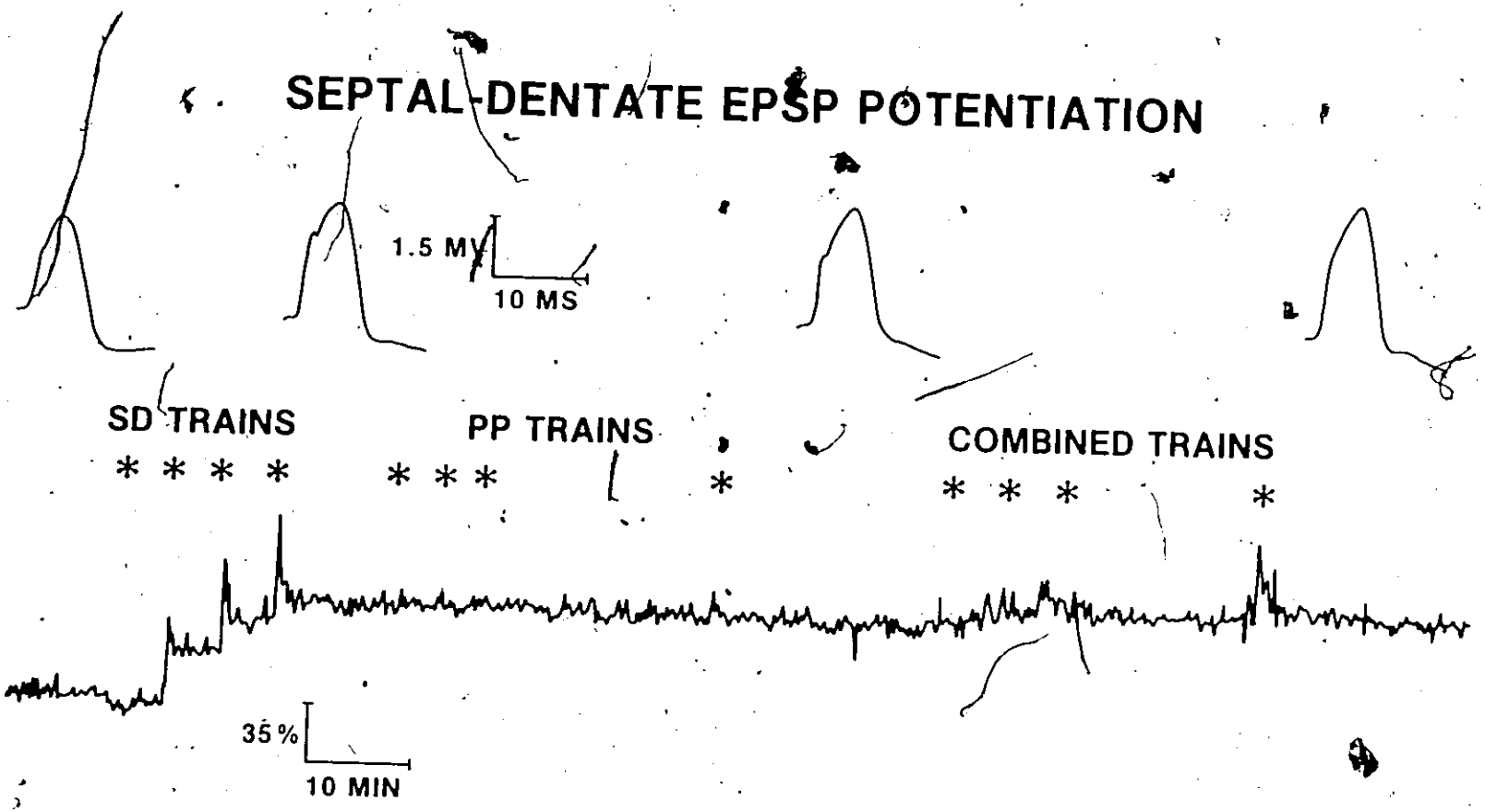

SEPTAL-DENTATE EPSP POTENTIATION
1.5 MV
10 MS
SD TRAINS
PP TRAINS
COMBINED TRAINS
35 %
10 MIN

Figure 5: Short-term heterosynaptic potentiation of the PP-GC population spike, in two different animals, as a result of SD-only trains.

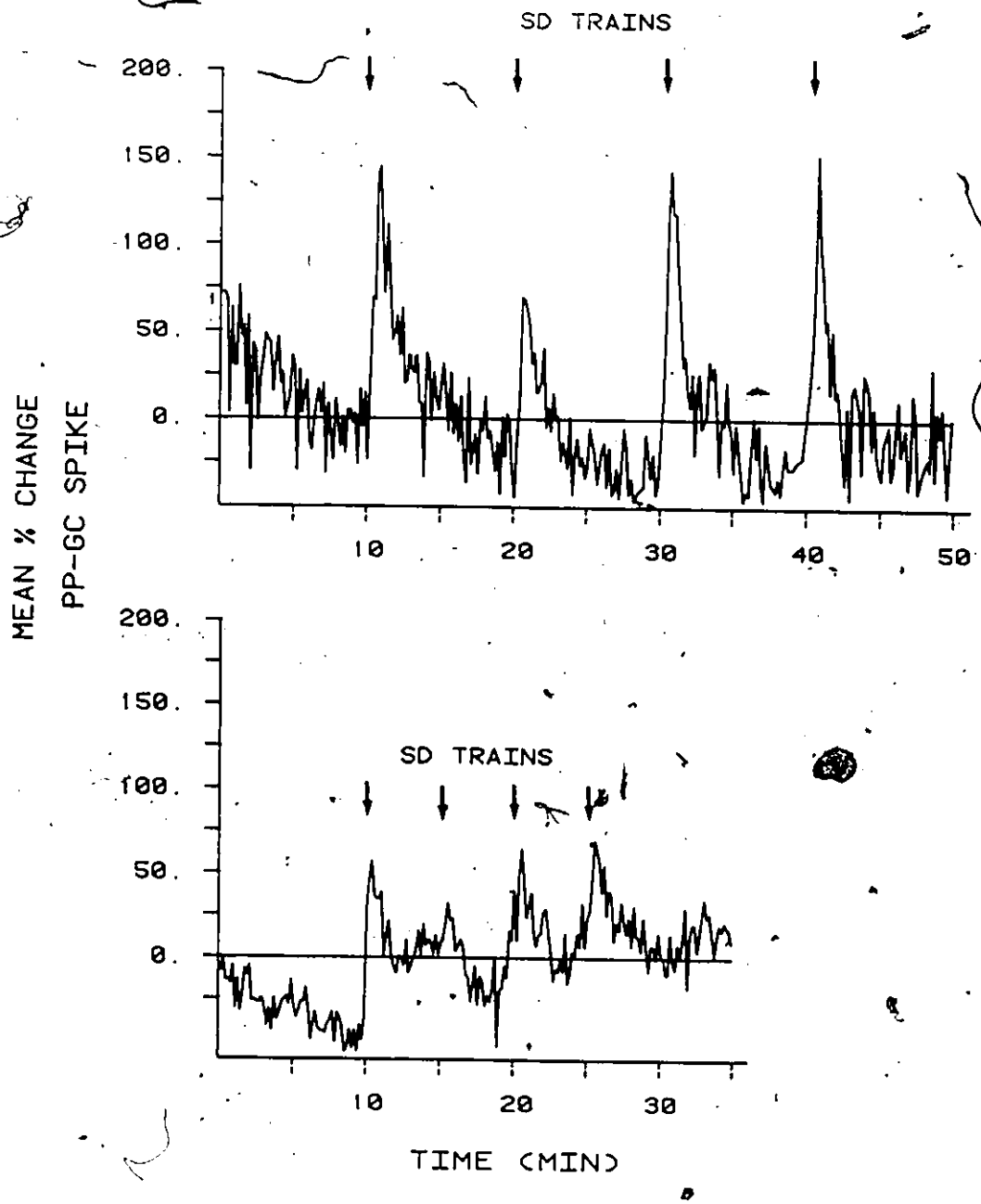
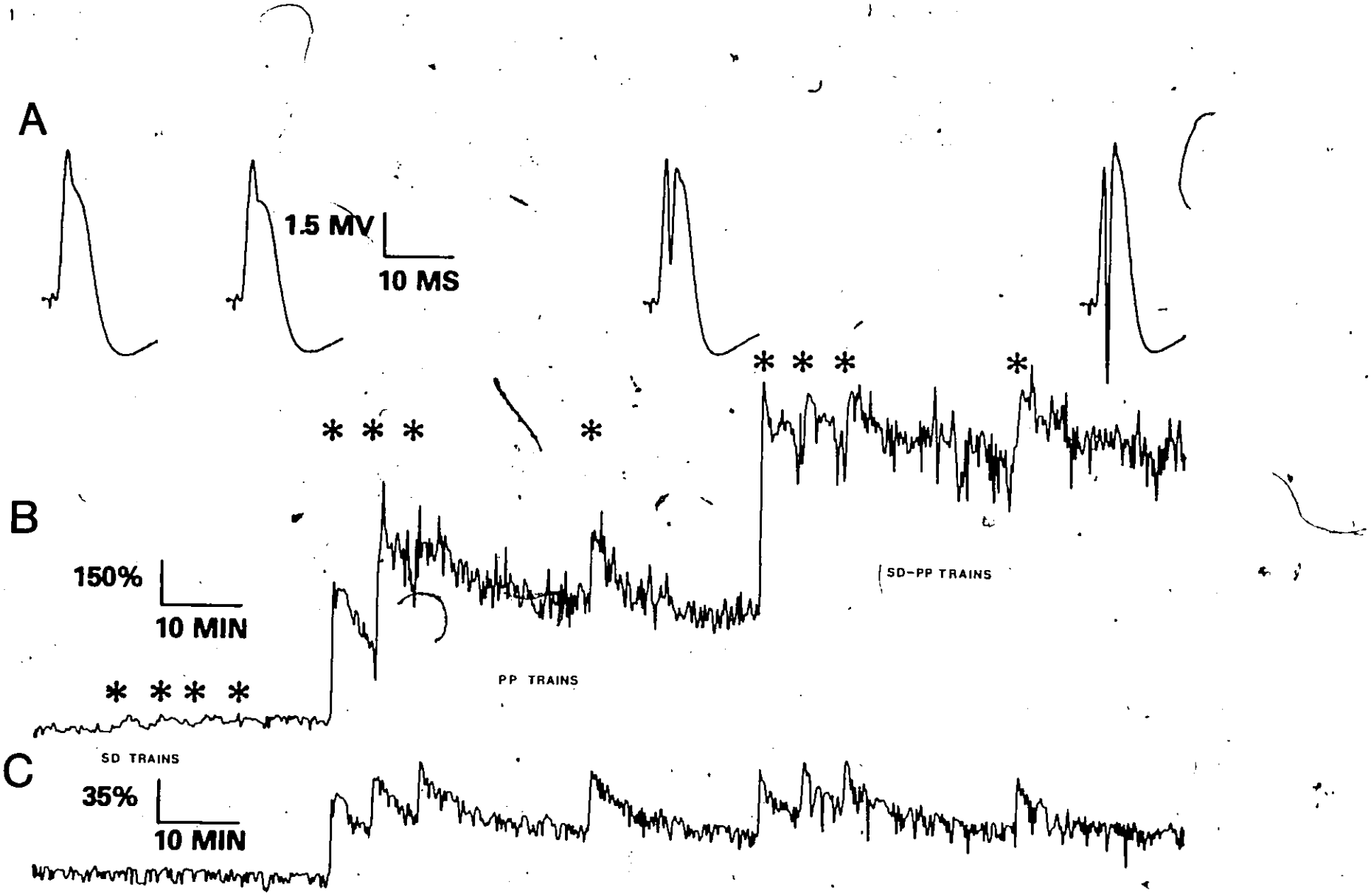

SD TRAINS
200.
150.
100.
50.
0.
10
20
30
40
50
MEAN % CHANGE
PP-GC SPIKE
200.
150.
100.
50.
0.
SD TRAINS
10
20
30
TIME (MIN)

Figure 6: LTP of PP-GC field responses. A. Sample average field potentials for the pretrain condition and for the last 2 min following application of the separate and combined SD and PP trains. B. Continuous record of the mean percent change in the PP-GC population spike. C. Continuous record of the mean percent change in the PP-GC population EPSP. For the population EPSP, the mean percent change in the control hemisphere was subtracted from that in the test hemisphere. Trains were applied at times indicated by the astericks (*).


A
1.5 MV
10 MS
B
150%
10 MIN
SD-PP TRAINS
PP TRAINS
SD TRAINS
C
35%
10 MIN

result of the combined trains (see Figure 4). It should be noted, however, that the cooperativity effect involved an increase in LTP of the PP-GC population spike and there was no comparable component in the SD-GC response.

Figure 7 is a summary figure for the animal shown in Figure 6, and represents the general findings of this experiment (the magnitude of LTP induced by the PP-only trains and the additional increment in LTP following the combined trains in this animal were close to the group mean). Briefly, SD-only trains resulted in a mean percent increase in the amplitude of the SD-GC population EPSP of 55 ± 5% ($\bar{X}$ ± SEM), 8 to 10 minutes after the last SD-only train set (see Figure 7A), no change in the EPSP of the PP-GC potential (see Figure 7B) and only a small transient effect on the PP-GC population spike (see Figure 7C). PP trains resulted in a mean percent increase in the amplitude of the PP-GC population spike of 451 ± 13% and a 35 ± 2% increase in the amplitude of the PP-GC field EPSP, but had no effect on the SD-GC response. Concurrent tetanization, resulted in a further increment in LTP of the PP-GC spike (1107 ± 22%) but no further increase in either the PP-GC EPSP (36 ± 3%) or the SD-GC response (60 ± 4%).

The results for all animals are summarized in Figure 8A. For the PP-GC population spike, the mean percent increase over the pretrain baseline, following application of the last 2 sets of PP-only trains, was 488 ± 134% and 499 ± 139%. Concurrent tetanization of both the SD and PP afferents increased the mean level of LTP to 819 ± 161% and 864 ± 166%, for the final two combined train sets. The magnitude of the

Figure 7: LTP of both PP-GC and SD-GC field responses from the same animal whose data appear in Figures 4 and 6. Allows direct comparison of separate and combined train effects on PP-GC and SD-GC field potentials. A. LTP of SD-GC population EPSP. B. PP-GC population EPSP. C. LTP of PP-GC population spike.

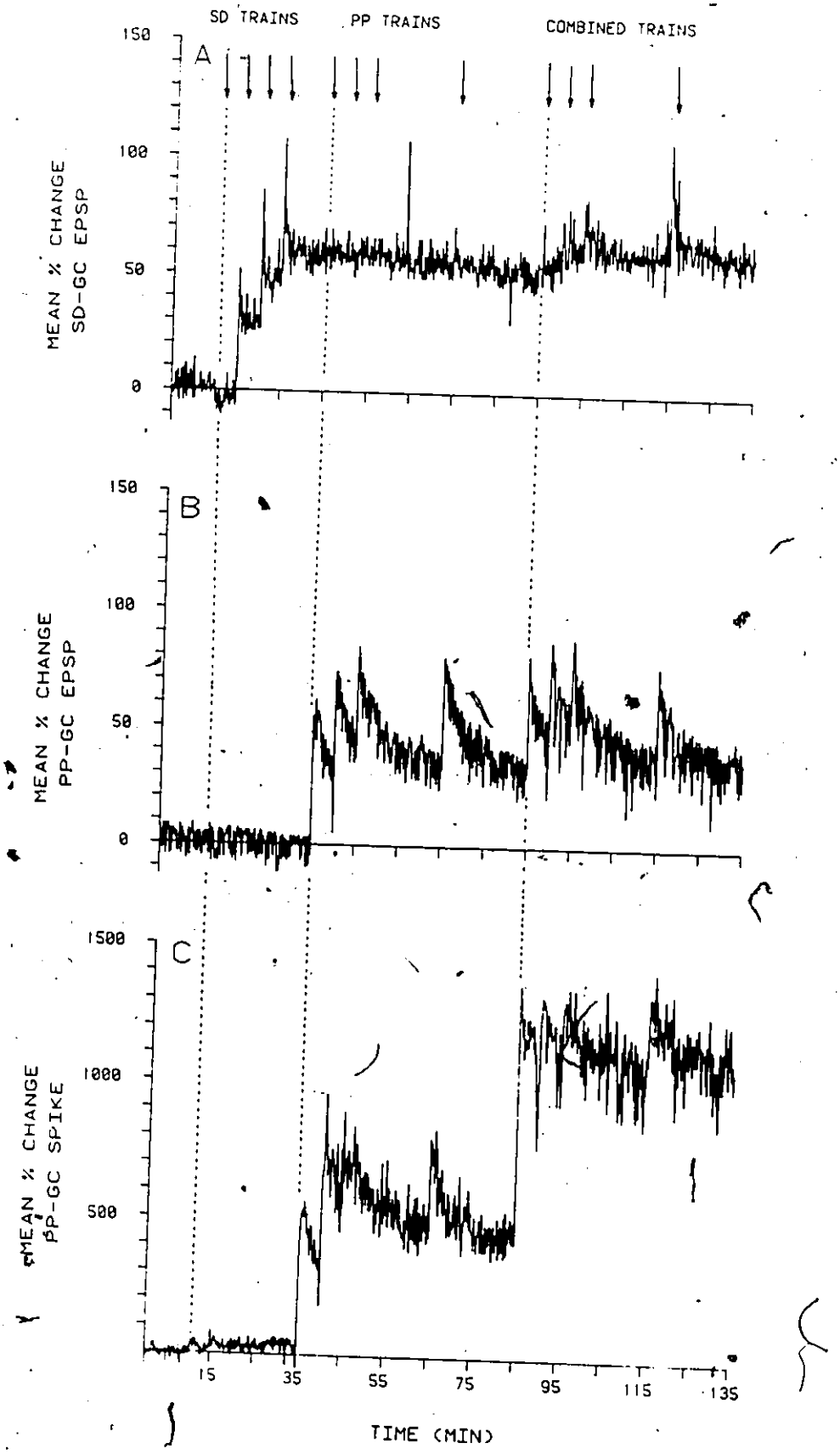
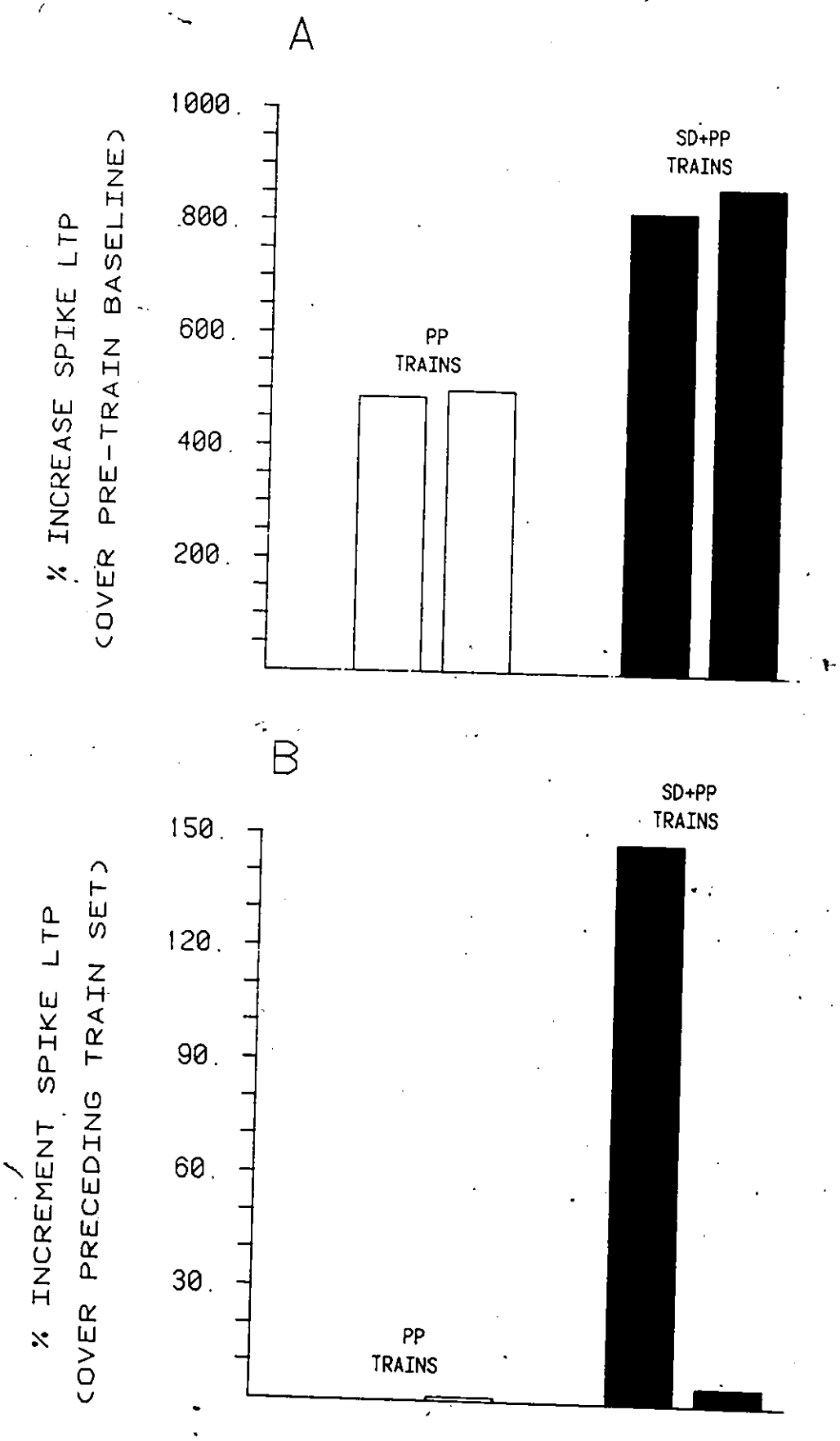

SD TRAINS
PP TRAINS
COMBINED TRAINS
A
B
C
150
100
50
0
MEAN % CHANGE
SD-GC EPSP
MEAN % CHANGE
PP-GC EPSP
1500
1000
500
MEAN % CHANGE
PP-GC SPIKE
15
35
55
75
95
115
135
TIME (MIN)

Figure 8: A. Mean percent increase in PP-GC spike LTP, over the pretrain baseline, produced by activation of either the PP alone or by the concurrent activation of the SD and PP afferents. The LTP effect following the last two sets of PP-only (blank bars) and combined SD and PP trains (solid bars) are shown. B. Mean percent increment in LTP levels, above that produced by the immediately preceding train set. Thus, additional LTP was calculated as the percent change, over the baseline level of LTP, 13 to 15 min after the previous train set. Repeating either the PP-only or the combined trains produced little additional increment in LTP.


A
% INCREASE SPIKE LTP
(OVER PRE-TRAIN BASELINE)
1000.
800.
600.
400.
200.
PP
TRAINS
SD+PP
TRAINS
B
% INCREMENT SPIKE LTP
(OVER PRECEDING TRAIN SET)
150.
120.
90.
60.
30.
PP
TRAINS
SD+PP
TRAINS

additional increment in LTP, following the combined trains was highly significant ($p<.001$; t-test for correlated means). The large degree of variability was likely related to differences in the magnitude of the LTP effect induced by the PP-only trains.

Another method of expressing these results is to consider the percent increment in LTP over that produced by the immediately preceding train set. Thus, LTP is now being measured from an already elevated baseline. Therefore, if subsequent train sets have no effect, the percent increment would be zero. When calculated for each animal, and then averaged, the final set of PP-only trains produced a mean percent increment of 1.34 ± 2.99% above that level of LTP observed following the next to last set of PP trains (see Figure 8B). The small magnitude of this additional increment clearly indicates that the level of LTP, produced by the PP-only trains, had saturated. There was an average additional increment of 147.90 ± 52.57% (range: 24.83 - 712.50%) in the level of LTP from the final set of PP-only trains to the second to last set of combined trains. When repeated, the fourth set of concurrent SD and PP trains produced an additional LTP increment of only 5.04 ± 2.68%. Thus, the largest increments in spike amplitude occurred between, rather than within a condition (i.e. from pretrain baseline to PP-only trains, and from PP-only trains to concurrent SD and PP trains).

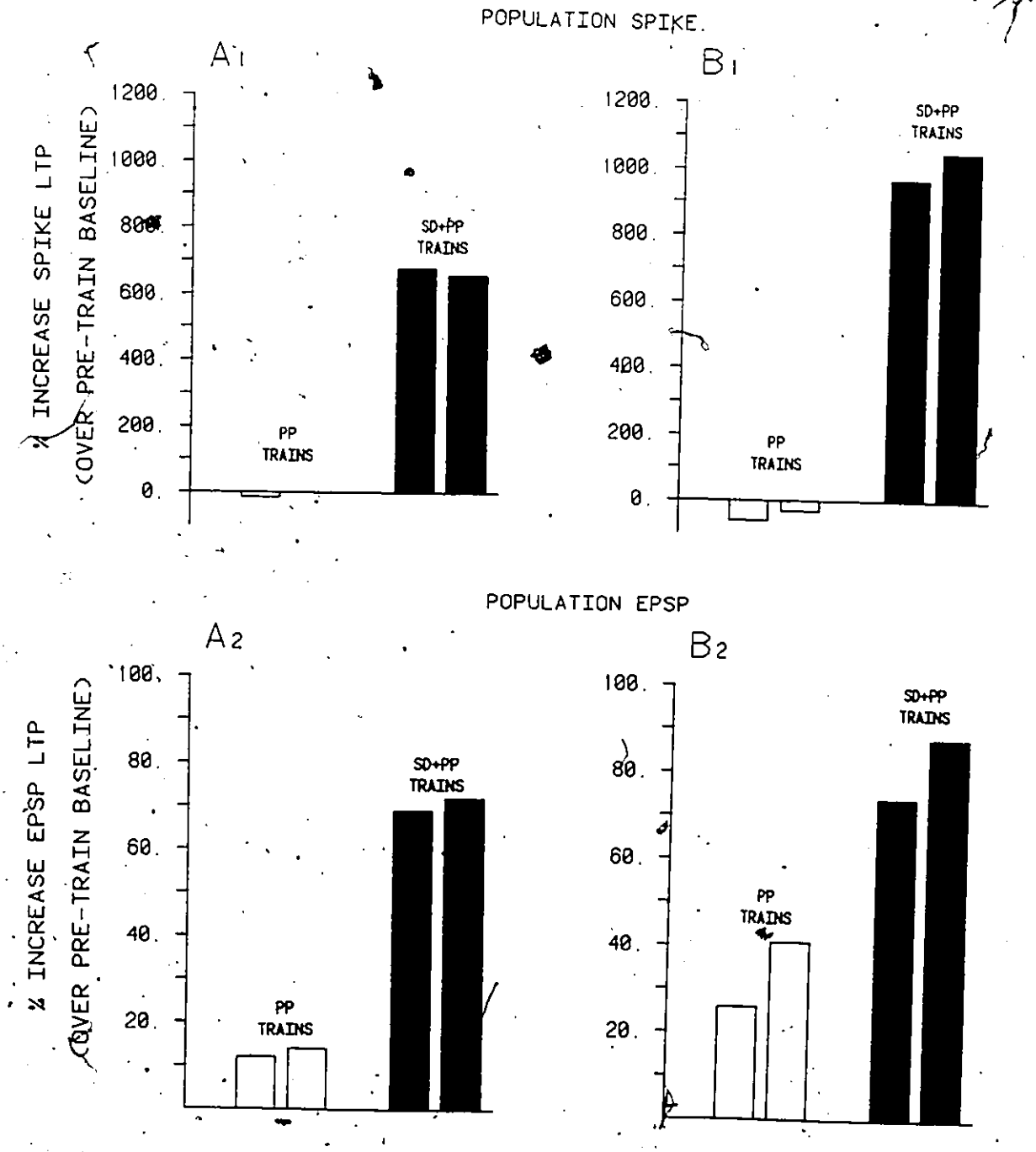
The data from two animals, shown in Figure 9, are of particular interest. In these animals, application of PP-only trains had no effect on the population spike amplitude (see Figure 9A1 and 9B1) although there was significant LTP of the population EPSP (14 ± 2% and 44 ± 4%;

see Figure 9A2 and 9B2, respectively). The dissociation between LTP of the PP-GC population EPSP and spike, in itself, is not unusual and has been noted by others (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; Wilson, 1981; Wilson, Levy and Steward, 1981). It is usually the population spike, however, which potentiates in the absence of EPSP potentiation.

Subsequent concurrent tetanization of both SD and PP afferents did result in significant LTP of the population spike in these two animals. The final level of LTP was 656 ± 12% (see Figure 9A1) and 1048 ± 26% (see Figure 9B1) and there was no overlap with the amplitude observed following the PP-only trains. There was, in addition, a significant further increase in the amplitude of the population EPSP. This was most evident in the first animal, whose level of EPSP LTP following the PP-only trains, had clearly saturated. In this particular animal, LTP of the population EPSP increased from 14 ± 2% to 72 ± 11% (see Figure 9A2).

Only 5 of the 17 animals showed a definite long-lasting additional increment in the amplitude of the PP-GC EPSP, that was above that produced by the PP-only trains. As noted above, part of the difficulty was ensuring that the level of EPSP LTP, produced by the PP-only trains, had saturated. In 8 of the remaining animals, in which the level of LTP of the EPSP had clearly saturated, there was no additional increase when the trains were subsequently applied together. In the remaining 4 animals the level of LTP of the EPSP had clearly not saturated. Overall, the mean percent increase in EPSP amplitude

Figure 9: Effect of combined SD and PP trains on PP-GC population spike and population EPSP, in the absence of spike LTP, following the PP-only trains. A1. Percent increase in spike LTP, animal 34. A2. Percent increase in EPSP LTP, animal 34. B1. Percent increase in spike LTP, animal 56. B2. Percent increase EPSP LTP, animal 56.


POPULATION SPIKE.
A1
B1
% INCREASE SPIKE LTP
(OVER PRE-TRAIN BASELINE)
1200.
1000.
800.
600.
400.
200.
0.
PP
TRAINS
SD+PP
TRAINS
POPULATION EPSP
A2
B2
% INCREASE EPSP LTP
(OVER PRE-TRAIN BASELINE)
100.
80.
60.
40.
20.

following the last PP-only train was 38 ± 9%, compared to 54 ± 12% following the last combined train set.

DISCUSSION

These results demonstrate that concurrent activation of the SD and PP afferents can increase LTP to a level above that produced by PP-only trains. While the SD input, by itself, is not capable of producing long-lasting changes in the PP-GC population response, when combined with the PP input, a significant increase is induced. The strength of these associative interaction effects was particularly apparent in the two animals that exhibited LTP of the population spike to the concurrent SD and PP trains, but not to the PP-only trains. In contrast, the magnitude of LTP for the SD-GC response was the same following both the SD-only and combined SD and PP trains.

In agreement with Fantie (1982), the effect of high-frequency coactivation of the SD and PP on the PP-GC EPSP was more variable. Fantie (1982) compared the level of LTP of the population EPSP following PP-only trains in one hemisphere with the effects of SD and PP coactivation in the other hemisphere. Although interaction effects were found, they were not found in all animals, and when observed, the magnitude of the effect was highly variable. It is possible that the variability may have been due to differences in the position of the PP stimulation electrodes. If the two PP stimulation electrodes did not activate the same number of afferents, then between hemisphere differences in the level of LTP would be expected on this basis alone

(McNaughton et al., 1978). In the present experiment, interaction effects were measured within a hemisphere, and the magnitude of the cooperativity effect compared directly against the effects of PP-only trains in the same hemisphere, so this was not a problem. The variability, however, was still present. This variability was also found in subsequent experiments, and for this reason this thesis shall be concerned mainly with the effect of coactivation on LTP of the PP-GC population spike.

These findings provide further support for LTP as a candidate mechanism of associative learning and memory, and for interactions between neural pathways in which there is no problem of current spread to the other input. In addition, the PP and SD afferents may carry different types of information to the hippocampus. Thus, it is possible that these two inputs play a role in the formation of neural associations necessary for learning and memory. Alternatively, the SD input may simply increase the strength of the memory trace formed by information carried via the PP. The SD input may also enhance the memory storage process by requiring fewer coactive PP afferents. This possibility is supported by the data from the two animals that exhibited LTP of the PP-GC spike to the concurrent SD and PP trains but not to the PP-only trains.

EXPERIMENT 2: HETEROSYNAPTIC COOPERATIVITY IN CHRONIC RATS: LTP DURATION

In the previous experiment, LTP of the PP-GC population spike, following the combined trains, was increased to a level above that observed following the application of PP trains by themselves. The effect on the EPSP was more variable, but did suggest that some additional increment of the synaptic response was associated with concurrent tetanization.

Laroche and Bloch (1982) have shown that tetanization of the mesencephalic reticular formation, immediately following PP-only trains, increases both the magnitude and duration of LTP of the PP-GC population spike. The duration was increased to approximately 5 days, but this is close to the LTP duration observed in most chronically prepared animals (Barnes, 1979; Racine et al., 1983; Robinson and Racine, 1984). The present experiment was designed to determine if the heterosynaptic interactions between SD and PP fibres would be confirmed in unanesthetized preparations, and whether or not these interactions prolonged the duration of LTP.

METHODS

Ten male Long-Evans Hooded rats (400-450 g) were anesthetized with 0.4 ml of sodium pentobarbitol (65 mg/ml). Burr holes, approximately 2 mm in diameter, were drilled for unilateral electrode implantation. Electrode construction and coordinates were as described

in the General Methods. When the optimal stimulation and recording sites were located, the electrodes were mounted in a plastic head cap and the cap and wires held in place by dental cement. Rats were then injected with Derapen-C (penicillin-G, 0.15 ml, im) and given at least 4 weeks to recover before starting the potentiation tests.

It was determined, from the initial group of 10 animals, that low intensity SD trains resulted in epileptiform afterdischarge in the dentate gyrus. This made it impossible to obtain uncontaminated measures of LTP. Due to the problem with afterdischarge, an additional group of eleven chronically prepared animals were potentiated while anesthetized with sodium pentobarbitol and subsequently tested for LTP duration in the awake state. Four animals initially received 8 sets of PP trains only. The remaining animals received 4 sets of PP trains followed by 4 sets of concurrent SD and PP trains (same train parameters as Experiment 1). Test pulses were applied alternately to the PP and SD at 5 s intervals. The test pulse frequency for each pathway was 0.1 Hz.

The decay of LTP was followed for a two week period in both groups. Following the decay all animals were tested under the opposite condition.

RESULTS

High-intensity SD trains (1200 μA) always evoked epileptiform afterdischarge in the dentate gyrus of unanesthetized chronics. Unanesthetized chronic animals were therefore tested at an SD train intensity of 100 μA. Even at this low intensity only one of the ten

unanesthetized chronic animals did not show an afterdischarge. For this animal, tetanization of the PP resulted in a 724 ± 36% increase in the amplitude of the PP-GC population spike but only a 1 ± 2% increase in the EPSP amplitude (13-15 min posttrain). Subsequent combined tetanization of both the PP and SD afferents resulted in an additional increment in the amplitude of the population spike, so that the final level of LTP was now 968 ± 42%. This represented an additional LTP increment of 34% above that produced by the PP-only trains (see Figure 10). This increment in spike LTP was accompanied by a small percent decrease in the EPSP to -7 ± 4%.

SD train intensities lower than 100 μA were not tested as it was believed that the number of activated SD afferents would be too few to produce a cooperativity effect (see Experiment 3). Therefore, in the second group of eleven chronics, high-intensity SD trains were delivered while the animals were anesthetized. In these animals PP-only trains produced significant LTP of both the PP-GC population spike (see Figure 11B) and EPSP (see Figure 11D). Repeated application of the PP trains saturated the magnitude of LTP in the population spike measure. Although the EPSP showed small, additional increments to successive PP trains there was never any sharp transition. In contrast, when the SD and PP trains were applied concurrently, there was a sharp additional increment in the amplitude of both the population spike (see Figure 11A) and EPSP (see Figure 11C). These increments were above that produced by the PP-only trains. The magnitude of these additional increments were highly significant (i.e. there was no overlap with the amplitude of the response observed following the PP-only trains).

Figure 10: Cooperativity between SD and PP inputs in unanesthetized animal with chronically implanted electrodes. A. Continuous record of mean percent change of the PP-GC EPSP. B. Continuous record of PP-GC population spike. C. Bar graph illustrating mean percent change in EPSP amplitude, over pretrain baseline, 13 to 15 min after each train set. D. Bar graph illustrating the mean percent change in spike amplitude, over pretrain baseline, 13-15 min after each train set. The SD train intensity was 100 μA.

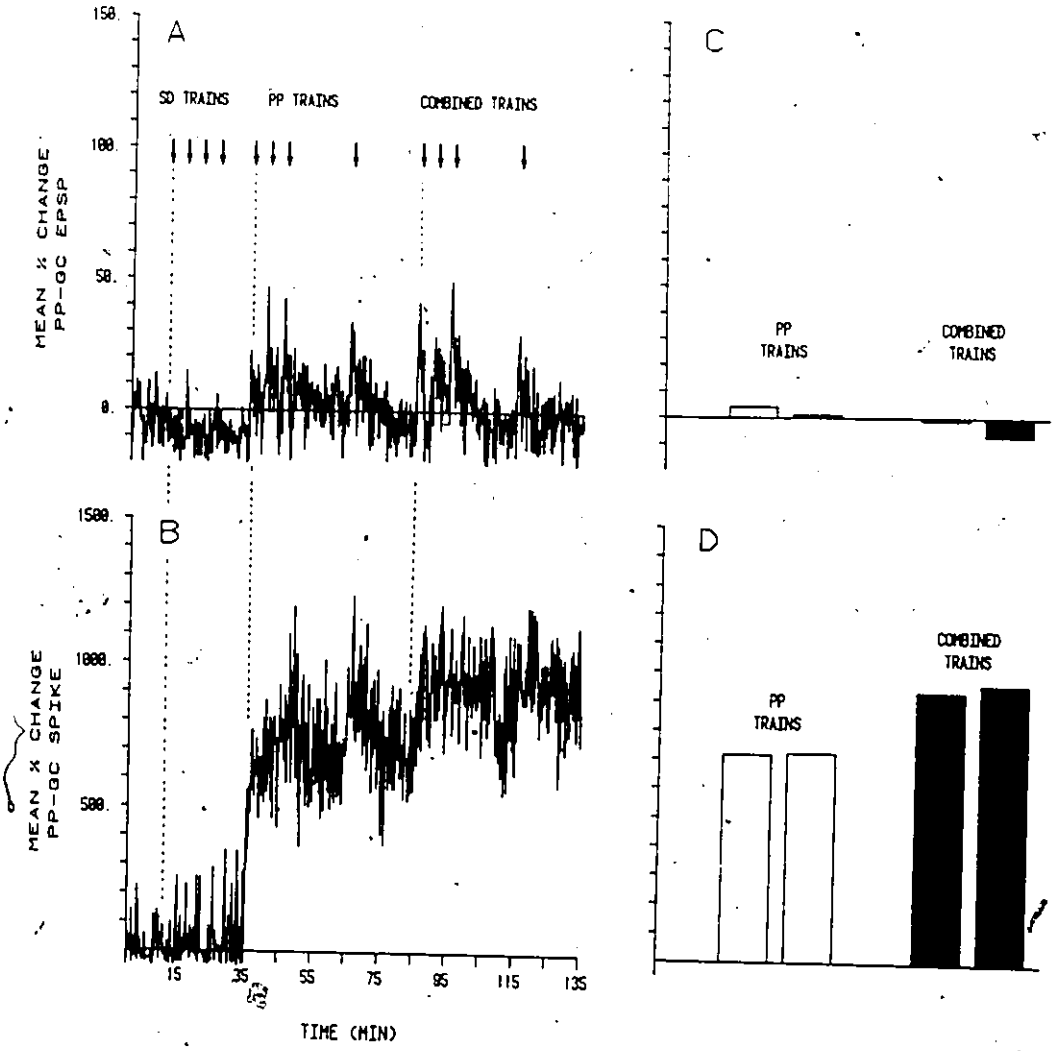

A
SD TRAINS
PP TRAINS
COMBINED TRAINS
150.
100.
50.
0.
MEAN % CHANGE PP-GC EPSP
B
1500.
1000.
500.
MEAN % CHANGE PP-GC SPIKE
15
35
55
75
95
115
135
TIME (MIN)
C
PP TRAINS
COMBINED TRAINS
D
PP TRAINS
COMBINED TRAINS

Figure 11: Cooperativity between SD and PP inputs in chronic, anesthetized rats. A. LTP of PP-GC spike to concurrent activation of the SD and PP afferents. B. LTP of the PP-GC spike to PP-only trains. C. LTP of the PP-GC EPSP to concurrent activation of the SD and PP afferents. D. LTP of the PP-GC EPSP to PP-only trains.

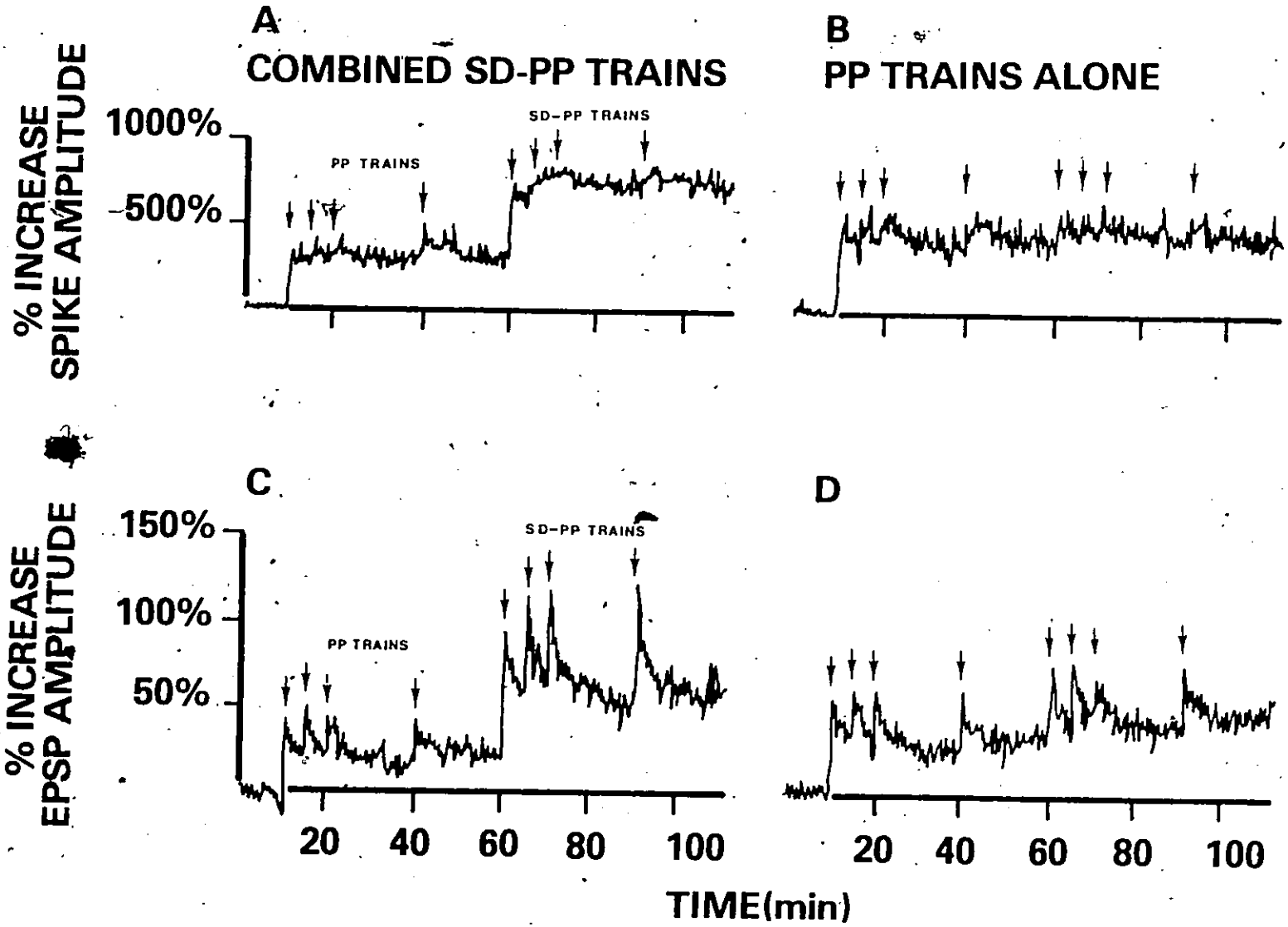

A
COMBINED SD-PP TRAINS
B
PP TRAINS ALONE
% INCREASE SPIKE AMPLITUDE
1000%
500%
PP TRAINS
SD-PP TRAINS
C
D
% INCREASE EPSP AMPLITUDE
150%
100%
50%
PP TRAINS
SD-PP TRAINS
20
40
60
80
100
TIME(min)

Figure 12 illustrates the effects of PP-only and combined SD and PP trains on the presumed SD-GC population EPSP. PP only trains had no effect on the amplitude of the SD-GC EPSP. When the trains were applied to both pathways, however, there was a significant increment in the EPSP which lasted for at least 15 min. This increment was presumably due only to the effect of the SD trains as the concurrent trains had no additional effect on this response in Experiment 1.

The decay of the increased PP-GC population spike amplitude was tested during the awake state, over a two week period, and the results are shown in Figure 13. To determine the time constants of decay, exponential functions were fit to the mean percent change of the population spike amplitude over this period. Animals in the concurrent condition maintained a higher level of LTP throughout the two week period and had slightly longer decay time constants (17 days vs. 14 days for the animals that received PP-only trains). The decay constants were not, however, significantly different. Chronic animals that receive PP-only trains in the unanesthetized state show comparable rates of decay (21 days: Barnes, 1979; 16 days: Robinson and Racine, 1984) indicating that the use of anesthetic did not affect the duration of spike LTP. Decay curves for either the PP-GC or SD-GC EPSP were poorly fit by exponential decay functions.

DISCUSSION

These results demonstrate that the cooperativity effect observed in the acute preparation also occurs in the chronic rat preparation.

Figure 12: LTP of the SD-GC population EPSP in rats with chronically implanted electrodes. Animals received either (A) PP trains followed by combined SD and PP trains, or (B) PP trains throughout testing. Trains were applied at times indicated by open circles (o).

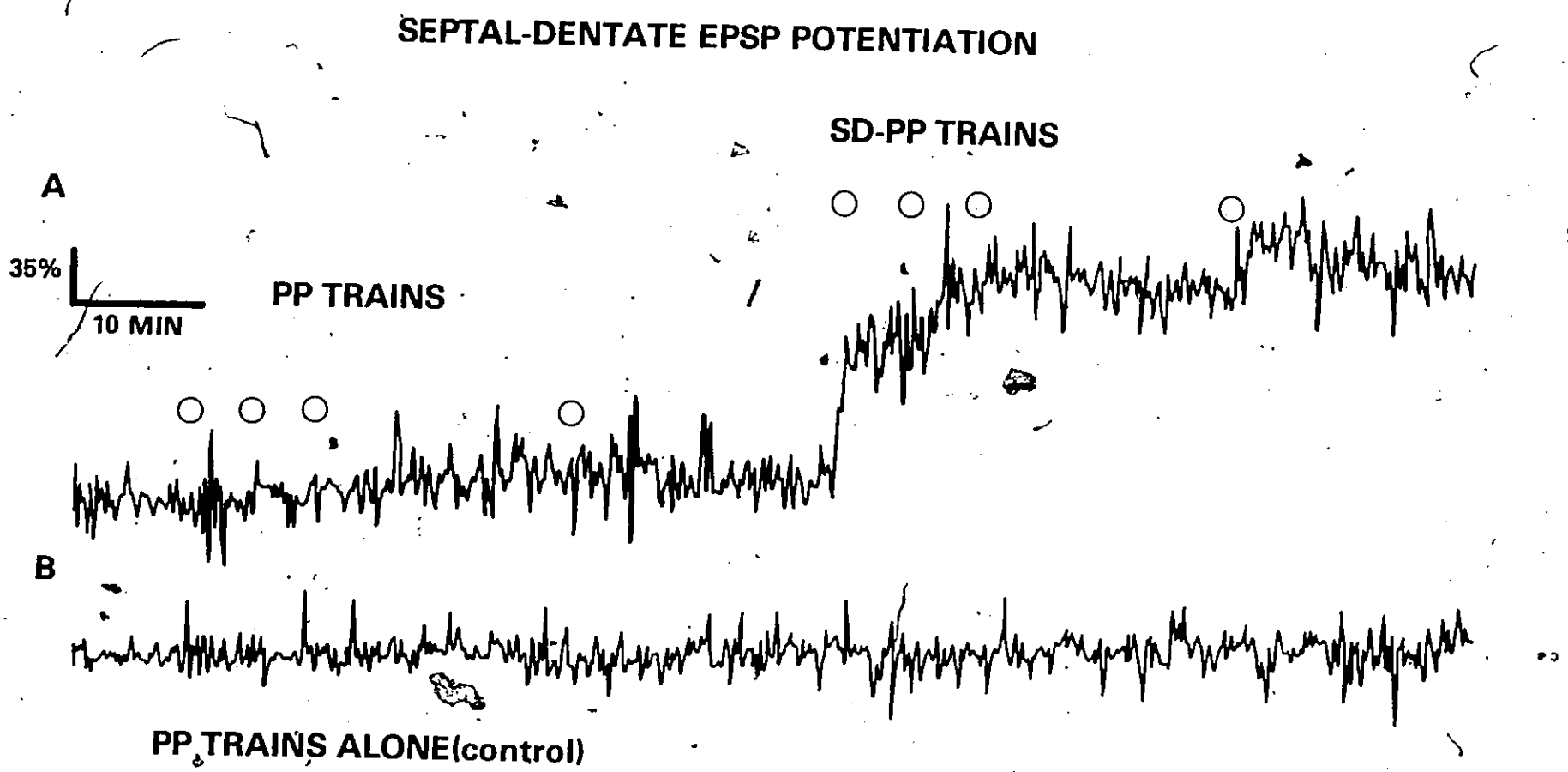
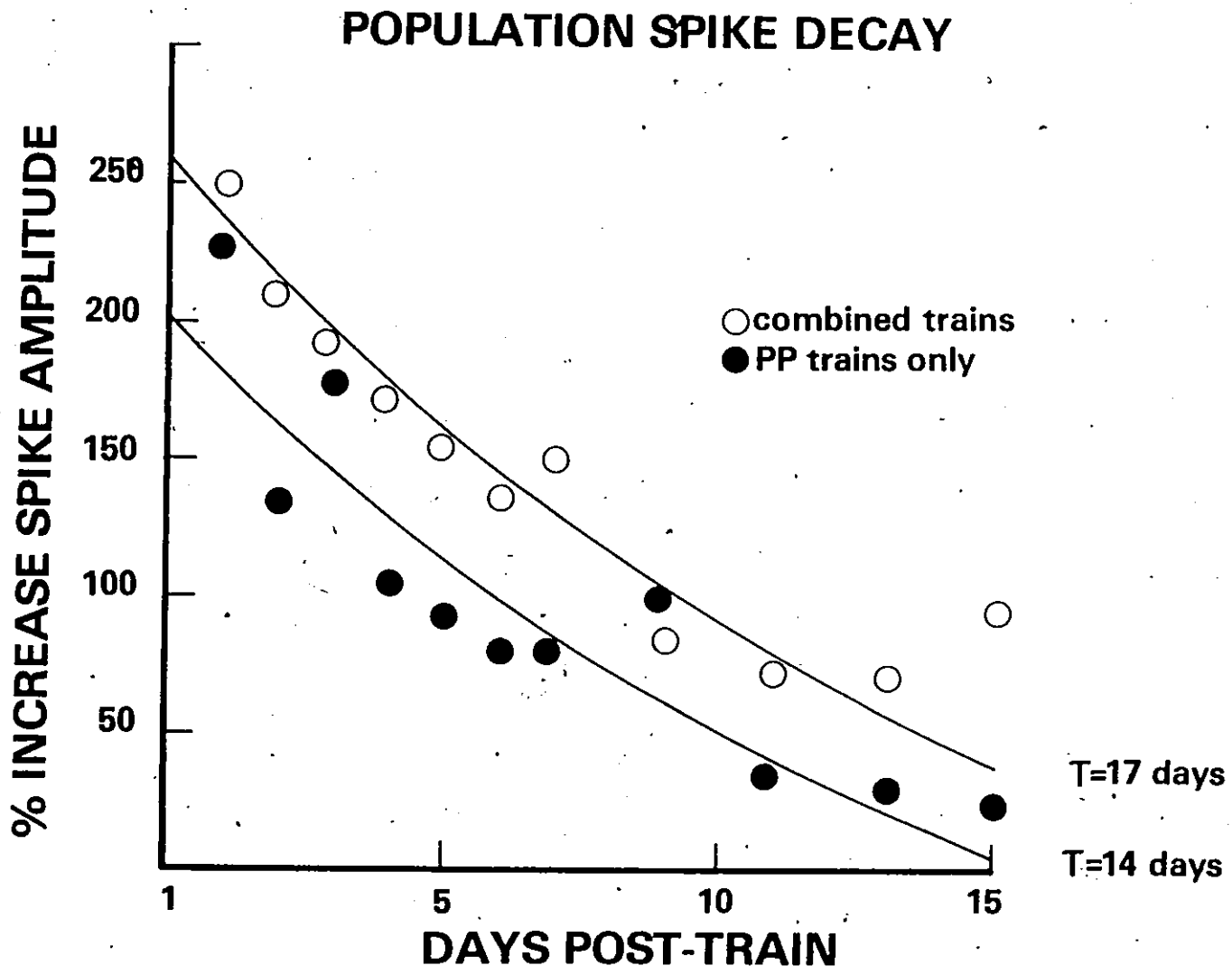

SEPTAL-DENTATE EPSP POTENTIATION
SD-PP TRAINS
A
35%
10 MIN
PP TRAINS
B
PP TRAINS ALONE(control)

Figure 13: Decay of LTP of the PP-GC population spike following either PP-only trains (•) or concurrent SD and PP trains (○).


POPULATION SPIKE DECAY
% INCREASE SPIKE AMPLITUDE
250
200
150
100
50
1
5
10
15
DAYS POST-TRAIN
○combined trains
●PP trains only
T=17 days
T=14 days

It was not possible to test the magnitude of these interaction effects with higher SD train intensities due to the problem with afterdischarge. The results from one animal, however, indicate that the cooperativity effect also occurs in the unanesthetized preparation.

Utilization of the chronic preparation, in addition, demonstrated that the heterosynaptically-induced increment in spike LTP was maintained for at least a 2 week period. Although the decay time constants were not significantly different from controls, they at least raised the possibility that concurrent tetanization of both the SD and PP afferents may also increase the duration of LTP.

EXPERIMENT 3: EFFECT OF SEPTAL TRAIN INTENSITY

Two methods have been utilized to demonstrate the cooperative nature of LTP of the PP-GC field response. The first method involves concurrent activation of two separate afferents to the dentate gyrus GCs. When the two components of the PP are activated, for example, there is significantly more LTP of the PP-GC field EPSP than is observed following tetanization of either pathway alone (McNaughton et al., 1978). The second method involves increasing the intensity of the PP trains, regardless of the particular pathway. As a result there are an increasing number of activated PP afferents, and this has the effect of incrementing the level of LTP above that produced by lower intensity trains (Bliss et al., 1983; McNaughton et al., 1978).

The previous two experiments have demonstrated the existence of heterosynaptic cooperative interactions between the SD and PP afferents

to the dentate gyrus. These experiments, however, utilized only the first method. This experiment examines whether the cooperativity effect also varied with SD train intensity. Specifically, would increasing the intensity of the SD trains increment the magnitude of the LTP cooperativity effect observed when the SD and PP trains were applied concurrently.

METHODS

Thirteen animals were prepared as previously described for acute stimulation and recording. Facilitation effects were examined prior to testing for train effects. To examine facilitation effects single pulses were applied concurrently to the SD and PP inputs, while varying the intensity of the SD pulse. PP pulse intensity was adjusted to produce a small but consistent GC population spike. A total of 7 SD intensities were examined in each animal. Ten pulse-pairs were delivered for each of the 7 SD intensity intervals.

From the resulting PP-GC facilitation curve, three intensities were chosen for the SD trains. The lowest SD train intensity was that which produced only minimal facilitation of the PP-GC population spike in the paired-pulse tests. The middle intensity resulted in approximately 50% of the maximum facilitation, and the highest intensity chosen resulted in maximum levels of spike facilitation.

Trains were then applied to the PP until the level of LTP produced by the PP-only trains had saturated. When the level of LTP

had saturated, trains were applied concurrently to the PP and SD inputs, starting with the lowest-intensity SD train and finishing with the highest intensity train. When the trains were applied concurrently, the magnitude of any additional LTP due to cooperativity effects was saturated before the intensity of the SD trains was further increased. Test pulse frequency was 0.1 Hz. The SD and PP train frequency and duration was 400 Hz and 50 ms, respectively. PP train intensity was set at spike threshold, as described in the General Methods.

RESULTS

Figure 14 illustrates the average effect of increasing the SD pulse intensity on facilitation of PP-GC population spikes. The magnitude of the facilitation effect increased with increasing SD pulse intensity. At the lowest SD pulse intensity the average magnitude of the facilitation was very small (26 ± 5%). Facilitation reached a maximum of 570 ± 50% with an SD pulse intensity of 1000 µA. Further increasing the SD pulse intensity to 1200 µA did not produce any further facilitation of the PP-GC population spike.

Figure 15 summarizes the results of applying SD trains of increasing intensity concurrently with the PP trains. The average additional increments in the level of spike LTP, produced when the low, medium and high intensity SD trains were applied concurrently with the PP trains, were 14% (±6%), 42% (±8%) and 66% (±8%), respectively. These increments were all calculated from the level of LTP produced by the

Figure 14: Effect of SD pulse intensity on facilitation of PP-GC population spikes. There was no delay between application of electrical pulses to the SD and PP afferents. Both the mean and SEM are shown.

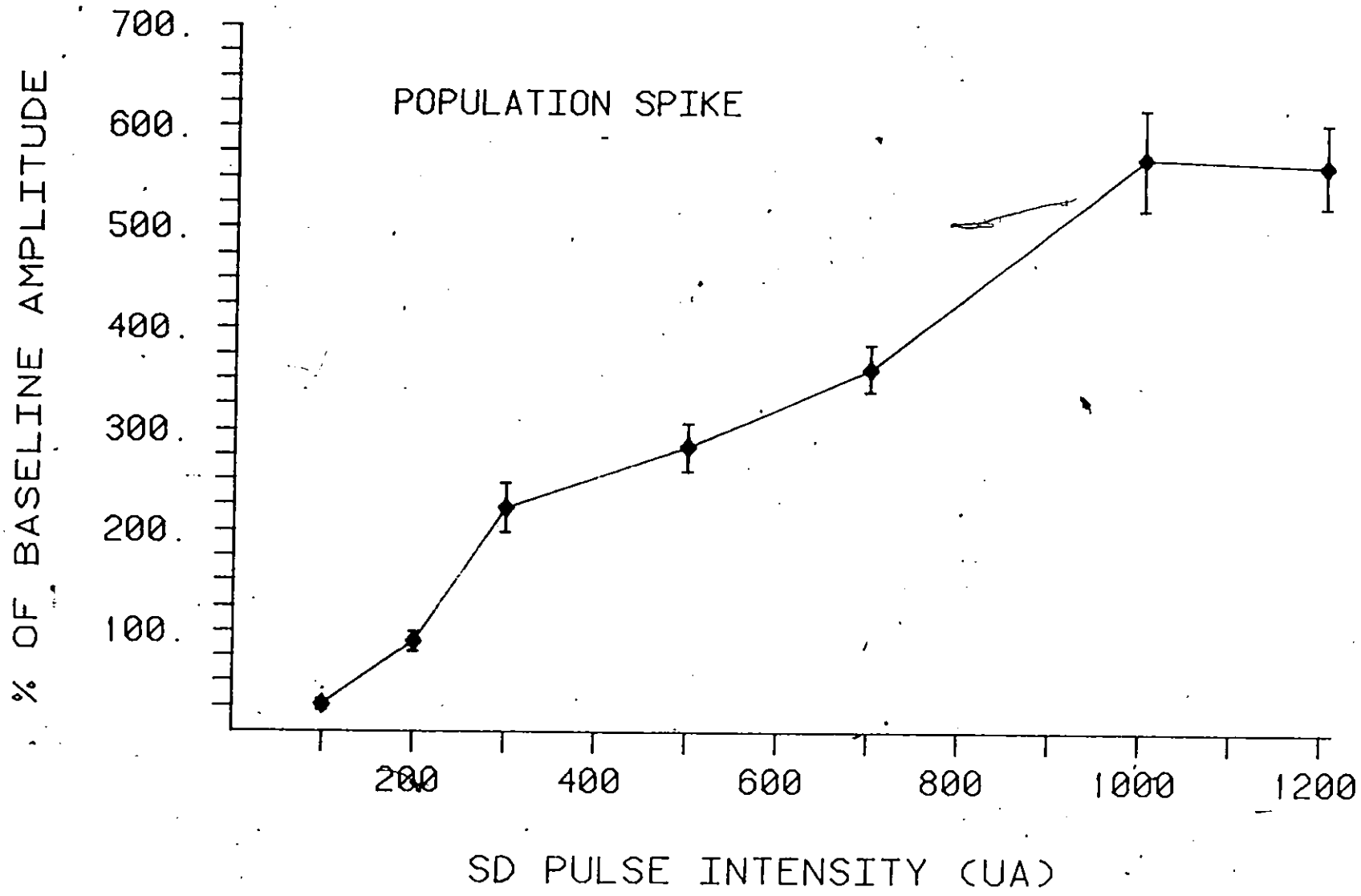
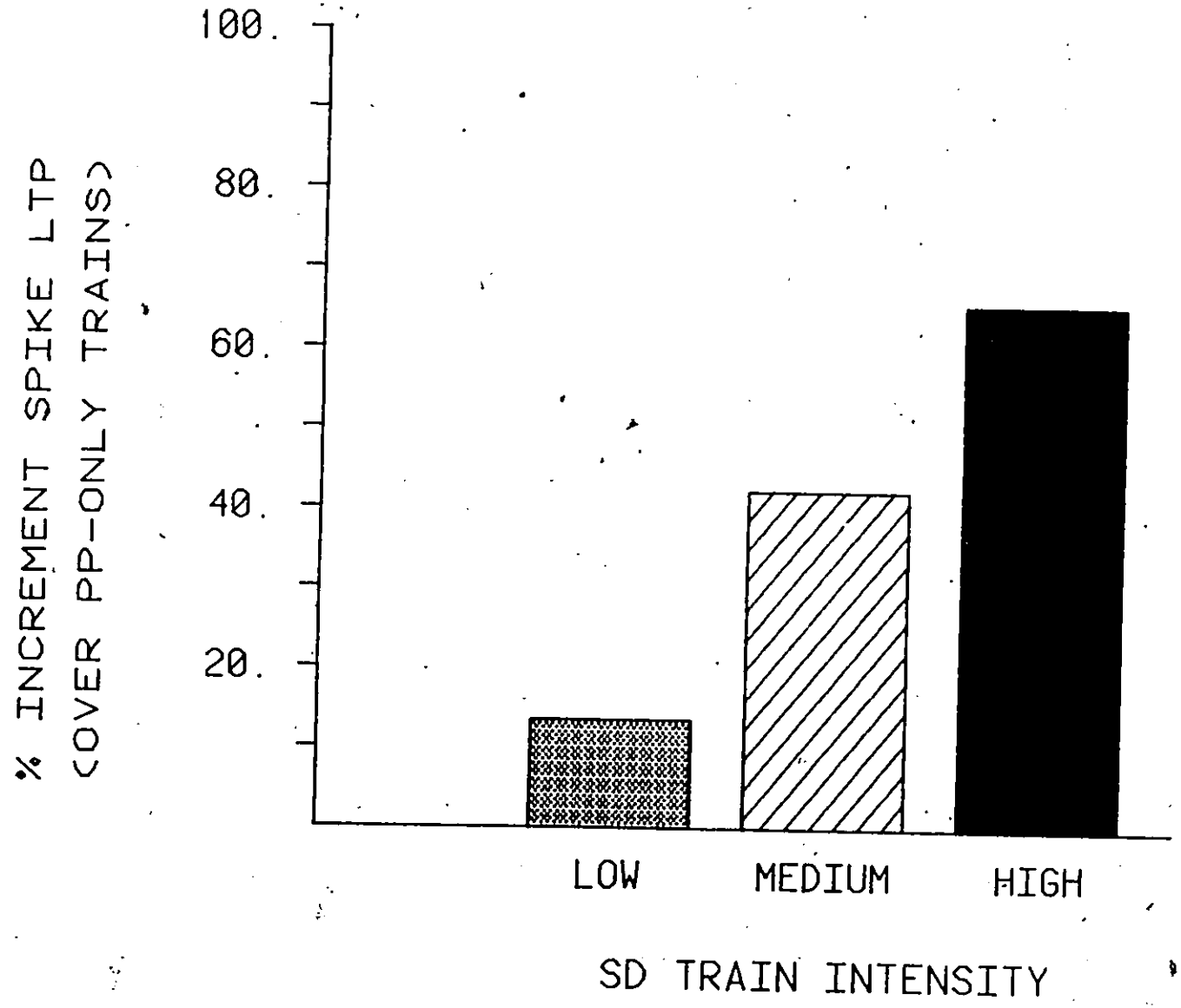

POPULATION SPIKE
% OF BASELINE AMPLITUDE
700.
600.
500.
400.
300.
200.
100.
200
400
600
800
1000
1200
SD PULSE INTENSITY (UA)

Figure 15: Average additional increment in the level of LTP of the PP-GC population spike, above that produced by the PP-only trains, as a function of SD train intensity. PP train intensity was held constant throughout testing. Low intensity SD trains (dotted bar), medium intensity SD trains (hatched bar) and high intensity SD trains (solid bar).


100.
80.
60.
40.
20.
% INCREMENT SPIKE LTP
(OVER PP-ONLY TRAINS)
LOW
MEDIUM
HIGH
SD TRAIN INTENSITY

PP-only trains. Four of the thirteen animals did not show any additional increment to the lowest SD train intensity, and 5 of the 13 showed an additional increment of less than 10%. Thus, only 4 animals showed a large increase in spike LTP, following the combined trains, with the SD train intensity at a minimum. Overall, the magnitude of the additional increment observed with the low intensity SD trains was not significantly different from that observed following the PP-only trains.

At the two higher intensities all animals showed an additional increment in the magnitude of the LTP effect, compared to the level observed following PP-only trains ($p<.01$, t-test for correlated means). Although an additional increment in LTP magnitude was not observed in all animals when SD train intensity was increased from medium to high levels, the observed increase in LTP was still significant ($p<.02$; t-test for correlated means).

DISCUSSION

It appears that the magnitude of the observed heterosynaptic cooperativity effect partially depends on the number of SD afferents that are coactive with the PP afferents. Increasing the intensity of the SD trains generally increased the magnitude of the observed cooperativity effect. The magnitude of this increase was significant when going from the low to medium ($p<.01$) and from the medium to high intensity SD trains ($p<.02$).

There did not appear to be any strict relationship between the magnitude of the LTP effect and the magnitude of the previously recorded facilitation effect. Although little or no facilitation (or LTP) was observed at low SD intensities, and facilitation (and LTP) effects increased with increasing SD intensity, large amplitude facilitation effects were not predictive of large LTP cooperativity effects.

The small magnitude of the additional increment in LTP (66% with high-intensity SD trains) deserves some comment. In Experiment 1, a 1200 μA SD train intensity produced an average additional increment in LTP magnitude of 154%. In that experiment the average level of LTP, to the PP-only trains, was 499 ± 139%. In the present experiment, however, PP-only trains produced an average increment of 922 ± 241%. The magnitude of the additional increment may depend on the magnitude of the LTP effect produced by the PP-only trains. If, for example, there is an upper limit to the magnitude of LTP, and this limit was approached following the PP-only trains, the combined trains may not be expected to have much effect. If so, this would account for the small percent increment in the present experiment.

CHAPTER IV. TEMPORAL CONSTRAINTS ON HETEROSYNAPTIC LONG-TERM POTENTIATION EFFECTS

The previous Chapter demonstrated the existence of a cooperative interaction between co-active SD and PP afferents in the production of LTP. An associative interaction which results in increased response strength is one of the primary requirements for any neuronal model of learning and memory. That is, more than one input must be activated for an association between stimuli to occur.

Of particular relevance to neural modelling of associative learning are those demonstrations of heterosynaptic interactions in which delays are interposed between the coactive afferents (Laroche and Bloch, 1982; Levy and Steward, 1983). As most classical conditioning experiments have delays between the stimuli that are to be associated (i.e. CS-UCS interval), this evidence serves to strengthen the attractiveness of LTP as a candidate neural mechanism for associative learning and memory.

There are, however, certain constraints on the above generalization. Temporal contiguity, for example, is one requirement that must be met before an association can form between activated inputs (see Mackintosh, 1974). The previous experiments satisfied this requirement in as much as the SD and PP afferents were simultaneously activated. These experiments, however, failed to answer two important questions. First, what would happen if delays were interposed between the two trains? Second, is their order of activation important or critical? This Chapter addresses both of these questions.

EXPERIMENT 1. EFFECT OF SD TRAINS PRECEDING THE PP TRAINS

This first experiment examines the effect of increasing the temporal interval between application of the SD and PP trains, with the SD trains being applied first. The objective was to first determine if the cooperativity effect was still observed with this order, and second to determine the maximum interval at which the cooperativity effect could still occur.

METHODS

Twenty-six male Long-Evans Hooded rats were prepared for surgery and electrodes were implanted in the PP, SD and the hilus of the fascia dentata using the same procedures as previously described.

The level of PP-GC LTP, following PP-only trains was saturated before applying trains to both the SD and PP afferents. A set of three combined trains were then applied with at least a 5 s interval between application of each SD-PP train pair. The intertrain interval for each pair was varied from 2000 to 0 ms. This range includes those intervals at which single SD pulses facilitate subsequent PP-GC population spikes (Alvarez-Leefmans and Gardner-Medwin, 1975; Fantie and Goddard, 1982) as well as longer intervals which do not support facilitation effects. The longer intervals were presented first. Animals were normally tested at only 3 or 4 of the following 8 intervals, 2000, 1000, 500, 100, 70, 50, 25 and 0 ms. Only two animals were tested at the 70, 50 and 25 ms intervals. In all but these two animals, the level of LTP resulting from the combined trains was saturated before further reducing the

intertrain interval. There were 15 min test periods between presentations of the combined trains. All other procedures and parameters were identical to those of Chapter III.

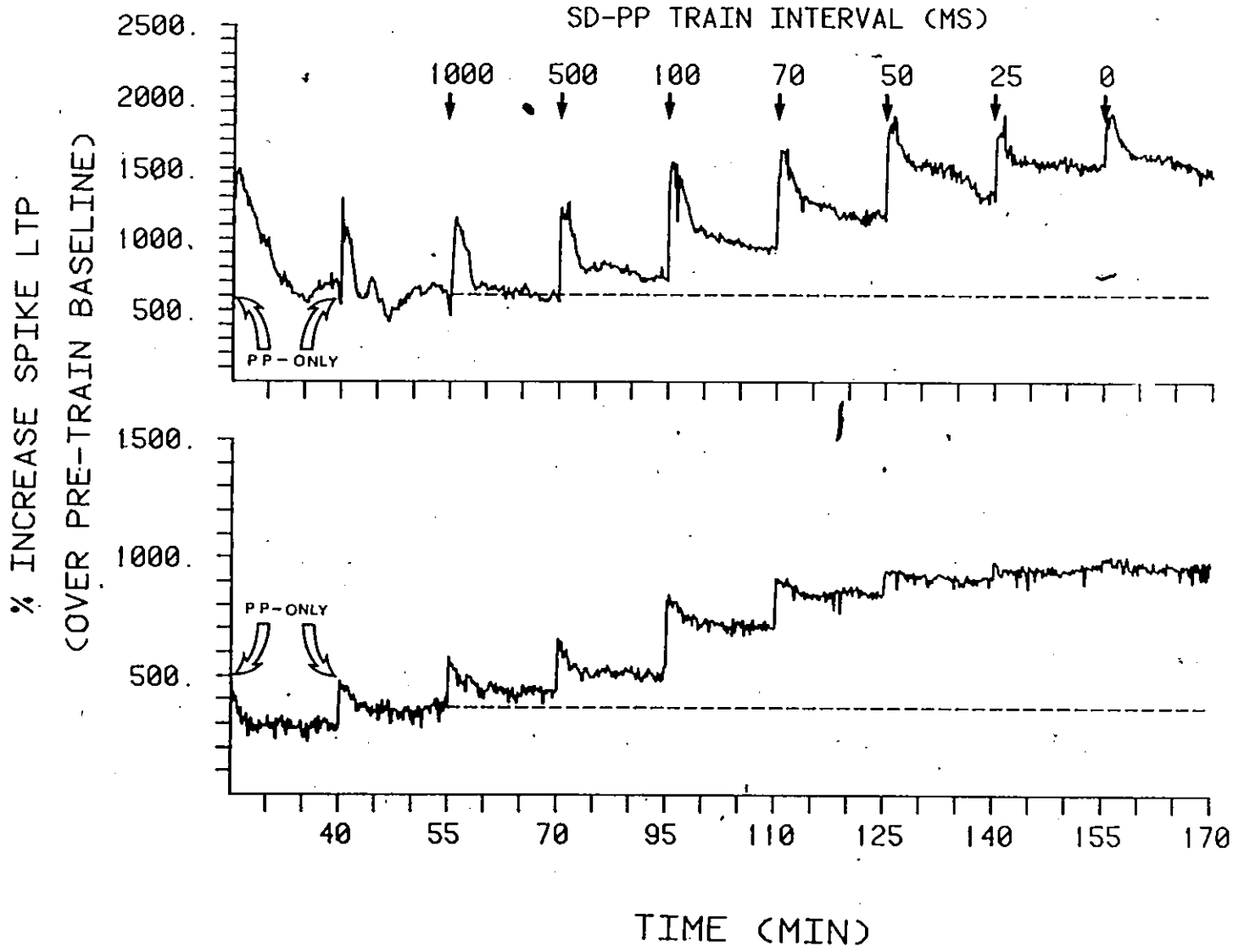
Following the combined train tests, some animals were again tested with PP-only trains, but the PP train intensity was increased. The final PP train intensity was generally 1200 μA which represented an increase of 2 to 12 times over the inital PP train intensity.

RESULTS

Figure 16 shows the data from two animals for which the SD trains preceded the PP trains. At the longest interval (1000 ms) there was a small decrease (632 ± 32% to 603 ± 27%) in the PP-GC population spike amplitude in one animal and a slight increase (366 ± 30% to 424 ± 18%; PP-only to combined trains) in the other. The findings were similar at an intertrain interval of 500 ms. When the interval was further decreased to 100 ms, however, both animals exhibited a significant increase in the amplitude of the population spike (749 ± 24% to 954 ± 17% and 496 ± 21% to 699 ± 11%; 500 ms to 100 ms delay). Further reductions, up to 25 ms, resulted in additional increments in the level of LTP, although the magnitude of these additional increments were not as great as observed at the 100 ms interval. Applying the trains concurrently did not produce any further increment in the level of LTP.

It is possible that the additional increments resulted from repeated application of the combined trains rather than from the

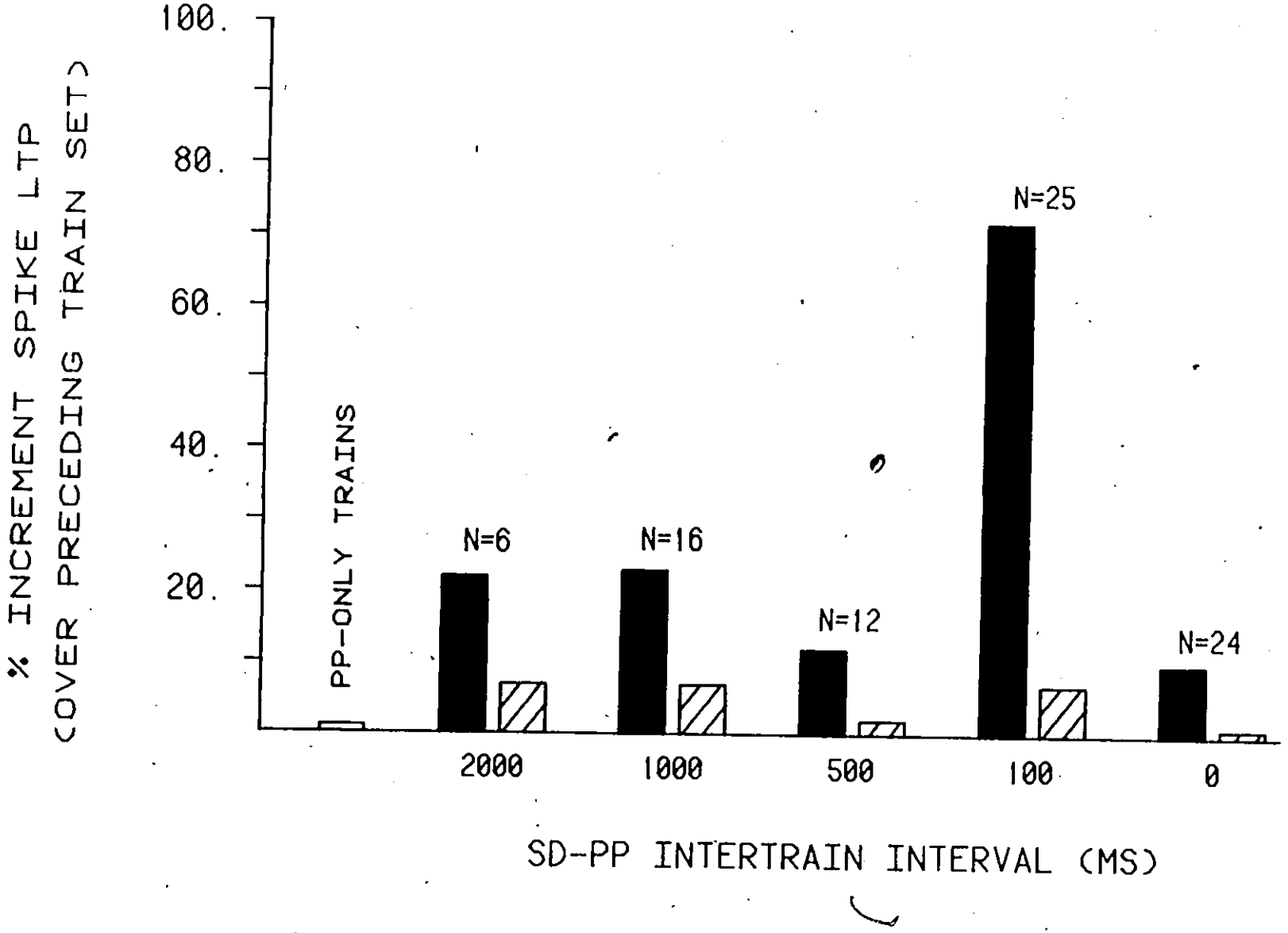
Figure 16: Effect of varying the interval between SD and PP trains (SD trains precede PP trains) on LTP cooperativity. Data are from two animals in which the combined trains were presented in a descending interval series from 1000 to 0 ms. Only the last two PP-only train sets, prior to the combined trains, are shown. Therefore, the baseline prior to any trains, is the X-axis (solid line). The baseline for the combined trains is the asymptotic LTP produced by PP-only trains (dashed line).


SD-PP TRAIN INTERVAL (MS)
1000
500
100
70
50
25
0
2500.
2000.
1500.
1000.
500.
PP-ONLY
PP-ONLY
1500.
1000.
500.
% INCREASE SPIKE LTP
(OVER PRE-TRAIN BASELINE)
40
55
70
95
110
125
140
155
170
TIME (MIN)

decreases in the temporal interval between their application. To test this possibility, combined trains were delivered at a set temporal interval, to 24 animals, until the effect on LTP saturated. The intertrain interval was then reduced and further trains applied. This reduced the possibility that additional LTP was due to cumulative train effects rather than to the decreases in the intertrain interval.

The results for all animals are illustrated in Figure 17. The data illustrate the percent increment in LTP over that observed following the preceding train set. The last PP-only train set produced a small non-significant increase in the level of LTP (1 ± 7%), indicating that the level of LTP had saturated. Subsequent combined trains, at the 2000 ms SD-PP intertrain interval induced a further increment in the level of LTP of 22 ± 8% (n=6). When repeated, the magnitude of the additional increment was 8 ± 3%. The level of LTP following the last 2000 ms presentation was just significantly different from that level observed following the PP-only trains ($p<.05$; t-test for correlated means). For SD-PP intertrain intervals of 1000 (n=16), 500 (n=12), 100 (n=25) and 0 ms (n=24), the initial additional increments in the level of LTP were 23 (± 9%), 12 (± 2%), 72 (± 15%) and 10% (± 4%), respectively. Thus, the largest additional increments occurred at 100 ms, even though this increment was calculated from an already elevated baseline. In contrast, the additional increments over the initial combined train presentations (at the same intervals) were 7 (± 5%), 2 (± 2%), 7 (± 3%) and 1% (±3%), respectively. Therefore, the LTP effect had generally saturated by the second pair of trains. Further decreasing

Figure 17: Average additional increment in spike LTP, over that produced by the immediately preceding interval, as a function of the interval between the SD and PP trains (solid bars). SD trains were always applied prior to the PP trains. When either the PP-only train or combined trains were repeated, at a given delay, there was little additional increment in LTP level (hatched bars).


% INCREMENT SPIKE LTP
(OVER PRECEDING TRAIN SET)
100.
80.
60.
40.
20.
PP-ONLY TRAINS
N=6
N=16
N=12
N=25
N=24
2000
1000
500
100
0
SD-PP INTERTRAIN INTERVAL (MS)

the intertrain interval, however, usually resulted in a further additional increment in the magnitude of LTP (see Figure 17). Eventually the magnitude of LTP appeared to reach asymptotic levels, as further increases in the PP train intensity (when PP trains were subsequently presented alone) failed to produce any additional increment in the level of LTP (data not shown).

DISCUSSION

These results demonstrate that the cooperativity effect, observed when the SD and PP trains are applied concurrently, also occurs with intertrain intervals up to 1000 ms. The magnitude of the additional increment does, however, depend on the intertrain interval. The largest effect occurred with intertrain delays of 100 ms or less.

The increments in LTP were likely due to the decreases in the intertrain interval, and not to cumulative train effects, for two reasons. First, when the LTP increment for a given intertrain interval had saturated, further decreasing the interval produced a larger additional increment than did repeating the combined trains at the same interval. Second, the final magnitude of the additional increment in LTP (167 ± 19%), over that produced by the PP-only trains, was remarkably similar to that produced in animals that only received the concurrent trains (153 ± 32%; Chapter III, Experiment 1).

EXPERIMENT 2: EFFECT OF PP TRAINS PRECEDING THE SD TRAINS

The previous experiment illustrated that when SD trains preceded the PP trains there was a large increase in the level of LTP. The magnitude of this increase was shown to depend on the intertrain interval. In the present experiment, the effect of the PP trains preceding the SD trains was examined to determine if the order of activation was also critical.

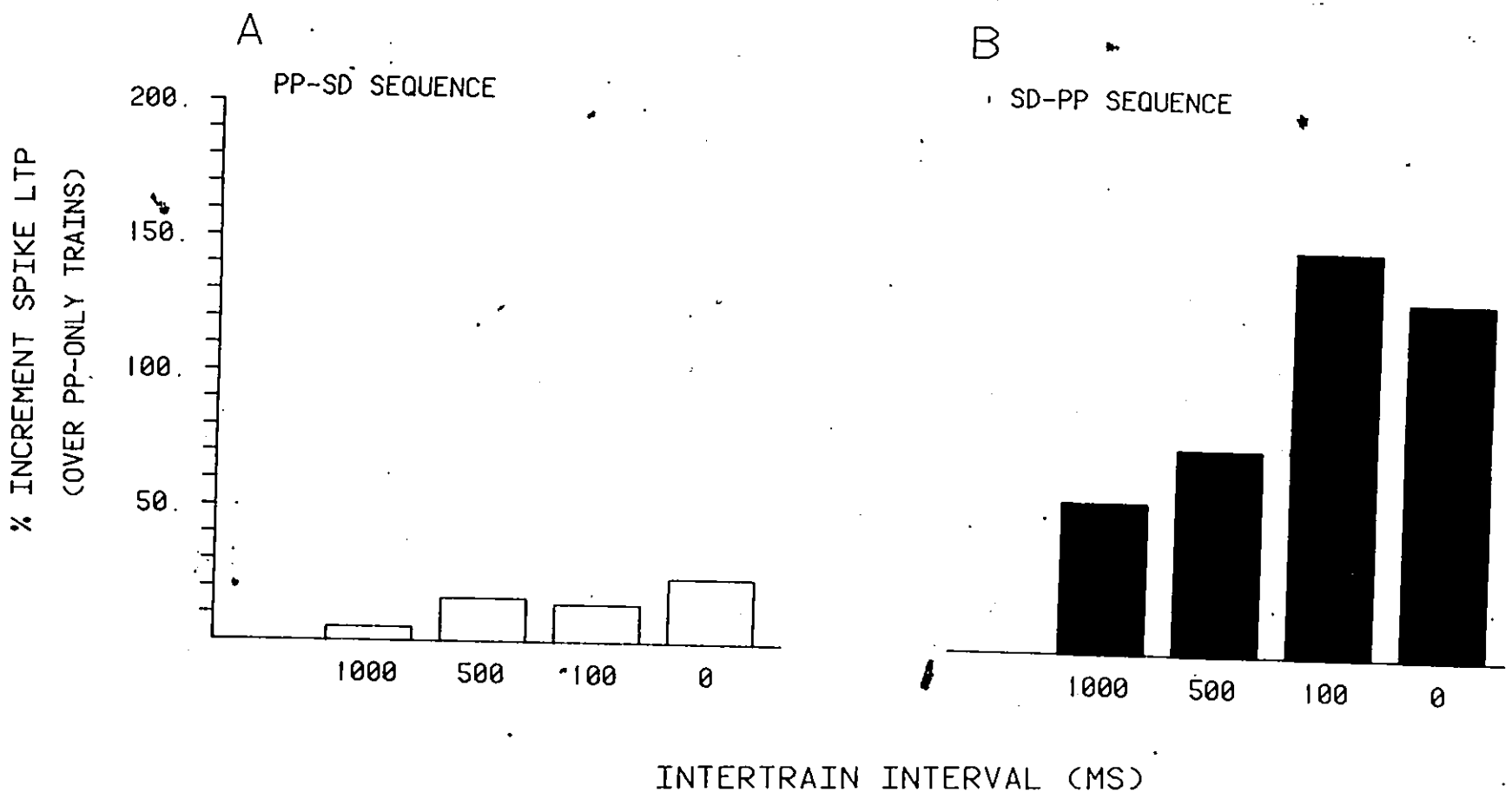
METHODS

Thirteen animals were prepared for acute stimulation and recording as previously described. Following saturation of the level of LTP produced by the PP trains alone, trains were presented to both the PP and SD afferents. For this experiment, however, the PP trains were applied before the SD trains. The intertrain intervals examined were 1000, 500, 100 and 0 ms. All other parameters were identical to Experiment 1.

RESULTS

Figure 18A illustrates the LTP cooperativity effect when the PP trains precede the SD trains. On average, the magnitude of the additional increment in LTP, above that produced when PP trains were presented alone, was extremely small. At intertrain intervals of 1000, 500, 100 and 0 ms, the additional increments were 5 (± 7%), 5 (± 9%),

Figure 18: Average additional increment in spike LTP, above that produced by the PP-only trains, as a function of the interval between application of the SD and PP trains. A. PP trains preceded SD trains. B. SD trains preceded PP trains.

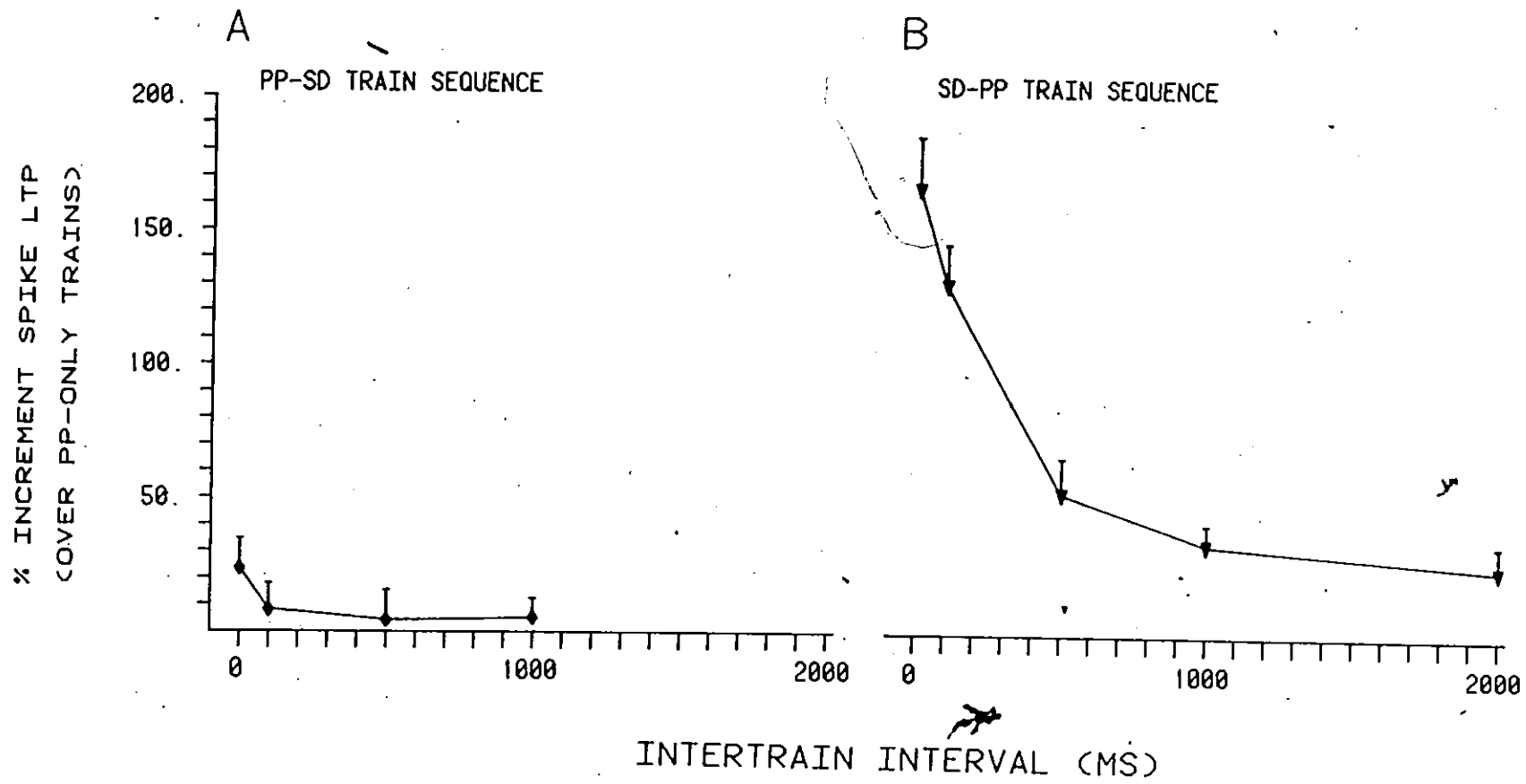

A
PP-SD SEQUENCE
B
SD-PP SEQUENCE
% INCREMENT SPIKE LTP
(OVER PP-ONLY TRAINS)
200.
150.
100.
50.
1000
500
100
0
1000
500
100
0
INTERTRAIN INTERVAL (MS)

8 (± 11%) and 23% (± 11%), respectively. In 5 of the 13 animals the magnitude of the population spike actually decreased when the combined trains were presented at the 1000 ms intertrain interval. If these animals were dropped from the average the resulting means were 22, 28, 30 and 41%. At the two longer intervals these means are comparable to those observed when the SD trains preceded the PP trains. The magnitude of the LTP effect, however, was much smaller at the shorter intervals (including 0 ms which was common to both experiments - compare Figure 18A to Figure 18B).

There are at least two explanations for this reduced cooperativity effect. First, the SD electrode may not have been in the optimal site for inducing the cooperativity effect. Second, this particular sequence may somehow inhibit the cooperativity effect even when the trains are subsequently applied concurrently. To distinguish between these two possibilities, the effect of the PP-SD sequence was examined in one hemisphere and compared to SD-PP interval effects in the other hemisphere. This should eliminate the first possibility and at least raise the probability that the second alternative is correct.

Figure 19 shows the between hemisphere difference for animals tested at intervals of 1000, 500, 100 or 0 ms. The graph illustrates the mean percent increment in spike amplitude over the level produced by the PP-only trains. When the PP trains preceded the SD trains, the average magnitude of the additional increments, were 5 (± 10%), 16 (±20%), 14 (±13%), and 24% (± 12%) for intertrain intervals of 1000, 500, 100 and 0 ms, respectively. Thus, the combined trains, even at the short intertrain intervals, had very little effect on the magnitude of

Figure 19: Comparison of LTP cooperativity effect in 5 animals. The effects of the PP-SD sequence were examined in one hemisphere (A) and compared to the effects of the SD-PP train sequence in the opposite hemisphere (B).


A
PP-SD TRAIN SEQUENCE
B
SD-PP TRAIN SEQUENCE
% INCREMENT SPIKE LTP
(OVER PP-ONLY TRAINS)
200.
150.
100.
50.
0
1000
2000
0
1000
2000
INTERTRAIN INTERVAL (MS)

LTP. Even when the SD and PP afferents were subsequently activated concurrently there was no increment in the magnitude of LTP. In the opposite hemisphere the trains were given in reverse order but at identical intervals and, in contrast, the magnitude of the additional increments were 56 (±14%), 76 (± 12%), 150 (± 15%) and 132% (± 16%), respectively. As in Experiment 1, the largest additional increments occurred at SD-PP intertrain intervals of 100 ms or less.

DISCUSSION

These results show that interactions between coactive SD and PP afferents to the dentate gyrus depend on their order of activation as well as their temporal interval. The largest interaction effects were observed when the SD trains were applied prior to the PP trains. When applied in this order, the magnitude of the additional increment in LTP depended on the interval between trains. Short intertrain intervals (less than 100 ms) resulted in large additional increments in the level of LTP. In contrast, at longer intertrain intervals (greater than 100 ms) the magnitude of the additional increment was significantly reduced. Whatever the intertrain interval, if the PP trains preceded the SD trains there was little or no evidence of a cooperativity effect. In fact, this sequence appeared to inhibit the appearance of a cooperativity effect when the trains were subsequently applied simultaneously.

There are a number of similarities between these heterosynaptic interactions and certain types of classical conditioning. First, the

intertrain intervals which support the heterosynaptic interactions between the SD and PP afferents are similar to those CS-UCS intervals which support classical conditioning in a variety of preparations (see Mackintosh, 1974). It should be noted, however, that simultaneous CS-UCS presentations do not generally support conditioned responding. The strong effect of simultaneous conditioning in the present study may have been due to the use of direct stimulation. Patterson (1970), when examining classical conditioning of the rabbit's nictitating membrane, found that strong conditioning occurred with a 50 ms CS-UCS interval when intracranial stimulation served as the CS. With a tone CS, however, this interval did not support conditioning. This reduction in interstimulus interval was thought to be due to bypassing the peripheral receptors and thus, shortening the conduction time. In the present experiment both the "CS" and "UCS" were intracranial stimulation, possibly accounting for the effectiveness of simultaneous activation. Also, with a zero intertrain delay the GCs are not activated simultaneously, as activity in the two pathways does not reach the GCs at the same time. These results, therefore, do not violate Rescorla's (1975) view that for conditioning to occur the CS must be predictive of the UCS. This parallel, however, assumes that the SD input is analogous to the CS.

These results also parallel the effects found in classical conditioning when the CS-UCS order is reversed (backward conditioning). If the UCS precedes the CS there is no behavioural conditioning, and subsequent behavioural conditioning to the CS is possible only with repeated presentations in the correct order. Similarly, there was

little evidence of a heterosynaptic cooperativity effect if the PP trains preceded the SD trains and, at the longer intervals, some animals exhibited a decreased response amplitude. This sequence also appeared to inhibit the cooperativity effect when the two trains were subsequently presented simultaneously. It is possible that if the SD trains were repeatedly applied prior to the PP trains, following the PP-SD sequence, that the cooperativity effect might appear. This possibility was not tested in the present experiment.

There is, however, a problem with the above correlations. Neural models of associative mechanisms (i.e. Hebb, 1949) and the results of behavioural conditioning experiments (see, Mackintosh, 1974), show that it is the weak input or CS which acquires associative strength. In the present experiments, the SD input would be considered the weak input, but it was the PP-GC response that increased as a result of the paired activation. The fact that PP-SD pairings produced little evidence of a cooperativity effect also argues against the SD input serving as a reinforcing stimulus. The SD input may, therefore, play a role in arousal rather than in learning per se (see Berger and Thompson, 1977; Berry and Thompson, 1978).

CHAPTER V. HETEROSYNAPTIC INTERACTIONS BETWEEN SEPTAL AND ENTORHINAL INPUTS TO THE DENTATE GYRUS: FACILITATION EFFECTS

As the SD and PP inputs to the dentate gyrus differ in terms of their terminal fields, it is likely that these interaction effects have a postsynaptic mechanism. It is, for example, possible that the SD trains interfere with the GC's recurrent inhibitory circuits and thereby increase the postsynaptic effect of each PP train. There is some evidence for this hypothesis. First, the majority of septal afferents terminate immediately below the GC layer (Lynch et al., 1978; Mosko et al., 1973), which is the site of the inhibitory basket cells. Second, Ben-Ari, Krnjevic, Reinhardt and Ropert (1981) and Krnjevic, Reiffenstein and Ropert (1981) have shown that in area CA1, ACh (the putative transmitter for septal terminals) may inhibit the release of gamma-aminobutyric acid (GABA) from inhibitory basket cell interneurons. Reduced GABA levels would result in relatively less inhibition. Third, both Douglas et al. (1982) and McNaughton et al. (1978) have demonstrated that the commissural input to the dentate gyrus, which is presumed to excite the inhibitory basket cell interneurons (Buzsaki and Czeh, 1981; Douglas et al., 1983), will reduce the magnitude of PP-GC LTP. If the SD input were to inhibit basket cell activity, an increase in LTP magnitude might occur. In further support of this hypothesis, Wigstrom and Gustafsson (1983) have recently shown that a reduction in the strength of the GC recurrent inhibitory circuits, induced by

treatment with drugs such as picrotoxin or bicuculline, increases the magnitude of LTP at the PP-GC synapse.

The following three experiments examined the immediate interactions (including depression/facilitation effects) between SD and PP inputs to the dentate gyrus. One of the most powerful tests for determining the strength of recurrent inhibitory circuits are the paired-pulse tests. Typically, paired-pulse effects depend on the interpulse interval (Adamec et al., 1981; Barnes, 1979; Racine and Milgram, 1983). At short interpulse intervals (20 to 50 ms) the amplitude of the population spike evoked by the second PP pulse (PP2) is depressed compared to the amplitude of the response evoked by the first pulse (PP1). From approximately 50 to 300 ms there is a period of relative facilitation of PP2. A second period of depression occurs at interpulse intervals from approximately 300 ms to 3s. The first period of inhibition is probably GABA-mediated (Ben-Ari, Krnjevic, Reiffenstein and Reinhardt, 1981) and results from basket cell activation following GC discharge (Adamec et al., 1981; Barnes, 1979; Tuff, Racine and Adamec, 1983). In contrast, the late depression is probably not GABA-mediated (Kehl and McLennan, 1983) but may depend on an increased conductance for K^+ (Newberry and Nicoll, 1984; Thalmann and Ayala, 1982). Facilitation is most likely due to increased levels of transmitter release.

EXPERIMENT 1. PAIRED-PULSE EFFECTS

To study these short-term interactions, I examined the effects of paired-pulse stimulation on the amplitude of the GC population response. Specifically, this experiment examined the effect of each of 4 possible combinations of paired stimuli (PP-PP; PP-SD; SD-SD; SD-PP) on field responses recorded in the dentate gyrus. The objectives were to determine 1) which pulse-pairs produced facilitation and/or depression effects, 2) if LTP cooperativity effects could be predicted by these short-term effects (Chapter III, Experiment 1), and 3) if the observed interactions were consistent with the proposed disinhibition hypothesis.

METHODS

This experiment used the same male Long-Evans Hooded rats that were used for Experiment 1 in Chapter III. The animals were prepared for surgery and implanted with electrodes as described in the General Methods sections. To study facilitation effects within a pathway (PP-PP; SD-SD), two stimuli of equal amplitude were delivered through the same electrode. The initial pulse served as a conditioning stimulus and the second pulse evoked the test response. To examine heterosynaptic facilitation effects (PP-SD; SD-PP) either the SD or PP pulse served as the conditioning pulse, while the test pulses were

applied to the PP or SD, respectively. These facilitation effects were examined prior to testing for LTP effects (Chapter III, Experiment 1).

PP stimulation was sufficient to consistently evoke a small GC population spike in response to a single PP volley. The SD intensity was maintained at 1200 μA. These intensities, were used to test all four combinations of paired-pulse stimulation.

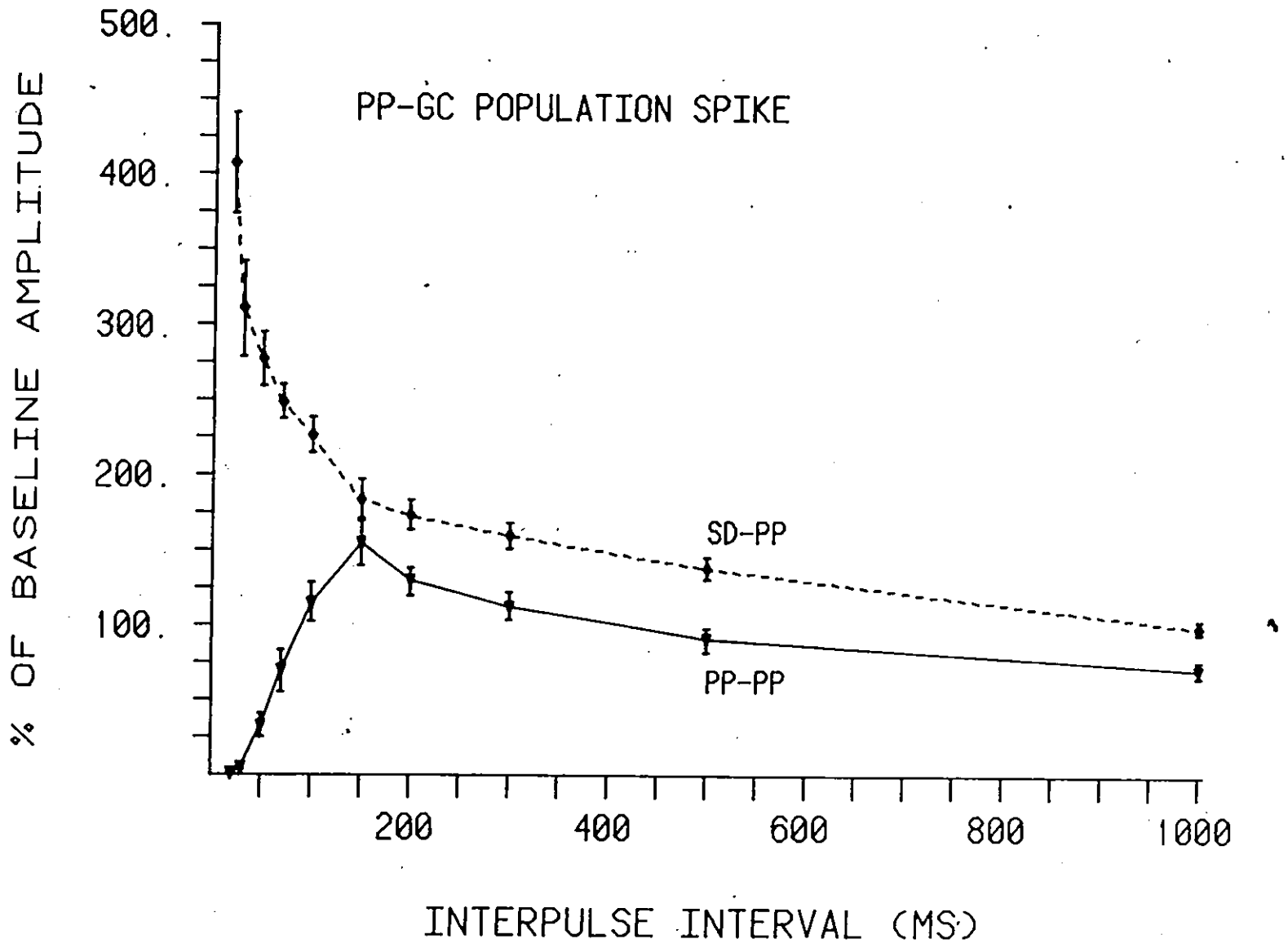
The interstimulus intervals for the pulse-pairs were 20, 30, 50, 70, 100, 150, 200, 300, 500 and 1000 ms. There was a 10 s interval between each pulse-pair. Five pulse-pairs were delivered at each interstimulus interval. The magnitude of the paired-pulse effects were calculated as the percent change in the amplitude of the test response over the conditioned response. If the conditioning pulse had no effect on the test response, the test response amplitude was recorded as 100% (of conditioning response, or baseline, amplitude). The data were then averaged for each interval, across all animals.

RESULTS

Figure 20 illustrates the effects of PP-PP and SD-PP pulse-pairings on the PP-GC population spike (the test response in these measures). The population EPSP was relatively unaffected by either the SD or PP conditioning pulse.

The PP-PP pulse-pairings resulted in the typical triphasic depression/facilitation/depression response curve. The period of early depression was from 20 to 100 ms and, at the two smallest intervals the test population spike was completely eliminated. Peak facilitation occurred at an interpulse interval of 150 ms (155 ± 15%; n=45). The

Figure 20: Effect of conditioning pulse on test PP-GC population spike as a function of the interpulse interval. Both homosynaptic depression and facilitation were observed when the conditioning pulse was applied to the PP (▽——▽). When the conditioning pulse was applied to the SD afferents, only heterosynaptic facilitation was observed (◇- -◇). The PP-GC population EPSP was relatively unaffected by either conditioning pulse.


PP-GC POPULATION SPIKE
% OF BASELINE AMPLITUDE
500.
400.
300.
200.
100.
SD-PP
PP-PP
200
400
600
800
1000
INTERPULSE INTERVAL (MS)

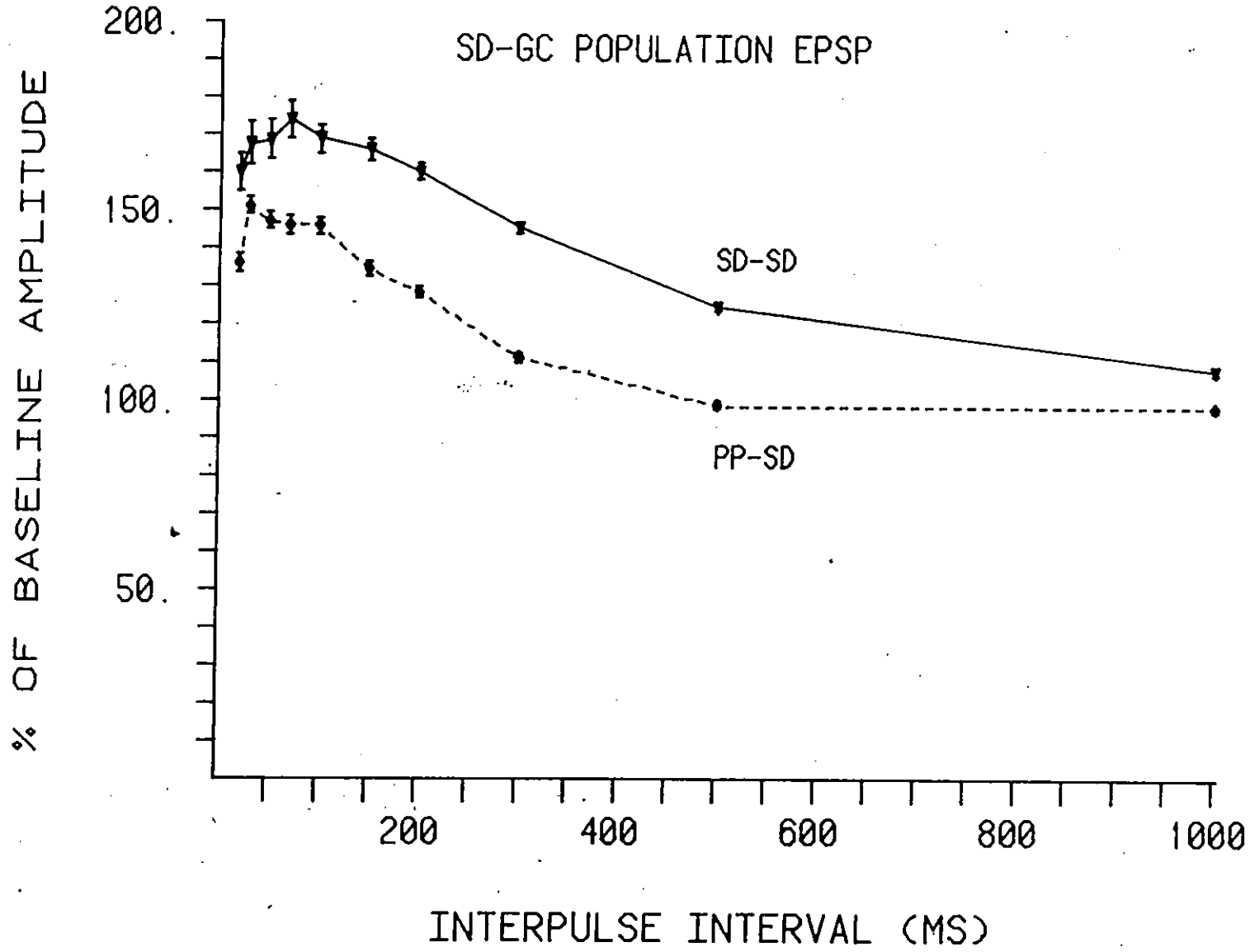
delay to peak facilitation may be due to activation of the recurrent inhibitory circuits (Racine and Milgram, 1983). The late depression phase occurred from 500 to 1000 ms (the largest interpulse interval examined). These results are similiar to those reported by Adamec et al. (1981), Barnes (1979), Racine and Milgram (1983) and Tuff et al. (1983).

In contrast, there was no early depression observed with the SD-PP pairings. SD-PP pairings showed a peak facilitation, of 408 ± 34% (n=70), at the 20 ms interpulse interval. In addition to its greater magnitude, heterosynaptic facilitation was also longer-lasting than homosynaptic facilitation. At an interstimulus interval of 500 ms, for example, the magnitude of heterosynaptic facilitation was 138 ± 7% compared to a 9 ± 2% depression with the homosynaptic pairings. There was no late depression with the SD-PP pairings.

Figure 21 illustrates the results of the paired-pulse tests when the SD-GC response was the test response. Only the initial positivity was measured, as there was no component of the response comparable to a population spike. Peak facilitation following the SD-SD pairings (175 ± 5%; n=85) was greater than that observed following the PP-SD pairings (151 ± 2%; n=60). These peak facilitation effects occurred at 70 ms and 30 ms, for the homosynaptic and heterosynaptic pairings, respectively. Homosynaptic facilitation effects were longer lasting than those observed for the heterosynaptic pairings.

Peak facilitation for either the homosynaptic (SD-SD) or heterosynaptic (PP-SD) paired-pulse tests did not occur at the shortest interpulse interval. The initial delay to peak facilitation may have

Figure 21: Effect of conditioning pulse on test SD-GC population EPSP as a function of the interpulse interval. Conditioning pulses were applied to either the SD afferents (▽——▽) or the PP afferents (◇- -◇). There was a delay to peak facilitation when the conditioning pulse was applied to either the SD or PP afferents.


SD-GC POPULATION EPSP
% OF BASELINE AMPLITUDE
200.
150.
100.
50.
SD-SD
PP-SD
200
400
600
800
1000
INTERPULSE INTERVAL (MS)

been due to transmitter depletion or to activation of the recurrent inhibitory circuits (Racine and Milgram, 1983). Whatever the mechanism, the initial depression, or delay to peak facilitation, was never as large as that observed when testing the PP-GC population spike.

DISCUSSION

Short-term interaction effects were found for all four pulse-pair combinations. The largest and longest-lasting effects occurred when the SD pulse served as the conditioning pulse for the PP-GC population spike. Although PP-GC spike facilitation could exceed 500%, the population EPSP measure did not appear to be affected. When testing the SD-GC response, the greatest facilitation effects also occurred when the conditioning pulse was applied to the SD pathway. Although short-term interaction effects were found for all 4 pulse-pair combinations, they did not appear to correlate with the amount of interaction produced by subsequent pairings of tetanic stimulation (see Chapter III, Experiment 1; also, see Chapter III, Experiment 3).

These results are similar to those previously reported for the PP-PP (Adamec et al., 1981; Barnes, 1979) and SD-PP pairings (Alvarez-Leefmans and Gardner-Medwin, 1975; Fantie and Goddard, 1982). In the dentate, however, the SD-SD and PP-SD pairings have not been previously studied. Tetanic and posttetanic potentiation of both the septal to CA1 input (Andersen et al., 1961b; DeFrance et al., 1978; Stanley, DeFrance and Marchand, 1979; Stanley et al., 1980) and the SD-GC pathway (Andersen et al., 1961a) have, however, been reported.

These groups independently demonstrated that short duration trains applied to the medial septum increased the amplitude of both the CA1 and GC responses to septal stimulation for a short period. Although these were potentiation (short-term), rather than facilitation effects, they nevertheless indicated that the pathways were capable of short-lasting changes in synaptic efficacy.

The fact that SD-PP pairings resulted in facilitation at the smallest interpulse intervals, whereas only depression was observed with the PP-PP pairings (see Figure 20) is consistent with the proposed SD-induced reduction in the strength of the inhibitory circuits. This possibility will be further investigated in the following experiment.

EXPERIMENT 2: TRIPLE-PULSE EFFECTS

In Experiment 1 the paired-pulse curves produced by the SD-PP and PP-PP pairings (see Figure 20) were mirror images of each other up to an interpulse interval of 150 ms. The PP pulse-pairs resulted in depression of the test population spike, whereas the SD-PP pairings produced only facilitation. This is consistent with the possibility that activation of the SD input may suppress or block GC inhibition. The associative interaction effects reported in Chapters III and IV could also be due to such a mechanism. As previously mentioned, if the SD trains interfered with the GC inhibitory circuits, the effect of the PP trains might be increased. If the SD input suppresses inhibitory interneurons, then it should also be possible to reduce or eliminate the

PP-PP paired-pulse depression by application of a single SD pulse just prior to the PP pulse-pair. This possibility was tested with a three-pulse experiment.

METHODS

Three conditions were examined: 1. SD-PP1-PP2 (SD pulse 3-5 ms prior to PP1), 2. PP1-SD---PP2 (SD pulse 3-5ms after PP1), and 3. PP1---SD-PP2 (SD pulse 3-5 ms prior to PP2). The duration (3-5 ms) between the SD and PP pulses varied according to PP1 spike latency. The effect of the three SD pulse paradigms on paired-pulse depression was compared against the effect of PP pulse-pairs restricted to the PP. All four tests were performed in each animal and within the same hemisphere.

In an initial group of 5 animals, the interval between the PP pulse-pairs was varied between 20 and 1000 ms, and 5 pairs were sampled at each interval. For the SD-PP1-PP2 paradigm, the intensity of PP1 was reduced to produce a population spike amplitude equal to that evoked by the conditioning pulse (PP1) in the other three conditions. For comparison purposes a non-corrected group was also tested.

In an additional group of 9 animals, interpulse intervals of 2000, 3000, 5000, and 10000 ms were also tested to determine the duration of the late depression. In this group, the interval between pulse-pairs was increased from 10 to 15 s.

The anesthetic used in this experiment was urethane (1.5 g/kg) as sodium pentobarbitol increases GABA-mediated recurrent inhibition in

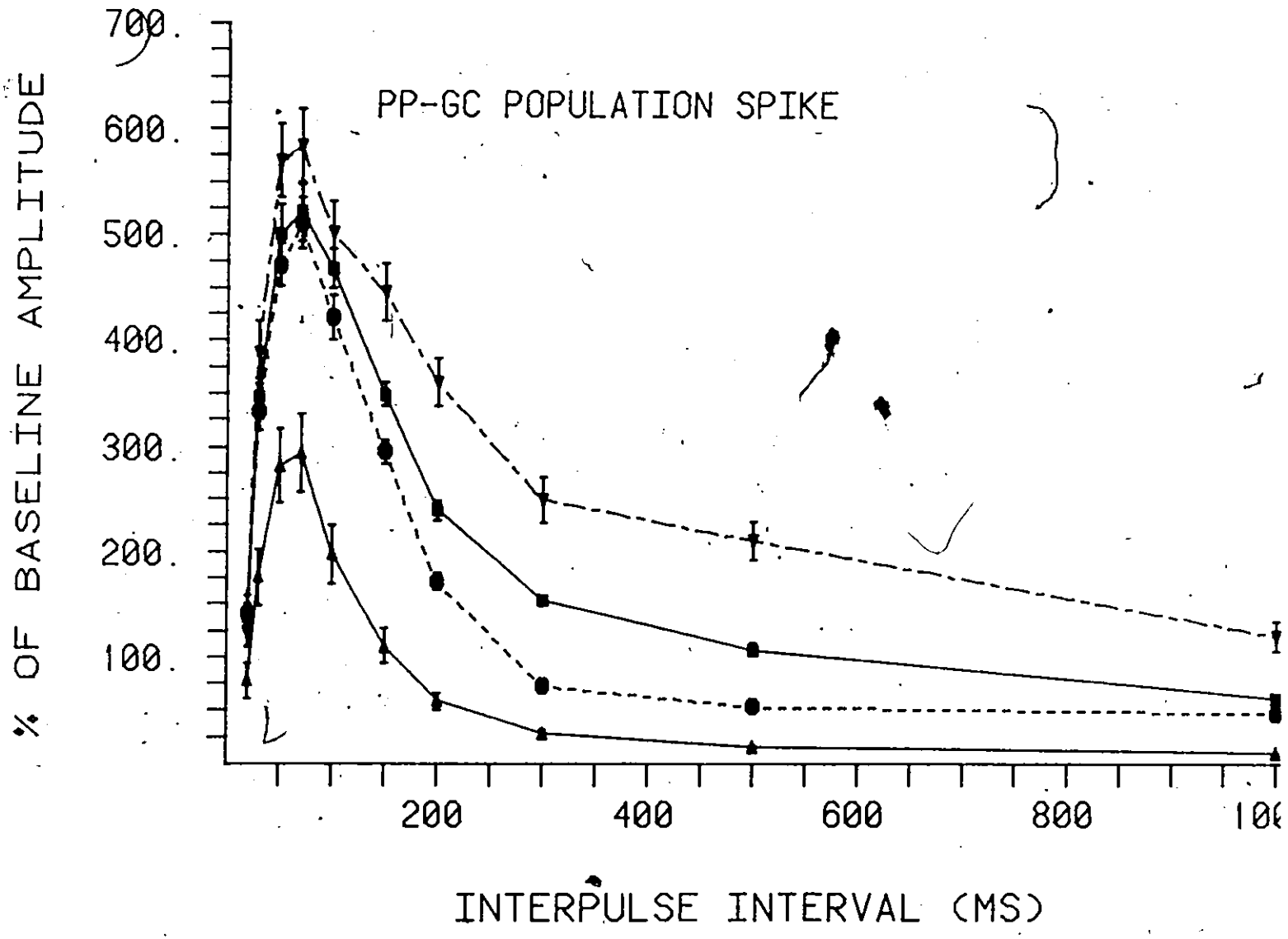
the hippocampus (Barnes, 1979; Nicoll, Eccles, Oshima and Rubia, 1975; Wilson and Racine, 1983b). The use of urethane leaves an apparently normal early inhibition, so if the SD pulse affected recurrent inhibition this effect would not be obscured.

RESULTS

Figure 22 illustrates the results from the initial group of 5 animals where the PP-PP interpulse interval was varied from 20 to 1000 ms. All four conditions resulted in the typical depression-facilitation-depression sequence. The maximum facilitation effect occurred at 70 ms, in all four conditions. For the SD-PP1-PP2, PP1-SD---PP2, PP1---SD-PP2 and the PP1-PP2 conditions the maximum facilitation was 294 (± 36%), 512 (± 25%), 585 (± 35%) and 521% (± 27%), respectively.

There was no late depression phase, for the PP1---SD-PP2 condition, although the level of facilitation did decrease to 126% (± 14%). The PP1-SD---PP2 and PP1-PP2 conditions were not significantly different from each other up to an interpulse interval of 70 ms. From 70 ms to 1000 ms, however, the magnitude of the late depression was greater for the PP1-SD---PP2 condition than was observed for tests with PP pairs alone. By 1000 ms this difference was quite small. The depression observed in the SD-PP1-PP2 condition was the result of not equating PP1 spike amplitude with the amplitude of the spike produced by the conditioning pulse in the other three conditions (non-corrected group). Figure 23A compares the SD-PP1-PP2 and PP1-PP2

Figure 22: Effect of SD pulse on PP-PP paired-pulse depression/facilitation. PP-PP paired-pulse (□——□), SD-PP1-PP2 (SD pulse before PP1; △——△), PP1-SD---PP2 (SD pulse after PP1;○- -○), PP1--SD-PP2 (SD pulse before PP2;▽- -▽).

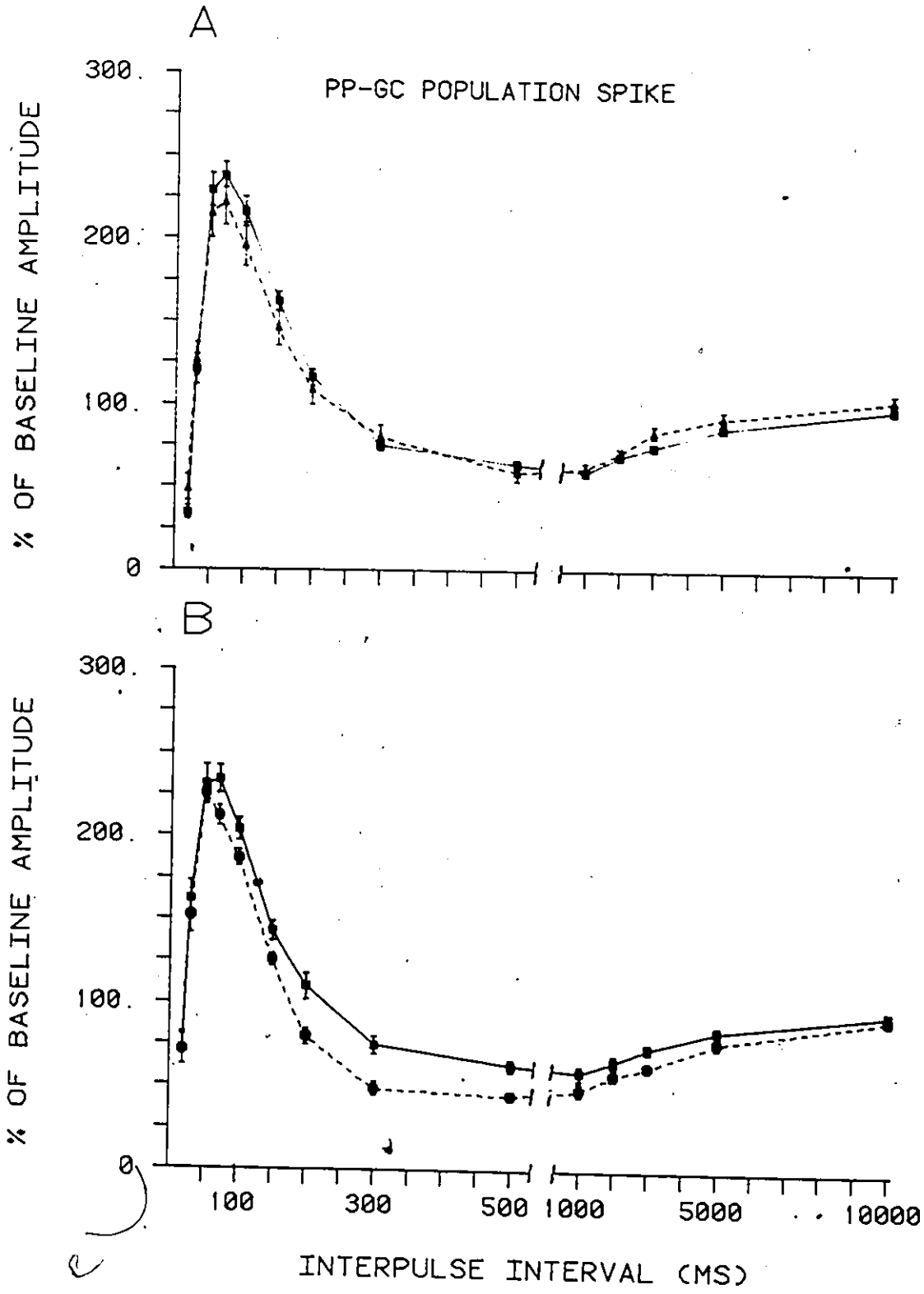

PP-GC POPULATION SPIKE
% OF BASELINE AMPLITUDE
700.
600.
500.
400.
300.
200.
100.
200
400
600
800
INTERPULSE INTERVAL (MS)

conditions, in the second group of animals, when the amplitude of PP1 was corrected for the effects of the SD pulse. Application of the SD pulse prior to PP1 had no effect on paired-pulse depression at the two shortest intervals and increased it slightly from 50 to 200 ms. In this second group of animals, the effect of the SD pulse following the conditioning pulse by 5 ms (PP1-SD---PP2) was also followed up to an interpulse interval of 10 s (see Figure 23B). When the SD pulse followed PP1, the magnitude of the late depression was significantly increased from 70 to 2000 ms but there was no effect on either the early depression or facilitation.

DISCUSSION

It appears that the SD input, when activated with single pulses, does not interfere with recurrent inhibition. If the SD input were mainly to basket cell interneurons, then application of the SD pulse prior to PP1 should have blocked the interneurons before they were activated by collateral discharge from the GCs. The SD pulse, however, had no effect on the magnitude of the early PP paired-pulse depression effects. These results, therefore, appear to be more consistent with a direct action of the SD terminals on GCs rather than an indirect action via inhibitory interneurons.

Figure 23: A. Comparison of PP-PP (□——□) and SD-PP1-PP2 (△- -△) paired-pulse depression/facilitation effects when the amplitude of PP1 was corrected for the effects of the SD pulse. B. Comparison of PP-PP (□——□) and PP1-SD---PP2 (○- -○) paired-pulse depression/facilitation effects at interpulse intervals up to 10 s.


A
PP-GC POPULATION SPIKE
% OF BASELINE AMPLITUDE
300.
200.
100.
0
B
% OF BASELINE AMPLITUDE
300.
200.
100.
0
100
300
500
1000
5000
10000
INTERPULSE INTERVAL (MS)

EXPERIMENT 3: EFFECT OF SEPTAL TRAINS ON GRANULE CELL RECURRENT INHIBITION

Although a single SD pulse, prior to PP pulse-pairs, had little effect on the early PP paired-pulse depression, it was possible that an SD train would affect the recurrent inhibitory circuits. It is known, for example, that single commissural volleys do not affect PP-GC LTP, whereas commissural trains reduce the magnitude of LTP (Douglas et al., 1982). This raises the possibility that SD trains, but not single pulses, may affect recurrent inhibitory circuits and thereby increase the effect of each PP pulse within the PP trains. To determine if the SD trains interfered with the GC inhibitory circuits, the effect of SD trains on PP paired-pulse tests was examined.

METHODS

Seventeen animals were prepared for acute recording and stimulation as previously described. Paired-pulse stimulation was applied to the PP, with the interval between the conditioning and test pulse being varied from 20 to 1000 ms (same intervals as Experiment 1). Five pulse-pairs were applied at each interval. Three sets of SD trains were then applied to the SD input (400 Hz, 50 ms, 100 μs by 1200 μA). Each train set consisted of 3 SD trains applied 1 s apart, with each set being separated by 5 min. Immediately after the last SD train set, pulse-pairs were again applied to the PP. If necessary the

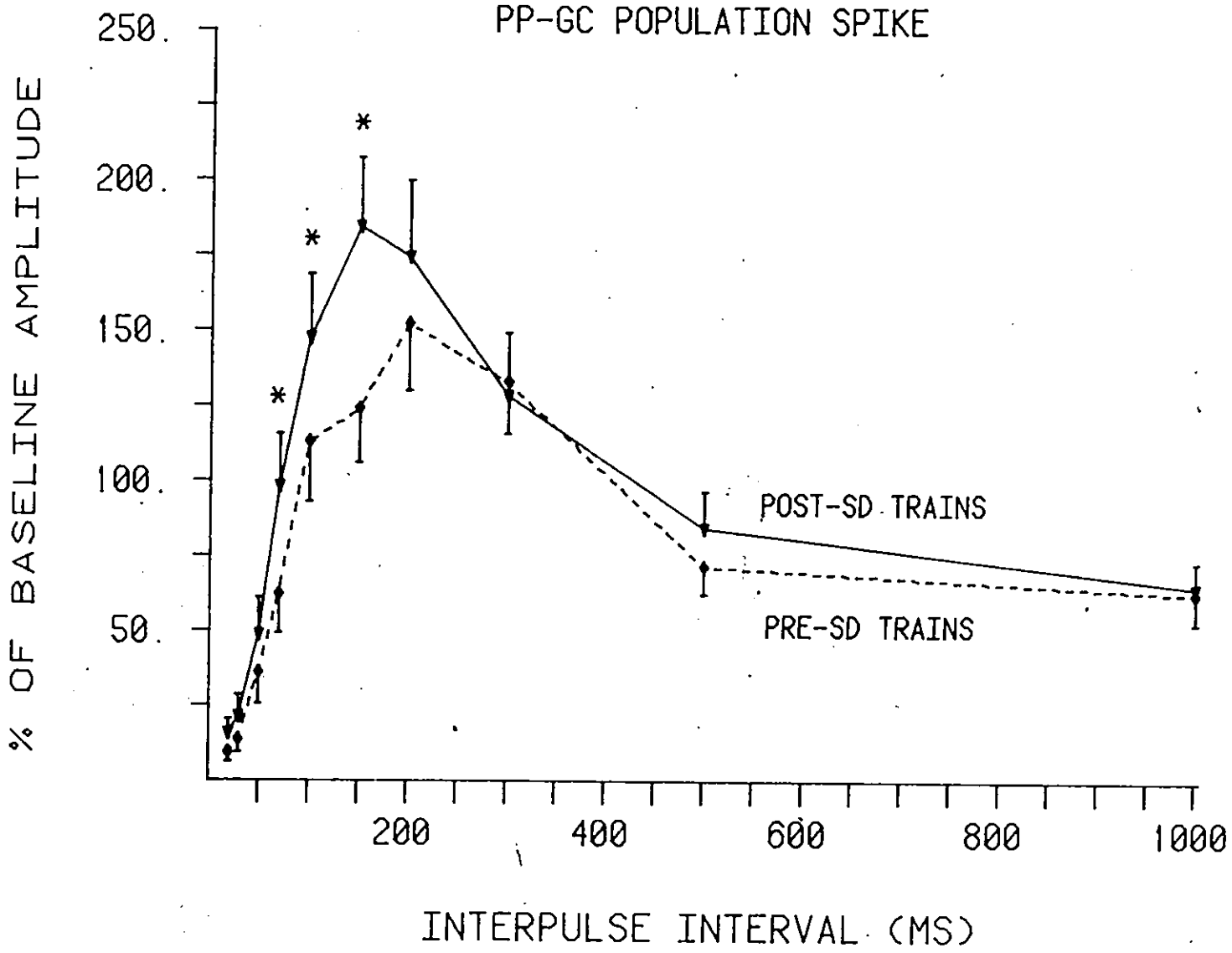
intensity of the PP pulses was adjusted so that the amplitude of the initial, or conditioning, PP population spike was similar to that evoked prior to the SD trains.

RESULTS

Figure 24 illustrates the effect of SD trains on the PP paired-pulse tests. Prior to the SD trains, the conditioning pulse resulted in depression of the test spike amplitude for interpulse intervals up to 100 ms. From 100 to 300 ms there was a period of relative facilitation that reached a maximum (153 ± 23%) at an interpulse interval of 200 ms. From 200 to 1000 ms the level of facilitation decreased, being replaced by a second phase of depression. The second depression was marked by a decrease in the amplitude of the test spike of 37% (± 8%) at 1000 ms.

Following the SD train sets, there was relatively less depression and/or greater facilitation at all interpulse intervals. The maximum level of facilitation increased to 185% (± 21%), at an interpulse interval of 150 ms. In contrast, prior to the SD trains, the maximum level of facilitation (153 ± 23%) was smaller and occurred at a later interval (200 ms). Thus, not only was the magnitude of the facilitation effect increased but the maximal level of facilitation also occurred at a shorter interpulse interval. The difference between the two paired-pulse curves was significant at interpulse intervals of 70, 100, and 150 ms ($p<.02$; t-test for correlated means). Although SD trains did not significantly affect the magnitude of the late

Figure 24: Effect of high-frequency SD trains on PP-PP paired-pulse depression/facilitation effects. Pre-SD trains (◇- -◇). Post-SD trains (▽——▽).


PP-GC POPULATION SPIKE
% OF BASELINE AMPLITUDE
250.
200.
150.
100.
50.
200
400
600
800
1000
INTERPULSE INTERVAL (MS)
POST-SD TRAINS
PRE-SD TRAINS
*
*
*

depression, there was a tendency for it to decrease following the SD trains.

DISCUSSION

These results suggest that at least part of the cooperativity effect may be due to a reduction in the level of recurrent inhibition. Both Ben-Ari et al. (1981) and Krnjevic et al. (1981) have suggested that trains of electrical pulses applied to the medial septum may reduce the release of GABA from inhibitory interneurons onto hippocampal pyramidal cells. This may result in an overall reduction in tonic inhibition and subsequent reduction in the threshold for GC discharge. This would explain the ability of SD trains, when presented alone, to induce a short-lasting potentiation of PP-GC population spike (Chapter III, Experiment 1).

CHAPTER VI. GENERAL DISCUSSION

These experiments demonstrate that interactions between the SD and PP afferents to the GCs of the dentate gyrus influence the magnitude of LTP. When concurrently activated, or when the SD input was activated within a certain temporal interval prior to PP activation, the magnitude of LTP was found to increase to a level above that produced by the PP-only trains.

This heterosynaptic cooperativity effect shares a number of the characteristics (see Chapter III) found for interactions between the medial and lateral, or crossed and ipsilateral, components of the PP (Levy and Steward, 1979; McNaughton et al., 1978). First, when the number of coactive afferents to the GCs was increased (i.e. concurrent SD and PP trains), the magnitude of the LTP effect also increased (see Experiments 1 and 2). Second, increments in the SD train intensity, a second method of increasing the number of coactive afferents, increased the magnitude of the heterosynaptic increase in LTP (see Experiment 3).

However, unlike other systems in which cooperative interaction effects have been examined, the SD and PP system was found to exhibit cooperativity effects even when long delays were interposed between the trains (see Chapter IV, Experiment 1). The medial and lateral components of the PP do not exhibit a cooperativity effect when any delay is interposed between their activation (McNaughton et al., 1978), and the crossed and ipsilateral systems will interact to increase the level of LTP only if the intertrain delay is no more than 20 ms (Levy

and Steward, 1983). In the present experiments, however, activation of the SD and PP afferents increased the magnitude of LTP even when the delay between the SD and PP trains was 1000 ms.

These delays are similar to those observed in some classical conditioning experiments and may, therefore, provide a possible basis for neural associations that are necessary for learning and memory. In addition, the magnitude of the additional increment was shown to depend on the width of the temporal window. As the duration of the interval between activation of the SD and PP inputs was increased, the magnitude of the additional increase was reduced. Similarly, as the CS-UCS interval is increased, behavioural conditioning generally becomes more difficult. These findings increase the attractiveness of the LTP phenomenon as a model for associative memory mechanisms.

There is, however, a wide range of CS-UCS intervals that support conditioning and the optimal interval depends on the behaviour as well as various stimulus conditions (see Mackintosh, 1974). Taste-aversion learning, for example, in which animals learn to associate tastes with subsequent illness, occurs with CS-UCS delays of several hours. Rat pups below 12 days of age, however, are unable to learn the association if there is a delay between the taste (CS) and illness (UCS) (Gemberling and Domjan, 1982; Gregg, Kittrell, Domjan, and Amsel, 1978). It is interesting that this is the approximate age at which high-frequency PP trains are first able to induce LTP effects in the dentate gyrus (Wilson and Racine, 1983a).

As mentioned in Chapter IV, the SD input could be considered the weak input (relative to the PP input) which should make it analogous to the CS. In behavioural conditioning experiments, however, it is the CS which acquires the associative strength, but in the present experiments it was the PP-GC response that increased as a result of the paired trains. As a model phenomenon for associative learning mechanisms there are a number of ways to consider these results. First, it is possible that the SD input, although producing a relatively weaker GC response, may actually be the neural representation, at that level, of a strong (e.g. biologically significant) input. Although numerically fewer than the PP terminals, if the major site of SD termination is the basket cell interneurons this could allow them to exert a strong effect on GC excitability (Lynch et al., 1978). If this were the case, however, the optimal ordering of the two inputs would appear to be reversed. This ordering would also seem to rule out a role for the SD input in reinforcement. While it is possible that the PP is actually a reinforcing input, this would again lead to the expectation that the SD-GC and not the PP-GC response should be potentiated. Another possibility, as suggested by others (Berger and Thompson, 1977; Berry and Thompson, 1978, 1979; Solomon, 1979, 1980), is that the SD input may be involved in attentional or arousal mechanisms. Berry and Thompson (1978), for example, found that rabbits acquired the nictitating membrane response at a faster rate when the frequency of hippocampal EEG was in the range of theta. The hippocampus receives a number of inputs from various brain stem nuclei that have been shown to play a role in

arousal. Many of these fibres pass through the medial septum before entering the hippocampus.

Whatever its role in normal function, there are several mechanisms that could mediate this cooperativity effect. Given that the majority of SD afferents appear to terminate immediately below the GC layer (Crutcher et al., 1982; Lewis and Shute, 1967; Lewis et al., 1967; Lynch et al., 1978; Mosko et al., 1973; Stanfield and Cowan, 1982) it is possible that the SD trains interfere with the GC recurrent inhibitory circuits (see, Assaf and Miller, 1978; Lynch et al., 1978; Winson, 1980). In support of this hypothesis, it was shown that SD trains decreased the magnitude of the early phase of depression observed in the paired-pulse tests (see Chapter V, Experiment 3). The results of Experiment 2 (Chapter V), however, suggested that the SD afferents may have been exerting their effect via a direct activation of the GCs. The main effect of the SD pulse on PP paired-pulse depression/facilitation effects appeared to be an increase in the magnitude of the late phase of depression. This late depression is most likely due to an increase in K^+ conductance (Thalmann and Ayala, 1982).

An increase in K^+ currents would be expected to decrease GC excitability, which seems to contradict the results of the train experiments. Assaf and Miller (1978), however, have shown that the magnitude of paired-pulse facilitation is positively correlated with the magnitude of GC inhibition. They suggested that this was due to the fact that facilitation effects were determined from population measures,

and that the period of inhibition would place a greater population of GCs in a similar state. A given afferent volley would therefore produce a larger amplitude population spike because an increased number of GCs would be in a similar state, thereby increasing the synchrony of discharge.

Nicoll and Alger (1981) first suggested that the increased K^+ conductance may depend on the rise in intracellular Ca^{++} that occurs following orthodromic activation. LTP also appears to depend on an increased intracellular Ca^{++} concentration (Lynch et al., 1983a,b). It is possible that the SD inputs activate Ca^{++} fluxes in GCs, thereby increasing both late hyperpolarization effects and LTP effects produced by PP activation. More recent evidence, however, has shown that the increased K^+ conductance in GC's occurs in the presence of EGTA and is therefore probably not Ca^{++} mediated (Lancaster and Wheal, 1984; Thalmann; 1984). It may, in fact be mediated by a chemical transmitter (Newberry and Nicoll, 1983; Segal, 1980).

There is an extensive body of literature showing that the septal projection is probably cholinergic. It was therefore reasonable to assume that these effects may have been cholinergically mediated. Iontophoretic application of ACh, to both the hippocampus and dentate gyrus, is known to increase the excitability of the principle cells in these regions (Bland, Kostopoulos and Phillis, 1974).

Two mechanisms have been proposed to account for the ACh-induced increase in excitability. First, ACh may decrease K^+ conductances (Benardo and Prince, 1982a,b,c; Ben-Ari et al., 1981; Dodd, Dingledine and Kelly, 1981; Krnjevic et al., 1981), responsible for repolarization

of the membrane, leaving the GCs in a more excitable state. Second, ACh may exert its excitatory influence by decreasing the level of GABA-mediated inhibition (Ben-Ari et al., 1981, Hounsgaard, 1978; Krnjevic et al., 1981). Specifically, ACh may act presynaptically to decrease GABA release from the inhibitory basket cell interneurons.

The triple-pulse experiments (Chapter V, Experiment 2), however, were consistent with an increase in K^+ currents, raising the possibility that the heterosynaptic LTP effect is not cholinergically mediated. In support of this possibility, Fantie and Goddard (1982) found that various cholinergic agonists and antagonists did not affect the magnitude of SD-PP paired-pulse spike facilitatation. In addition, the muscarinic antagonist, atropine (50 mg/kg), does not block the short-lasting heterosynaptic increase in the population spike, that is sometimes observed following SD-only trains, and does not block the additional increase in LTP observed when the trains are subsequently applied concurrently (Robinson, unpublished observations).

It is possible that the cooperativity and paired-pulse effects were due to activation of fibres of passage. One subset of these fibers is noradrenergic, but Fantie and Goddard (1982) showed that noradrenaline depletion did not affect SD-PP paired-pulse spike facilitation. Assaf and Miller (1978) showed that paired stimulation of the raphe nucleus (which gives rise to serotonergic fibers) and PP (raphe stimulation served as the conditioning pulse) increased the magnitude of the PP-GC population spike. It is therefore possible that the cooperativity effect is due to the activation of serotonergic fibres of passage.

Segal (1980) has shown that application of serotonin to the pyramidal cell layer, of area CA1, causes a hyperpolarization and a decrease in input resistance. The decreased input resistance was thought to be due to activating K^+ channels (i.e. an increased conductance for K^+). These findings are consistent with both the results of Assaf and Miller (1978) and the increased magnitude of the late depression found in the triple-pulse experiment. To determine between the various possibilities would require the use of intracellular recording techniques.

Whatever the mechanism, these results have provided another demonstration of associative interactions between coactive afferents leading to an increase in the magnitude of LTP. The anatomical and neurochemical differences between these two pathways make it an attractive system in which to study these interactions. In addition, the SD and PP afferents probably convey different types of information to the dentate gyrus. Finally, the allowable temporal delays for these interactions are similar to those found for certain types of behavioural conditioning. As already mentioned, however, these interactions may reflect an arousal-like mechanism rather than an associative mechanism.

REFERENCES

Abraham, W. C. & Goddard, G. V. Modulation of synaptic transmission and LTP in rat dentate gyrus by stimulation in and near the locus coeruleus. Society for Neuroscience Abstracts, 1982, 8, 482.

Abraham, W. C. & Goddard, G. V. Asymmetric relationships between synaptic long-term potentiation and heterosynaptic depression. Nature, 1983, 305, 717-719.

Adamec, R. E., McNaughton, B., Racine, R. & Livingston, K. E. Effects of diazepam on hippocampal excitability in the rat: action in the dentate. Epilepsia, 1981, 22, 205-215.

Ajmone-Marsan, C. & Matthies, H. (Eds.), Neuronal plasticity and memory formation. New York: Raven Press, 1982.

Alger, B. & Teyler, T. Long-term and short-term plasticity in the CA1, CA3, and dentate regions of the rat hippocampal slice. Brain Research, 1976, 110, 463-480.

Alvarez-Leefmans, F. J. & Gardner-Medwin, A. R. Influences of the septum on the hippocampal dentate area which are unaccompanied by field potentials. Journal of Physiology, 1975, 249, 14P-16P.

Andersen, P. Organization of hippocampal neurons and their interconnections. In R. L. Isaacson & K. H. Pribram (Eds.), The hippocampus, Volume 1: Structure and development. New York: Plenum Press, 1975.

Andersen, P. Possible cellular basis for prolonged changes of synaptic efficiency - a simple case of learning. Progress in Brain Research, 1983, 58, 419-426.

Andersen, P., Blackstad, T. W. & Lomo, T. Location and identification of excitatory synapses on hippocampal pyramidal cells. Experimental Brain Research, 1966, 1, 236-248. (a)

Andersen, P., Bland, B. H. & Dudar, J. D. Organization of the hippocampal output. Experimental Brain Research, 1973, 17, 152-168.

Andersen, P., Bliss, T. V. P. & Skrede, K. K. Unit analysis of hippocampal population spikes. Experimental Brain Research, 1971, 13, 208-221. (a)

Andersen, P., Bliss, T. V. P. & Skrede, K. K. Lamellar organization of hippocampal excitatory pathways. Experimental Brain Research, 1971, 13, 222-238. (b)

Andersen, P., Bruland, H. & Kaada, B. R. Activation of the dentate area by septal stimulation. Acta Physiologica Scandinavica, 1961, 51, 17-28. (a)

Andersen, P., Bruland, H. & Kaada, B. R. Activation of the field CA1 of the hippocampus by septal stimulation. Acta Physiologica Scandinavica, 1961, 51, 29-40. (b)

Andersen, P., Eccles, J. C. & Loyning, Y. Location of postsynaptic inhibitory synapses on hippocampal pyramidals. Journal of Neurophysiology, 1964, 27, 592-607. (a)

Andersen, P., Eccles, J. C. & Loyning, Y. Pathway of postsynaptic inhibition in the hippocampus. Journal of Neurophysiology, 1964, 27, 608-619. (b)

Andersen, P., Holmqvist, B. & Voorhoeve, P. Entorhinal activation of dentate granule cells. Acta Physiologica Scandinavica, 1966, 66, 448-460. (b)

Andersen, P., Sundberg, S. H., Sveen, O., Swann, J. W. & Wigstrom, H. Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea pigs. Journal of Physiology, 1980, 302, 463-482.

Andersen, P., Sundberg, S., Sveen, O. & Wigstrom, H. Specific long-lasting potentiation of synaptic transmission in hippocampal slices. Nature, 1977, 266, 736-737.

Assaf, S. Y., Mason, S. T. & Miller, J. J. Noradrenergic modulation of neuronal transmission between the entorhinal cortex and the dentate gyrus of the rat. Journal of Physiology, 1979, 292, 52P.

Assaf, S. Y. & Miller, J. J. Neuronal transmission in the dentate gyrus: role of inhibitory mechanisms. Brain Research, 1978, 151, 587-592.

Azmitia, E. C. & Segal, M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. Journal of Comparative Neurology, 1978, 179, 641-668.

Baimbridge, K. G. & Miller, J. J. Calcium uptake and retention during long-term potentiation of neuronal activity in the rat hippocampal slice preparation. Brain Research, 1981, 221, 299-305.

Barnes, C. A. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. Journal of Comparative and Physiological Psychology, 1979, 93, 74-104.

Barnes, C. A. & McNaughton, B. L. Where is the cognitive map? Society for Neuroscience Abstracts, 1983, 9, 649.

Baudry, M. & Lynch, G. Hypothesis regarding the cellular mechanisms responsible for long-term synaptic potentiation in the hippocampus. Experimental Neurology, 1980, 68, 202-204.

Baudry, M., Oliver, M., Creager, R., Wieraszko, A. & Lynch, G. Increase in glutamate receptors following repetitive stimulation in hippocampal slices. Life Sciences, 1980, 27, 325-330.

Benardo, L. S. & Prince, D. A. Cholinergic excitation of mammalian hippocampal pyramidal cells. Brain Research, 1982, 249, 315-331. (a)

Benardo, L. S. & Prince, D. A. Ionic mechanisms of cholinergic excitation in mammalian hippocampal pyramidal cells. Brain Research, 1982, 249, 333-344. (b)

Benardo, L. S. & Prince, D. A. Cholinergic pharmacology of mammalian hippocampal pyramidal cells. Neuroscience, 1982, 7, 1703-1712. (c)

Ben-Ari, Y., Krnjevic, K., Reiffenstein, R. J. & Reinhardt, W. Inhibitory conductance changes and action of y-aminobutyrate in rat hippocampus. Neuroscience, 1981, 6, 2445-2463.

Ben-Ari, Y., Krnjevic, K., Reinhardt, W. & Ropert, N. Intracellular observations on the disinhibitory action of acetylcholine in the hippocampus. Neuroscience, 1981, 6, 2475-2484.

Ben-Barak, J. & Dudai, Y. Cholinergic binding sites in rat hippocampal formation: properties and ontogenesis. Brain Research, 1979, 166, 245-257.

Berger, B. & Levy, W. B. Shared characteristics of potentiation and depression in the EC-DG system. Society for Neuroscience Abstracts, 1983, 9, 1221.

Berger, T. W., Alger, B. & Thompson, R. F. Neuronal substrate of classical conditioning in the hippocampus. Science, 1976, 192, 483-485.

Berger, T. W., Laham, R. I. & Thompson, R. F. Hippocampal unit-behavior correlations during classical conditioning. Brain Research, 1980, 193, 229-248.

Berger, T. W. & Thompson, R. F. Limbic system interrelations: functional division among hippocampal-septal connections. Science, 1977, 197, 587-589.

Berry, S. D. & Thompson, R. F. Prediction of learning rate from the hippocampal electroencephalogram. Science, 1978, 200, 1298-1300.

Berry, S. D. & Thompson, R. F. Medial septal lesions retard classical conditioning of the nictitating membrane response in rabbits. Science, 1979, 205, 209-211.

Berthier, N. E. & Moore, J. W. Spatial differential conditioning of the nictitating membrane response in hippocampectomized rabbits. Physiological Psychology, 1980, 8, 451-454.

Blackstad, T. Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. Journal of Comparative Neurology, 1956, 105, 417-538.

Bland, B. H., Kostopoulos, G. K. & Phillis, J. W. Acetylcholine sensitivity of hippocampal formation neurons. Canadian Journal of Physiology and Pharmacology, 1974, 52, 966-971.

Bliss, T. V. P. Synaptic plasticity in the hippocampus. Trends in Neuroscience, 1979, 2, 42-45.

Bliss, T. V. P. & Dolphin, A. C. What is the mechanism of long-term potentiation in the hippocampus? Trends in Neuroscience, 1982, 5, 289-290.

Bliss, T. V. P. & Gardner-Medwin, A. Long lasting potentiation of synaptic transmission in the dentate area of the unanesthetized rabbit following stimulation of the perforant path. Journal of Physiology, 1973, 232, 357-374.

Bliss, T. V. P., Goddard, G. V. & Riives, M. Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. Journal of Physiology, 1983, 334, 475-491.

Bliss, T. V. P. & Lomo, T. Long lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. Journal of Physiology, 1973, 232, 331-356.

Brons, J. F. & Woody, C. D. Long-term changes in excitability of cortical neurons after Pavlovian conditioning and extinction. Journal of Neurophysiology, 1980, 44, 605-615.

Brown, T. H. & McAfee, D. A. Long-term synaptic potentiation in the superior cervical ganglion. Science, 1982, 215, 1411-1413.

Buzsaki, G. Long-term potentiation of the commissural path-CA1 pyramidal cell synapse in the hippocampus of the freely moving rat. Neuroscience Letters, 1980, 19, 293-296.

Buzsaki, G., Bors, L., Nagy, F. & Eidelberg, E. Spatial mapping, working memory, and the fimbria-fornix system. Journal of Comparative and Physiological Psychology, 1982, 96, 26-34.

Buzsaki, G. & Czeh, G. Commissural and perforant path interactions in the rat hippocampus. Experimental Brain Research, 1981, 43, 429-438.

Buzsaki, G. & Eidelberg, E. Commissural projection to the dentate gyrus of the rat: evidence for feed-forward inhbition. Brain Research, 1981, 230, 346-350.

Buzsaki, G. & Eidelberg, E. Direct afferent excitation and long-term potentiation of hippocampal interneurons. Journal of Neurophysiology, 1982, 48, 597-607.

Buzsaki, G., Grastyan, E., Czopf, J., Kellenyi, L. & Prohaska, O. Changes in neuronal transmission in the rat hippocampus during behavior. Brain Research, 1981, 225, 235-247.

Carew, T. J., Hawkins, R. D. & Kandel, E. R. Differential classical conditioning of a defensive withdrawal reflex in Aplysia californica. Science, 1983, 219, 397-400.

Carew, T. J., Walters, E. T. & Kandel, E. R. Classical conditioning in a simple withdrawal reflex in Aplysia californica. Journal of Neuroscience, 1981, 1, 1426-1437.

Castellucci, V. & Kandel, E. R. A quantal analysis of the synaptic depression underlying habituation of the gill-withdrawal reflex in in Aplysia. Proceedings of the National Academy of Sciences, 1974, 71, 5004-5008.

Chang, J. J. & Gelperin, A. Rapid taste-aversion learning by an isolated molluscan central nervous system. Proceedings of the National Academy of Sciences, 1980, 77, 6204-6206.

Ciba Foundation Symposium 58; Functions of the Septo-hippocampal System, 1978.

Clark, G. A., Berger, T. W. & Thompson, R. F. The role of entorhinal cortex during classical conditioning: evidence for entorhinal-dentate facilitation. Society for Neuroscience Abstracts, 1978, 4, 217.

Conrad, L. C. A., Leonard, C. M. & Pfaff, D. W. Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study. Journal of Comparative Neurology, 1974, 156, 179-206.

Creager, R., Dunwiddie, T. & Lynch, G. Paired-pulse and frequency facilitation in the CA1 region of the in vitro rat hippocampus. Journal of Physiology, 1980, 299, 409-424.

Crunelli, V., Forda, S. & Kelly, J. S. Blockade of animo acid-induced depolarizations and inhibition of excitatory post-synaptic potentials in rat dentate gyrus. Journal of Physiology, 1983, 341, 627-640.

Crutcher, K. A., Kesner, R. P. & Novak, J. M. Medial septal lesions, radial arm maze performance, and sympathetic sprouting: a study of recovery of function. Brain Research, 1983, 262, 91-98.

Crutcher, K. A., Madison, R. & Davis, J. N. A study of the rat septohippocampal pathway using anterograde transport of horseradish peroxidase. Neuroscience, 1981, 6, 1961-1973.

Davies, P. & Melvill Jones, G. An adaptive neural model compatible with plastic changes induced in the human vestibulo-ocular reflex by prolonged optical reversal of vision. Brain Research, 1976, 103, 546-550.

Davis, W. J. & Gillette, R. Neural correlate of behavioral plasticity in command neurons of Pleurobranchaea. Science, 1978, 199, 801-804.

Deadwyler, S. A., Dudek, F. E., Cotman, C. W. & Lynch, G. Intracellular responses of rat dentate granule cells in vitro: post-tetanic potentiation to perforant path stimulation. Brain Research, 1975, 88, 80-85.

Deadwyler, S. A., West, J. R., Cotman, C. W. & Lynch, G. S. A neurophysiological analysis of commissural projections to dentate gyrus of the rat. Journal of Neurophysiology, 1975, 38, 167-184.

Deadwyler, S. A., West, M. O. & Robinson, J. H. Evoked potentials from the dentate gyrus during auditory stimulus generalization in the rat. Experimental Neurology, 1981, 71, 615-624. (a)

Deadwyler, S. A., West, M. O. & Robinson, J. H. Entorhinal and septal inputs differentially control sensory-evoked responses in the rat dentate gyrus. Science, 1981, 211, 1181-1183. (b)

DeFrance, J. F. (Ed.), The septal nuclei. New York: Plenum Press, 1976.

DeFrance, J. F., Stanley, J. C., Marchand, J. E. & Chronister, R. B. Cholinergic mechanisms and short-term potentiation. In Ciba Foundation Symposium, 58, Functions of the Septo-hippocampal System. 1978.

Desmond, N. C. & Levy, W. B. Synaptic correlates of associative potentiation/depression: an ultrastructural study in the hippocampus. Brain Research, 1983, 265, 21-30.

Dodd, J., Dingledine, R. & Kelly, J. S. The excitatory action of acetylcholine on hippocampal neurones of the guinea pig and rat maintained in vitro. Brain Research, 1981, 207, 109-127.

Dolphin, A. C. The excitatory amino-acid antagonist γ-D-glutamylglycine masks rather than prevents long-term potentiation of the perforant path. Neuroscience, 1983, 10, 377-383.

Dolphin, A. C., Errington, M. L. & Bliss, T. V. P. Long-term potentiation of the perforant path in vivo is associated with increased glutamate release. Nature, 1982, 297, 496-498.

Douglas, R. J. The hippocampus and behavior. Psychological Bulletin, 1967, 67, 416-442.

Douglas, R. M. Long-lasting synaptic potentiation in the rat dentate gyrus following brief high-frequency stimulation. Brain Research, 1977, 126, 361-365.

Douglas, R. M. & Goddard, G. V. Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. Brain Research, 1975, 86, 205-215.

Douglas, R. M., Goddard, G. V. & Riives, M. Inhibitory modulation of long-term potentiation: evidence for a postsynaptic locus of control. Brain Research, 1982, 240, 259-272.

Douglas, R. M., McNaughton, B. L. & Goddard, G. V. Commissural inhibition and facilitation of granule cell discharge in fascia dentata. Journal of Comparative Neurology, 1983, 219, 285-294.

Dudar, J. D. The effect of septal nuclei stimulation on the release of acetylcholine from the rabbit hippocampus. Brain Research, 1975, 83, 123-133.

Dudek, F. E., Deadwyler, S. A., Cotman, C. W. & Lynch, G. Intracellular responses from granule cell layer in slices of rat hippocampus: perforant path synapse. Journal of Neurophysiology, 1976, 39, 384-393.

Dunwiddie, T. V. & Lynch, G. The relationship between extracellular calcium concentrations and the induction of hippocampal long-term potentiation. Brain Research, 1979, 169, 103-110.

Dunwiddie, T., Madison, D. & Lynch, G. Synaptic transmission is required for initiation of long-term potentiation. Brain Research, 1978, 150, 413-417.

Ellen, P. & Delouche, J. Hippocampal lesions and spontaneous alternation behaviour in the rat. Physiology and Behaviour, 1968, 3, 857-860.

Fantie, B. D. Augmentation of dentate granule cell population spikes after high-frequency trains delivered to the septum and the perforant path. Society for Neuroscience Abstracts, 1982, 7, 315.

Fantie, B. D. & Goddard, G. V. Septal modulation of the population spike in the fascia dentata produced by perforant path stimulation in the rat. Brain Research, 1982, 252, 227-237.

Fifkova, E. & Andersen, C. L. Stimulation-induced changes in dimensions of stalks of dendritic spines in the dentate molecular layer. Experimental Neurology, 1981, 74, 621-627.

Fillenz, M. Hypothesis for a neuronal mechanism involved in memory. Nature, 1972, 238, 41-43.

Fonnum, F. Topographical and cellular localization of choline acetyltransferase in rat hippocampal region. Journal of Neurochemistry, 1970, 17, 739-750.

Galiana, H. L. & Outerbridge, J. S. A bilateral model for central neural pathways in vestibuloocular reflex. Journal of Neurophysiology, 1984, 51, 210-241.

Gardner-Medwin, A. R. Modifiable synapses necessary for learning. Nature, 1969, 223, 916-919.

Gemberling, G. A. & Domjan, M. Selective associations in one-day old rats: taste-toxicosis and texture-shock aversion learning. Journal of Comparative and Physiological Psychology, 1982, 96, 105-113.

Goddard, G. Component properties of the memory machine: Hebb revisited. In P. W. Jusczyk & R. M. Klein (Eds.), The nature of thought: essays in honour of D. O. Hebb. Hillsdale, N. J.: Lawrence Erlbaum Associates, 1980.

Goddard, G. V., McIntyre, D. C. & Leech, C. K. A permanent change in brain function resulting from daily electrical stimulation. Experimental Neurology, 1969, 25, 295-330.

Goddard, G. V. & Riives, M. Inhibitory modulation of LTP in the hippocampal dentate area of awake rats. Society for Neuroscience Abstracts, 1981, 7, 773.

Goldowitz, D. W., White, E., Steward, O., Cotman, C. W. & Lynch, G. S. Anatomical evidence for a projection from the entorhinal cortex to the contralateral dentate gyrus of the rat. Experimental Neurology, 1975, 47, 433-441.

Gormezano, I. Investigations of defense and reward conditioning in the rabbit. In A. H. Black & W. F. Prokasy (Eds.), Classical conditioning. II. Current research and theory. New York: Appleton-Century-Crofts, 1972.

Gottlieb, D. I. & Cowan, W. M. Autoradiographic studies of the commisural and ipsilateral association connections of the hippocampus and dentate gyrus of the rat. I. The commissural connections. Journal of Comparative Neurology, 1973, 149, 383-422.

Gray, J. A. The neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system. New York: Oxford University Press, 1982.

Gregg, B., Kittrell, E. M. W., Domjan, M. & Amsel, A. Ingestional aversion learning in preweanling rats. Journal of Comparative and Physiological Psychology, 1978, 92, 785-795.

Harley, C. W., Lacaille, J-C. & Milway, S. Potentiation of the perforant path evoked potential in the dentate gyrus by locus coeruleus stimulation. Society for Neuroscience Abstracts, 1982, 8, 483.

Haubenreiser, J., Hansen, E. & Haschke, W. Hippocampal evoked potentials, operant conditioning, and spatial discontiguity of CS-US (a preliminary report). In C. Ajmone-Marsan & H. Matthies (Eds.), Neuronal plasticity and memory formation. New York: Raven Press, 1982.

Hawkins, R. D., Abrams, T. W., Carew, T. J. & Kandel, E. R. A cellular mechanism of classical conditioning in Aplysia: activity-dependent amplification of presynaptic facilitation. Science, 1983, 219, 400-405.

Hebb, D. O. The organization of behavior. New York: Wiley, 1949.

Hjorth-Simonsen, A. Projection of the lateral part of the entorhinal area to the hippocampus and fascia dentata. Journal of Comparative Neurology, 1972, 146, 219-232.

Hjorth-Simonsen, A. & Juene, B. Origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. Journal of Comparative Neurology, 1972, 144, 215-232.

Hjorth-Simonsen, A. & Laurberg, S. Commissural connections of the dentate area in the rat. Journal of Comparative Neurology, 1977, 174, 591-606.

Hoehler, F. K. & Thompson, R. F. The effect of temporal single alternation on learned increases in hippocampal unit activity in classical conditioning of the rabbit nictitating membrane response. Physiological Psychology, 1979, 4, 345-351.

Hoehler, F. K. & Thompson, R. F. Effects of the interstimulus (CS-UCS) interval on hippocampal unit activity during classical conditioning of the nictitating membrane response of the rabbit (Oryctolugus cuniculus). Journal of Comparative and Physiological Psychology, 1980, 94, 201-215.

Hounsgaard, J. Presynaptic inhibitory action of acetylcholine in area CA1 of the hippocampus. Experimental Neurology, 1978, 62, 787-797.

Isaacson, R. L. The limbic system. New York: Plenum Press, 1974.

Isaacson, R. L. & Pribram, K. H. (Eds.), The hippocampus, Volume 1: Structure and development. New York: Plenum Press, 1975. (a)

Isaacson, R. L. & Pribram, K. H. (Eds.), The hippocampus, Volume 2: Neurophysiology and behavior. New York: Plenum Press, 1975. (b)

Ishikawa, K., Ott, T. & McGaugh, J. L. Evidence for dopamine as a transmitter in dorsal hippocampus. Brain Research, 1982, 232, 222-226.

Jaffard, R. & Jeantet, Y. Posttraining changes in excitability of the commissural path-CA1 pyramidal cell synapse in the hippocampus of mice. Brain Research, 1981, 220, 167-172.

Kairiss, E. W., Racine, R. J. & Smith, G. K. The development of the interictal spike during kindling in the rat. Brain Research, 1984. (in press)

Kandel, E. R. Cellular basis of behavior. San Francisco: W. H. Freeman and Company, 1976.

Kandel, E. R. Cellular aspects of learning. In M. A. B. Brazier (Ed.), Brain mechanisms in memory and learning: from the single neuron to man. New York: Raven Press, 1979.

Kandel, E. R. & Schwartz, J. H. Molecular biology of learning: modulation of transmitter release. Science, 1982, 218, 433-443.

Kehl, S. J. & McLennan, H. Evidence for a bicuculline-insensitive long-lasting inhibition in the CA3 region of the rat hippocampal slice. Brain Research, 1983, 279, 278-281.

Koda, L. Y. & Bloom, F. E. A light and electron microscopic study of noradrenergic terminals in the dentate gyrus. Brain Research, 1977, 120, 327-335.

Kohler, C. & Steinbusch, H. Identification of serotonin and non-serotonin-containing neurons of the mid-brain raphe projecting to the entorhinal area and the hippocampal formation. A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain. Neuroscience, 1982, 7, 951-975.

Komatsu, Y., Toyama, K., Maeda, J. & Sakaguchi, H. Long-term potentiation investigated in a slice preparation of striate cortex of young kittens. Neuroscience Letters, 1981, 26, 269-274.

Krasne, F. B. Invertebrate systems as a means of gaining insight into the nature of learning and memory. In M. R. Rosenzweig & E. L. Bennett (Eds.), Neural mechanisms of learning and memory. Cambridge: MIT Press, 1976.

Krnjevic, K., Reiffenstein, R. J. & Ropert, N. Disinhibitory action of acetylcholine in the rat's hippocampus: extracellular observations. Neuroscience, 1981, 6, 2465-2474.

Krnjevic, K. & Ropert, N. Septo-hippocampal pathway modulates hippocampal activity by a cholinergic mechanism. Canadian Journal of Physiology and Pharmacology, 1981, 59, 911-914.

Krug, M., Brodemann, R. & Ott, T. Blockade of long-term potentiation in the dentate gyrus of freely moving rats by the glutamic acid antagonist GDEE. Brain Research, 1982, 249, 57-62.

Lancaster, B. & Wheal, H. V. The synaptically evoked late hyperpolarisation in hippocampal pyramidal cells is resistant to intracellular EGTA. Neuroscience, 1984, 12, 267-275.

Laroche, S. & Bloch, V. Conditioning of hippocampal cells and long-term potentiation: an approach to mechanisms of posttrial memory facilitation. In C. Ajmone-Marsan & H. Matthies (Ed.), Neuronal plasticity and memory formation. New York: Raven Press, 1982.

Laurberg, S. Commissural and intrinsic connections of the rat hippocampus. Journal of Comparative Neurology, 1979, 184, 685-708.

Lee, K. S., Schottler, F., Oliver, M. & Lynch, G. Brief bursts of high-frequency stimulation produce two types of structural change in rat hippocampus. Journal of Neurophysiology, 1980, 44, 247-258.

Leung, L. S. Behavior-dependent evoked potentials in the hippocampal CA1 region of the rat. I. Correlation with behavior and EEG. Brain Research, 1980, 198, 95-117.

Levy, W. B. & Steward, O. Synapses as associative memory elements in the hippocampal formation. Brain Research, 1979, 175, 233-245.

Levy, W. B. & Steward, O. Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. Neuroscience, 1983, 8, 791-797.

Lewis, P. R. & Shute, C. C. D. The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. Brain, 1967, 90, 521-540.

Lewis, P. R., Shute, C. C. D. & Silver, A. Confirmation from choline acetylase analysis of a massive cholinergic innervation to the rat hippocampus. Journal of Physiology, 1967, 191, 215-224.

Lomo, T. Some properties of a cortical-excitatory synapse. In P. Andersen & J. K. S. Jansen (Eds.), Excitatory synaptic mechanisms. Denmark: Universitetsforlaget, 1970.

Lomo, T. Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation. Experimental Brain Research, 1971, 12, 18-45. (a)

Lomo, T. Potentiation of monosynaptic EPSP's in the perforant path-dentate granule cell synapse. Experimental Brain Research, 1971, 12, 46-63. (b)

Loy, R., Koziell, D. A., Lindsey, J. D. & Moore, R. Y. Noradrenergic innervation of the adult rat hippocampal formation. Journal of Comparative Neurology, 1980, 189, 699-710.

Lynch, G., Halpain, S. & Baudry, M. Effects of high frequency synaptic stimulation on glutamic receptor binding studied with a modified in vitro hippocampal slice preparation. Brain Research, 1982, 244, 101-111.

Lynch, G., Halpain, S. & Baudry, M. Structural and biochemical effects of high-frequency stimulation in the hippocampus. In W. Seifert (Ed.), Neurobiology of the hippocampus. New York: Academic Press, 1983. (a)

Lynch, G., Larson, J., Kelso, S., Barrionuevo, G. & Schottler, F. Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature, 1983, 305, 719-721. (b)

Lynch, G., Rose, G. & Gall, C. Anatomical and functional aspects of the septo-hippocampal projections. In Ciba Foundation Symposium, 58, Functions of the Septo-hippocampal System. 1978.

Mackintosh, N. J. The psychology of animal learning. New York: Academic Press. 1974.

McCormick, D. A., Lavond, D. G., Clark, G. A., Kettner, R. E., Rising, C. E. & Thompson, R. F. The engram found? Role of the cerebellum in classical conditioning of nictitating membrane and eyelid responses. Bulletin of the Psychonomic Society, 1981, 18, 103-105.

McLennan, H. & Miller, J. J. The hippocampal control of neuronal discharges in the septum of the rat. Journal of Physiology, 1974, 237, 607-624.

McNaughton, B. Evidence for two physiologically distinct perforant pathways to the fascia dentata. Brain Research, 1980, 199, 1-19.

McNaughton, B. L. Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. Journal of Physiology, 1982, 324, 249-262.

McNaughton, B. L. Activity dependent modulation of hippocampal synaptic efficacy: some implications for memory processes. In W. Seifert (Ed.), Neurobiology of the hippocampus. New York: Academic Press, 1983.

McNaughton, B. L. & Barnes, C. A. Physiological identification and analysis of dentate granule cell response to stimulation of the medial and lateral perforant pathways in the rat. Journal of Comparative Neurology, 1977, 175, 439-454.

McNaughton, B. L., Barnes, C. A. & Andersen, P. Synaptic efficacy and EPSP summation in granule cells of rat fascia dentata studied in vitro. Journal of Neurophysiology, 1981, 46, 952-966.

McNaughton, B. L., Douglas, R. M. & Goddard, G. V. Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. Brain Research, 1978, 157, 277-293.

McNaughton, N. & Miller, J. J. Synapse-specific long-term potentiation (LTP) in the septo-hippocampal system of the rat. Neuroscience Letters (Suppl.), 1979, 3, S72.

Meibach, R. C. & Siegal, A. Efferent connections of the hippocampal formation. Brain Research, 1977, 124, 197-224.

Misgeld, U., Sarvey, J. M. & Klee, M. R. Heterosynaptic postactivation potentiation in hippocampal CA3 neurons: long-term changes of the postsynaptic potentials. Experimental Brain Research, 1979, 37, 217-229.

Monaghan, D. T., Holets, V. R., Toy, D. W. & Cotman, C. W. Anatomical distributions of four pharmacologically distinct ^{3}H-l-glutamate binding sites. Nature, 1983, 306, 176-178.

Monmaur, P. & Thomson, M. A. Topographic organization of septal cells innervating the dorsal hippocampal formation of the rat: special reference to both the CA1 and dentate Ø generators. Experimental Neurology, 1983, 82, 366-378.

Moore, R. Y. & Halaris, A. E. Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat. Journal of Comparative Neurology, 1975, 164, 171-184.

Morris, R. G. M. An attempt to dissociate "spatial-mapping" and "working memory" theories of hippocampal function. In W. Seifert (Ed.), Neurobiology of the hippocampus. New York: Academic Press, 1983.

Mosko, S., Lynch, G. & Cotman, G. W. The distribution of septal projections to the hippocampus of the rat. Journal of Comparative Neurology, 1973, 152, 163-174.

Nadler, J. V., Cotman, C. W. & Lynch, G. S. Subcellular distribution of transmitter-related enzyme activities in discrete areas of the rat dentate gyrus. Brain Research, 1974, 79, 465-475.

Nadler, J. V., Vaca, K. W., White, W. F., Lynch, G. S. & Cotman, C. W. Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents. Nature, 1976, 260, 538-540.

Nafstad, P. An electronmicroscopic study on the termination of the perforant path fibers in the hippocampus and the fascia dentata. Zeitschrift fur Zellforschung, 1967, 46, 532-542.

Neuman, R. S. & Harley, C. W. Long-term potentiation of the dentate gyrus population spike by norepinephrine. Brain Research, 1983, 273, 162-165.

Newberry, N. R. & Nicoll, R. A. A bicuculline-resistant inhibitory post-synaptic potential in rat hippocampal pyramidal cells in vitro. Journal of Physiology, 1984, 348, 239-254.

Nicoll, R. A. & Alger, B. E. Synaptic excitation may activate a calcium dependent potassium conductance in hippocampal pyramidal cells. Science, 1981, 212, 957-959.

Nicoll, R. A., Eccles, J. C., Oshima, T. & Rubia, F. Prolongation of hippocampal inhibitory post-synaptic potentials by barbiturates. Nature, 1975, 258, 625-627.

O'Keefe, J. Two spatial systems in the rat brain - implications for the neural basis of learning and memory. Progress in Brain Research, 1983, 58, 453-464. (a)

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

O'Keefe, J. & Black, A. H. Single units and lesion experiments on the sensory inputs to the hippocampal cognitive map. In Ciba Foundation Symposium, 58, Functions of the Septo-Hippocampal System. 1978.

O'Keefe, J. & Nadel, L. The hippocampus as a cognitive map. Oxford: Clarendon Press, 1978.

O'Keefe, J., Nadel, L., Keightley, S. & Kill, D. Fornix lesions selectively abolish place learning in the rat. Experimental Neurology, 1975, 48, 152-166.

O'Keefe, J., Nadel, L. & Willner, J. Tuning out irrelevancy? Comments on Solomon's temporal mapping view of the hippocampus. Psychological Bulletin, 1979, 86, 1280-1289.

Olton, D. S. The function of septo-hippocampal connections in spatially organized behaviour. In Ciba Foundation Symposium, 58, Functions of the Septo-Hippocampal System. 1978.

Olton, D. S. Memory functions and the hippocampus. In W. Seifert (Ed.), Neurobiology of the hippocampus. New York: Academic Press, 1983.

Olton, D. S., Becker, J. T. & Handelmann, G. E. Hippocampal function: working memory or cognitive mapping? Physiological Psychology, 1980, 8, 239-246.

Olton, D. S., Walker, J. A. & Wolf, W. A. A disconnection analysis of hippocampal function. Brain Research, 1983, 233, 241-253.

Orr, W. B. & Berger, T. W. Hippocampal lesions disrupt discrimination reversal learning of the rabbit nictitating membrane response. Society for Neuroscience Abstracts, 1981, 7, 648.

Ott, T., Ruthrich, K., Reymann, K., Lindinau, L. & Matthies, H. Direct evidence for the participation of changes in synaptic efficacy in the development of behavioral plasticity. In C. Ajmone-Marsan & H. Matthies (Eds.), Neuronal plasticity and memory formation. New York: Raven Press, 1982.

Patterson, M. M. Classical conditioning of the rabbit's (ORYCTOLAGUS CUNICULUS) nictitating membrane response with fluctuating ISI and intracranial CS. Journal of Comparative and Physiological Psychology, 1970, 72, 193-202.

Penfield, W. & Milner, B. Memory deficit produced by bilateral lesions in the hippocampal zone. Archives of Neurology and Psychiatry, 1958, 79, 475-497.

Penfield, W. & Perot, P. The brain's record of auditory and visual experience. Brain, 1963, 86, 595-697.

Potempska, A., Gradkowska, M. & Oderfeld-Nowak, B. Early changes in acetylcholine pools in the hippocampus of the rat brain after septal lesions. Journal of Neurochemistry, 1975, 24, 787-789.

Racine, R. J. Kindling: the first decade. Neurosurgery, 1978, 3, 234-252.

Racine, R. J. & Burnham, W. M. The kindling model. In H. V. Wheal & P. A. Schwartzkroin (Eds.), The electrophysiology of epilepsy. London: Academic Press, 1984. (in press)

Racine, R. J. & Milgram, N. W. Short-term potentiation phenomena in the rat limbic forebrain. Brain Research, 1983, 260, 201-216.

Racine, R. J., Milgram, N. W. & Hafner, S. Long-term potentiation in the rat limbic forebrain. Brain Research, 1983, 260, 217-231.

Racine, R. J., Wilson, D. A., Gingell, R. & Sunderland, D. Long-term potentiation in the interpositus and vestibular nuclei. Society for Neuroscience Abstracts, 1984, 10, 79.

Raisman, G. The connexions of the septum. Brain, 1966, 89, 317-348.

Raisman, G., Cowan, W. M. & Powell, T. P. S. The extrinsic afferent, commissural and association fibres of the hippocampus. Brain, 1965, 88, 963-996.

Rescorla, R. A. Pavlovian excitatory and inhibitory conditioning. In W. K. Estes (Ed.), Handbook of learning and cognitive processes: Volume 2, Conditioning and behavior theory. Hillsdale, N.J.: Lawrence Erlbaum Associates, 1975.

Roberts, G. W., Woodhams, P. L., Polak, J. M. & Crow, T. J. Distribution of neuropeptides in the limbic system of the rat: the hippocampus. Neuroscience, 1984, 11, 35-77.

Robinson, G. B. & Racine, R. J. Long-term potentiation in the dentate gyrus: cooperativity between septal and entorhinal afferents. Society for Neuroscience Abstracts, 1982, 8, 742. (a)

Robinson, G. B. & Racine, R. J. Heterosynaptic interactions between septal and entorhinal inputs to the dentate gyrus: long-term potentiation effects. Brain Research, 1982, 249, 162-166. (b)

Robinson, G. B. & Racine, R. J. Long-term potentiation in the dentate gyrus: effects of noradrenaline depletion in the awake rat. Brain Research, 1984. (in press)

Ropert, N. & Krnjevic, K. Septo-hippocampal modulation: intracellular observations in situ. Society for Neuroscience Abstracts, 1983, 9, 968.

Ruthrich, H., Matthies, H. & Ott, T. Long-term changes in synaptic excitability of hippocampal cell populations as a result of training. In C. Ajmone-Marsan & H. Matthies (Eds.), Neuronal plasticity and memory formation. New York: Raven Press, 1982.

Sahley, C., Rudy, J. W. & Gelperin, A. An analysis of associative learning in a terrestrial mollusc 1. higher-order conditioning, blocking and a transient US pre-exposure effect. Journal of Comparative Physiology, 1981, 144, 1-8.

Sastry, B. R., Chirwa, S. S., Goh, J. W., Maretic, H. & Pandanoboina, M. M. Verapamil counteracts depression but not long-lasting potentiation of the hippocampal population spike. Life Sciences, 1984, 34, 1075-1086.

Sastry, B. R. & Goh, J. W. Long-lasting potentiation in hippocampus is not due to an increase in glutamate receptors. Life Sciences, 1984, 34, 1497-1501.

Sastry, B. R., Goh, J. W. & Pandanoboina, M. M. Verapamil counteracts the masking of long-lasting potentiation produced by 2-amino-5-phosphonovalerate. Life Sciences, 1984, 34, 323-329.

Schwartzkroin, P. A. & Wester, K. Long-lasting facilitation of a synaptic potential following tetanization in the in vitro hippocampal slice. Brain Research, 1975, 89, 107-119.

Scoville, W. B. & Milner, B. Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery and Psychiatry, 1957, 20, 11-19.

Segal, M. Excitability changes in rat hippocampus during conditioning. Experimental Neurology, 1977, 55, 105-119.

Segal, M. The action of serotonin in the rat hippocampal slice preparation. Journal of Physiology, 1980, 303, 423-439.

Segal, M. Norepinephrine modulates reactivity of hippocampal cells to chemical stimulation in vitro. Experimental Neurology, 1982, 77, 86-93.

Segal, M., Dudai, Y. & Amsterdam, A. Distribution of an α-bungarotoxin-binding cholinergic nicotinic receptor in rat brain. Brain Research, 1978, 148, 105-119.

Segal, M. & Landis, S. Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. Brain Research, 1974, 78, 1-15.

Seifert, W. (Ed.), Neurobiology of the hippocampus. New York: Academic Press, 1983.

Sharp, P. E., McNaughton, B. L. & Barnes, C. A. Spontaneous synaptic enhancement in hippocampi of rats exposed to a spatially complex environment. Society for Neuroscience Abstracts, 1983, 9, 647.

Shelton, D. L., Nadler, J. V. & Cotman, C. W. Development of high-affinity choline uptake and associated acetylcholine synthesis in the rat fascia dentata. Brain Research, 1979, 163, 263-275.

Skrede, K. K. & Malthe-Sorenssen, D. Increased resting and evoked release of transmitter following repetitive electrical tetanization in hippocampus: a biochemical correlate to long-lasting synaptic potentiation. Brain Research, 1981, 208, 436-441.

Skrede, K. K. & Westgaard, R. H. The transverse hippocampal slice: a well-defined cortical structure maintained in vitro. Brain Research, 1971, 35, 589-593.

Solomon, P. R. Role of the hippocampus in blocking and conditioned inhibition of the rabbit's nictitating membrane response. Journal of Comparative and Physiological Psychology, 1977, 91, 407-417.

Solomon, P. R. Temporal versus spatial information processing theories of hippocampal function. Psychological Bulletin, 1979, 86, 1272-1279.

Solomon, P. R. A time and a place for everything? Temporal processing views of hippocampal funotion with special reference to attention. Physiological Psychology, 1980, 8, 254-261.

Solomon, P. R. & Moore, J. W. Latent inhibition and stimulus generalization of the classically conditioned nictitating membrane response in rabbits (Oryctolagus cuniculus) following dorsal hippocampal ablation. Journal of Comparative and Physiological Psychology, 1975, 89, 1192-1203.

Stanfield, B. B. & Cowan, W. M. The sprouting of septal afferents to the dentate gyrus after lesions of the entorhinal cortex in adult rats. Brain Research, 1982, 232, 162-170.

Stanley, J. C., DeFrance, J. F. & Marchand, J. E. Tetanic and posttetanic potentiation in the septohippocampal pathway. Experimental Neurology, 1979, 64, 445-451.

Stanley, J. C., DeFrance, J. F. & Marchand, J. E. Characteristics of tetanic and post-tetanic potentiation in the septohippocampal and hippocampal commissural systems in the acute rabbit. Journal of Neurobiology, 1980, 11, 193-208.

Steward, O. Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. Journal of Comparative Neurology, 1976, 167, 285-314.

Steward, O., White, W. F. & Cotman, C. W. Potentiation of the excitatory synaptic action of commissural, associational and entorhinal afferents to dentate granule cells. Brain Research, 1977, 134, 551-560.

Storm-Mathisen, J. Quantitative histochemistry of acetylcholinesterase in rat hippocampal region correlated to histochemical staining. Journal of Neurochemistry, 1970, 17, 739-750.

Storm-Mathisen, J. Glutamic acid and excitatory nerve endings: reduction of glutamic acid uptake after axotomy. Brain Research, 1977, 120, 379-386. (a)

Storm-Mathisen, J. Localization of transmitter candidates in the brain: the hippocampal formation as a model. Progress in Neurobiology, 1977, 8, 119-181. (b)

Storm-Mathisen, J. Localization of putative transmitters in the hippocampal formation (with a note on the connection to septum and hypothalamus). In Ciba Foundation Symposium, 58, Functions of the Septo-hippocampal System. 1978.

Storm-Mathisen, J. & Fonum, F. Quantitative histology of glutamate decarboxlase in the rat hippocampal region. Journal of Neurochemistry, 1971, 18, 1105-1111.

Swanson, L. W. The anatomical organization of septo-hippocampal projections. In Ciba Foundation Symposium, 58, Functions of the Septo-hippocampal System. 1978.

Swanson, L. W. & Cowan, W. M. Autoradiographic studies of the development and connections of the septal area in the rat. Advances in Behavioral Biology, 1976, 20, 37-64.

Swanson, L. W., Teyler, T. J. & Thompson, R. F. Hippocampal long-term potentiation: mechanisms and implications for memory. Neurosciences Research Program Bulletin, 1982, 20, 613-769.

Swanson, L. W., Wyss, F. M. & Cowan, W. M. An autoradiographic study of the organization of intrahippocampal association pathways in the rat. Journal of Comparative Neurology, 1978, 181, 681-716.

Szerb, J. C., Hadhazy, P. & Dudar, J. D. Release of (^{3}H) acetylcholine from rat hippocampal slices: effect of septal lesion and of graded concentrations of muscarinic agonists and antagonists. Brain Research, 1977, 128, 285-291.

Teyler, T. J. & Discenna, P. Long-term potentiation as a candidate mnemonic device. Brain Research Reviews, 1984, 7, 15-28.

Thalmann, R. H. Reversal properties of an EGTA-resistant late hyperpolarization that follows synaptic stimulation of hippocampal neurons. Neuroscience Letters, 1984, 46, 103-108.

Thalmann, R. H. & Ayala, G. F. A late increase in potassium conductance follows synaptic stimulation of granule neurons of the dentate gyrus. Neuroscience Letters, 1982, 29, 243-248.

Thompson, R. F., Berger, T. W., Berry, S. D., Hoehler, F. K., Kettner, R. E. & Weisz, D. J. Hippocampal substrate of classical conditioning. Physiological Psychology, 1980, 8, 262-279.

Thompson, R. F., Mamounas, L. A., Lynch, G. & Baudry, M. Increased glutamate receptor binding in hippocampus following classical conditioning of the rabbit eyelid response. Society for Neuroscience Abstracts, 1983, 9, 830.

Thompson, R. F., Patterson, M. M. & Berger, T. W. Associative learning in the mammalian nervous system. In T. Teyler (Ed.), Brain and learning. Stanford: Greylock Publishers, 1978.

Tuff, L. P., Racine, R. J. & Adamec, R. The effects of kindling on GABA-mediated inhibition in the dentate gyrus of the rat. I. Paired-pulse depression. Brain Research, 1983, 277, 79-90.

Turner, R. W., Baimbridge, K. G. & Miller, J. J. Calcium-induced long-term potentiation in the hippocampus. Neuroscience, 1982, 7, 1411-1416.

Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiologica Scandinavica (Suppl. 367), 1971, 83, 1-48.

Valentino, R. J. & Dingledine, R. Presynaptic inhibitory effect of acetylcholine in the hippocampus. Journal of Neuroscience, 1981, 1, 784-792.

Vanderwolf, C. H. Hippocampal electrical activity and voluntary movement in the rat. Electroencephalography and Clinical Neurophysiology, 1969, 26, 407-418.

Vanderwolf, C. H. & Leung, L. W. S. Hippocampal rhythmical slow activity: a brief history and the effects of entorhinal lesions and phencyclidine. In W. Seifert (Ed.), Neurobiology of the hippocampus. New York: Academic Press, 1983.

Vanderwolf, C. H. & Ossenkopp, K. P. Are there patterns of brain slow wave activity which are specifically related to learning and memory. In C. Ajmone-Marsan & H. Matthies (Eds.), Neuronal plasticity and memory formation. New York: Raven Press, 1982.

Van Harreveld, A. & Fifkova, E. Swelling of dendritic spines in the fascia dentata after stimulation of the perforant path fibers as a mechanism of post-tetanic potentiation. Experimental Neurology, 1975, 49, 736-749.

Walters, E. T. & Byrne, J. H. Associative conditioning of single sensory neurons suggest a cellular mechanism for learning. Science, 1983, 219, 405-408.

Weisz, D. J., Solomon, P. R. & Thompson, R. F. The hippocampus appears necessary for trace conditioning. Bulletin Psychonomic Society, Abstract, 1980, 193, 244.

Wheal, H. V. & Miller, J. J. Pharmacological identification of acetylcholine and glutamate excitatory systems in the dentate gyrus of the rat. Brain Research, 1980, 182, 145-155.

White, W. F., Nadler, J. V. & Cotman, C. W. Analysis of short-term plasticity at the perforant path-granule cell synapse. Brain Research, 1979, 178, 41-53.

White, W. F., Nadler, J. V., Hamberger, A., Cotman, C. W. & Cummins, J. T. Glutamate as transmitter of hippocampal perforant path. Nature, 1977, 270, 356-357.

Wigstrom, H. & Gustafsson, B. Large long-lasting potentiation in the dentate gyrus in vitro during blockade of inhibition. Brain Research, 1983, 275, 153-158.

Wigstrom, H., McNaughton, B. L. & Barnes, C. A. Long-term synaptic enhancement in hippocampus is not regulated by post-synaptic membrane potential. Brain Research, 1982, 233, 195-199.

Wilson, D. A. & Racine, R. J. Transient and long-term potentiation in the neocortex of the rat. Society for Neuroscience Abstracts, 1981, 7, 69.

Wilson, D. A. & Racine, R. J. The postnatal development of post-activation potentiation in the rat neocortex. Developmental Brain Research, 1983, 7, 271-276. (a)

Wilson, D. A. & Racine, R. J. The effects of NA-pentobarbital and urethane on synaptic activity in the dentate gyrus of the postnatal rat. Society for Neuroscience Abstracts, 1983, 9, 1141. (b)

Wilson, R. C. Changes in translation of synaptic excitation to dentate granule cell discharge accompanying long-term potentiation. I. Differences between normal and reinnervated dentate gyrus. Journal of Neurophysiology, 1981, 46, 324-338.

Wilson, R. C., Levy, W. B. & Steward, O. Functional effects of lesion-induced plasticity: long-term potentiation in the normal and lesion-induced temporodentate circuits. Brain Research, 1979, 176, 65-78.

Wilson, R. C., Levy, W. B. & Steward, O. Changes in translation of synaptic excitation to dentate granule cell discharge accompanying long-term potentiation. II. An evaluation of mechanisms utilizing dentate gyrus dually innervated by surviving ipsilateral and sprouted crossed temporodentate inputs. Journal of Neurophysiology, 1981, 46, 339-355.

Winson, J. Influence of raphe nuclei on neuronal transmission from perforant pathway through dentate gyrus. Journal of Neurophysiology, 1980, 44, 937-950.

Winson, J. Reticular formation influence on neuronal transmission from perforant pathway through dentate gyrus. Brain Research, 1981, 225, 37-49.

Winson, J. & Abzug, C. Neuronal transmission through hippocampal pathways dependent on behavior. Journal of Neurophysiology, 1978, 41, 716-732.

Yamamoto, C. & Chujo, T. Long-term potentiation in thin hippocampal sections studied by intracellular and extracellular recordings. Experimental Neurology, 1978, 58, 242-250.

Yamamoto, C., Matsumoto, K. & Takagi, M. Potentiation of excitatory postsynaptic potentials during and after repetitive stimulation in thin hippocampal sections. Experimental Brain Research, 1980, 38, 469-477.

Yamamoto, C. & Sawada, S. Important factors in induction of long-term potentiation in thin hippocampal sections. Experimental Neurology, 1981, 744, 122-130.

Yamamura, H. I., Kuhar, M. J. & Snyder, S. H. In vivo identification of muscarinic cholinergic receptor binding in rat brain. Brain Research, 1974, 80, 170-178.

Yamamura, H. I. & Snyder, S. H. Postsynaptic localization of muscarinic cholinergic receptor binding in the hippocampus. Brain Research, 1974, 78, 320-326. (a)

Yamamura, H. I. & Snyder, S. H. Muscarinic cholinergic binding in rat brain. Proceedings of the National Academy of Sciences, 1974, 71, 1725-1729. (b)

Zimmer, J. Ipsilateral afferents to the commissural zone of the fascia dentata demonstrated in decommissurated rats by silver impregnation. Journal of Comparative Neurology, 1971, 142, 393-416.