Order Number 8821086

The relationship of hippocampal theta rhythm and long-term potentiation

Greenstein, Yoram J., Ph.D.

City University of New York, 1988

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print _____
3. Photographs with dark background ✓
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages _____
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received _____
16. Other _____

_____

_____

THE RELATIONSHIP OF HIPPOCAMPAL THETA RHYTHM AND LONG-TERM POTENTIATION

by

YORAM J. GREENSTEIN

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1988

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirements for the degree of Doctor of Philosophy.

4/26/88 — Thomas E. Frumkes

Date — Thomas E. Frumkes, Ph.D.
Chair of the Examining Committee

April 29, 1988 — Herbert D. Saltzstein

Date — Herbert D. Saltzstein, Ph.D.
Executive Officer

Richard J. Bodnar, Ph.D.

Jonathan Winson, Ph.D.

Thom Verhave, Ph.D.

Emmanuel Landau, M.D.

Supervisory Committee

The City University of New York

Abstract

THE RELATIONSHIP OF HIPPOCAMPAL THETA RHYTHM AND LONG-TERM POTENTIATION

by

Yoram J. Greenstein

Advisor: Professor T.E. Frumkes

A leading candidate for a physiological model of memory is hippocampal Long-Term Potentiation (LTP), a long-term enhancement of synaptic efficacy that results from intense tetanic stimulation of hippocampal afferents. This synaptic enhancement can last for hours and even days. However, successful induction of LTP requires long trains of intense, tetanic stimuli that are not within the physiological range. Theta rhythm is a physiological phenomenon unique to the hippocampus. Hippocampal cells fire at theta rhythm periodicity (approximately 5-7 Hz) and are phase-locked to theta. The dissertation investigated the influence of stimulation of hippocampal afferents at theta rhythm frequency or stimulation at different phases of the rhythm upon the induction of LTP. Stimulating the perforant path at theta rhythm periodicity (200 msec inter-train interval; 5 Hz) preferentially induced LTP in the dentate gyrus, as compared to other inter-train intervals. Tetanizing the perforant path or Schaffer collaterals preferentially induced LTP in the dentate gyrus or CA1 area, respectively, if the stimulation was administered on the positive phase of the local theta rhythm. Stimulation on the negative phase caused no change or depressed the field potential. The data, taken in conjuction with studies of freely-moving rats suggest that theta rhythm plays a modulatory role in the induction of LTP, and suggests a mnemonic function for the rhythm.

Acknowledgments

I would like to thank the following individuals for the help that was given me and that enabled me to complete this dissertation:

My Dissertation Committee: the Chairman, Professor Frumkes provided excellent guidance and was totally dedicated to my research; Professor Bodnar carefully reviewed the manuscript and provided continuous encouragement throughout my studies; and Professors Landau and Verhave critically reviewed the text and suggested important revisions.

Mr. Herb Cohen has provided invaluable help in building and maintaining the electronic circuitry that was crucial for the success of the experiment. Mr. Owen Smith has written the software that has made the data collection and analysis possible. These two gentlemen have contributed much time and effort to make these studies successful.

Ms. Didi Arrisian and Ms. Elizabeth R. Oquendo have provided excellent assistance in the histological analysis.

Mr. Mark Grudman assisted during the early stages of the data collection.

Ms. Gwen Smith read the manuscript and provided many useful suggestions, in addition to continuous moral support.

Prof. Hiroshi Asanuma, colleagues and staff of the Neurophysiology Laboratory at Rockefeller University made me feel at home, with their help and encouragement.

The studies described in this dissertation were designed and carried out with the meticulous supervision and excellent training of Dr. Jonathan Winson and Dr. Constantine Pavlides. Their assistance made this endeavor possible.

This dissertation is dedicated to my family.

Table of Contents

Table of Contents (Continued from previous page)

List of Tables

List of Figures

List of Figures (continued from previous page)

List of Abbreviations

APV - D-2-amino-5-phosphonovalerate

CA - Cornus Ammoni

DG - dentate gyrus

EC - entorhinal cortex

ESP - evoked synaptic potential

mf - Mossy fibers

MPP - medial perforant path

PP - perforant path

SCH - Schaffer collaterals

Overview

The search for the neurobiological basis of learning and memory has focused upon anatomical, physiological and chemical levels of inquiry. A leading candidate for a physiological model of memory is Long-Term Potentiation (LTP), a long-term enhancement of synaptic efficacy that results from intense tetanic stimulation of afferents to the hippocampal formation. This synaptic enhancement can last for hours and even days. However, successful induction of LTP requires long trains of intense, tetanic stimuli that are not within the physiological range. Theta rhythm is a physiological phenomenon unique to the hippocampus. Should theta rhythm be involved in the induction of LTP, a physiological normal mechanism might be revealed for LTP induction. The dissertation investigates the influence of stimulation of hippocampal afferents at theta rhythm periodicity or stimulation at different phases of the rhythm upon the induction of LTP. For this series of studies, extracellular field potentials were evoked by a constant current pulse. The slope of the evoked synaptic potential (ESP) and the amplitude of the population spike were analyzed to detect any changes in synaptic efficacy.

1. The Anatomy of the Hippocampal Formation

Overview

The hippocampal formation (entorhinal and subicular cortices, hippocampus, and dentate gyrus) is the focus of sensory specific and multimodal afferents, arriving from temporal, parietal and frontal association cortices. These afferents converge on the different components of the formation, either directly or indirectly, through various subcortical structures. The majority of the afferents converge on the entorhinal cortex, which projects to the hippocampus proper via the dentate gyrus, by means of the perforant pathway. A circuit is then formed in which information flows from the dentate to the hippocampus and back to entorhinal cortex (via subicular complex). The hippocampus projects directly to a subcortical structure (lateral septum), and indirectly, through the subicular complex, to various basal forebrain nuclei. Finally, the circuitry of the system is completed by projections from subicular and entorhinal cortices back to association cortex.

Introduction

The hippocampal formation is a supra modal, association area, that receives information from higher-order cortical association areas (Swanson, 1983). The pioneering neural tracing studies of Pandya and Kuypers (1969) and Jones and Powell (1970) have shown that sensory information projects initially from primary sensory cortical areas to local, unimodal association areas, of the temporal, parietal and occipital cortex. At subsequent levels of the projection, information is carried to more remote, polymodal association areas, located in the

superior temporal gyrus, intra-parietal sulcus, inferior parietal lobule and orbito-frontal cortex. In each level of the projection there is increasing convergence of information, and topographical organization is gradually lost. The medial temporal lobe is the last station in the chain. The polymodal association areas project to the parahippocampal gyrus and the perirhinal cortex which are the transition areas between neocortex and the hippocampal formation (Amaral, 1987).

The hippocampal formation consists of several structures: The hippocampus proper (Cornus Ammoni), dentate gyrus, subicular complex and entorhinal cortex. All of these structures have a simpler laminar structure than neocortex and are therefore classified as archicortex. The entorhinal cortex is the final link between the neocortex and archicortex. After the transition between the two cortices a bulging appears, marking the parasubiculum. At the end of the bulging, some cell layers coalesce and the presubiculum is apparent. Then, a single layer of cells is observed, indicating the beginning of the subiculum, which is the first of single-cell-layered fields of the hippocampus proper (Nauta and Freitag (1986). Figure 1 shows the structure of the hippocampal formation, based on histological study.

The Entorhinal Cortex Two divisions exist in the entorhinal cortex (EC): lateral and medial, with a transition zone between them (Mitchell and Ranck, 1980). There are six layers in the entorhinal cortex, but moving more medially the layers shrink (Carpenter and Sutin, 1983). The entorhinal cortex projects to and also receives projections from the hippocampus. Olfactory input is relayed from the olfactory bulb to the

Figure 1

A) A low-power photomicrograph of a Luxol fast blue-cresyl violet-stained horizontal section through the temporal region of the rat hippocampus at the level marked by the line in B. Scale: 250 μM. (From: Swanson and Cowan, 1977.)

Abbreviations:

ab - angular bundle

alv - alveus

cp - cerebral peduncle

fi - fimbria

ot - optic tract

pp - perforant path

CA1 - CA1 field of the hippocampus

CA3 -CA3 field of the hippocampus

CA4 - CA4 field of the hippocampus (DG hilus)

DG - dentate gyrus

ENT - entorhinal cortex
l- lateral, m- medial

PARA - parasubiculum

PERI - perirhinal area

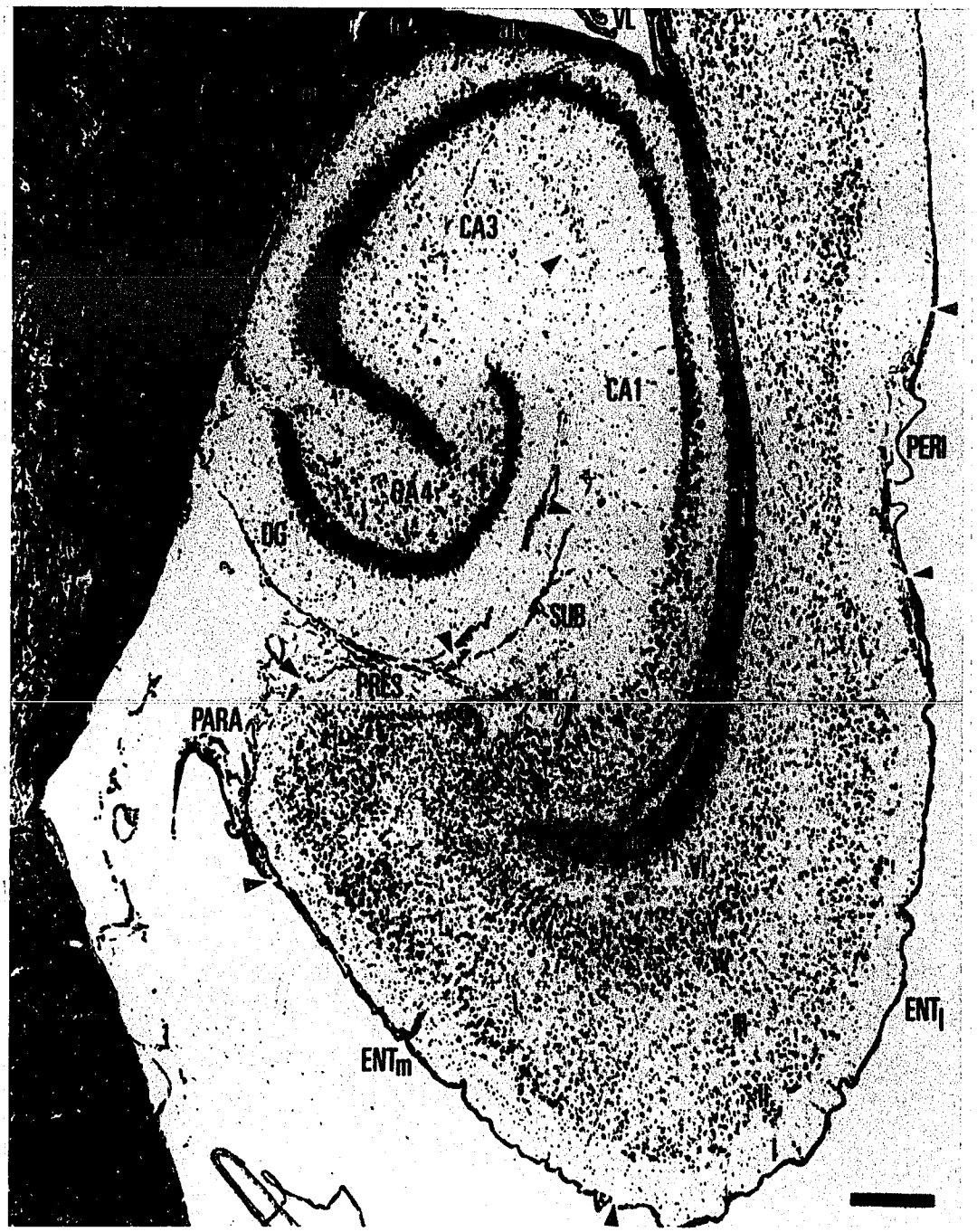
PRES - presubiculum

SUB - subiculum

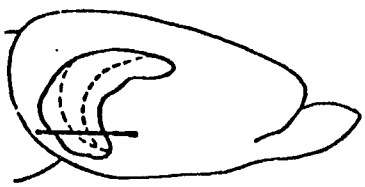
VL - lateral ventricle

Figure 1

A



B



hippocampus through the entorhinal cortex: Tracing studies using tritiated amino acids have shown reciprocal connections between the lateral EC and the primary olfactory cortex, pyriform cortex, lateral olfactory tract, olfactory tubercle and anterior olfactory nucleus (Steward and Scoville, 1976; Wyss, 1981). Both lateral and medial ECs receive projections from association areas in inferior temporal gyrus and orbitofrontal cortex in the rat (Swanson, 1982) and from the ventral part of temporal lobe and caudo-ventral parts of frontal lobe in the monkey (Van Heusen, Pandya and Butters, 1972). A HRP tracing study, (Beckstead, 1978) indicates that the following areas also project to both divisions of the EC: claustrum, medial septum, diagonal band of Broca, ventral tegmental area, locus coeruleus and dorsal raphe. Stimulating the amygdala produces EPSPs in layers III, IV and V in the EC, indicating the existence of an excitatory input from the amygdala to the EC (Finch, Wong, Derian et al., 1986). The EC receives projections from the pulvinar and also from the paraventricular nuclei of the hypothalamus. There are also reciprocal connections between the EC and perirhinal cortex, posterior parts of the parahippocampal gyrus, peri-amygdaloid cortex and cingulate gyrus (Amaral, 1987; Van Hoesen, 1982). The major efferents of the EC are to the hippocampus proper and the dentate gyrus. Autoradiographic studies have shown that layer II of the EC projects ipsilaterally to the dentate gyrus in proxymo-distal gradient, so that the medial EC sends the perforant path (PP) to the mid-third part of the dendritic tree of the dentate gyrus granule cells, while the lateral EC projects, through the lateral PP, to the outer third of the granule cells dendritic tree. Projections from layer III of the medial EC terminate on Regio Superior, near the CA1-CA2

transition area, whereas the lateral EC sends projections to a more remote area, near the CA1-subicular transition area (Steward, 1976; Steward and Scoville, 1976). The EC projections to the CA1 field are bilateral. More recent evidence indicates that the lateral EC projects to the whole cortical mantle, to the striatum-accumbens complex, caudate, putamen, amygdala, medial pre-frontal cortex, cingulate gyrus and insula. This projection suggests that the hippocampus may modulate locomotor activity through the lateral EC (Swanson and Kohler, 1986).

The Subicular Complex Three divisions are distinguished in the subicular complex: subiculum, presubiculum and parasubiculum. The transition from the parahippocampal gyrus to the subicular complex is characterized by reduction of the six layers to three layers, marking the change from neocortex to archicortex (Carpenter and Sutin, 1983). Since the subicular complex bends around the angular bundle, its lamination is less distinct. There are three layers in the subiculum proper: a) the molecular layer, which is continuous with the superficial layer of the neocortex; b) a pyramidal layer, whose dendrites project to the molecular layer, and whose axons form the alveus; c) a polymorphic layer, which is the deepest one and is similar to the neocortical polymorphic layer. The presubiculum is found medial to the subiculum, and consists of a dense layer of pyramidal cells and a more superficial plexiform layer. The parasubiculum consists of a plexiform layer and a primary cell layer. This structure marks the transition from the subicular complex to the EC, which is found more laterally (Amaral, 1987). Projections from the amygdala and claustrum to the subicular complex have been documented. Temporal and parietal

cortices and the cingulate gyrus also project to the subicular complex. The subicular complex projects to cingulate, entorhinal and retrosplenial cortices and to prefrontal and temporal association areas (Irle and Markowitsch, 1982). Most hippocampal projections to cortical and subcortical regions are made through the subicular complex (Van Hoesen, 1982). Sub-cortical regions that were labeled when tritiated amino acids were injected to the subicular complex included: anterior and lateral-dorsal thalamus, mamillary bodies, nucleus accumbens, lateral septum, and ventro-medial nucleus of the hypothalamus (Meibach and Siegel, 1977; Rosene and Van hoesen, 1977)

The Hippocampus The hippocampus is a bilateral, symmetrical "S"-shaped folding of the cortical mantle. It folds also into a "C"-shaped (seen coronally) structure along the inferior horn of the lateral ventricle (Swanson, 1982). Figure 2 shows the location and structure of the hippocampus.

Several fields can be distinguished in the hippocampus. Ramon Y Cajal (1893) distinguished between two fields. The first contained large pyramidal cells (Regio Inferior) and the other one was populated by smaller pyramidal cells cells (Regio Superior). Lorente De No (1934) offered a different map of the hippocampus. He distinguished among four fields: CA1, which corresponded to the Regio Superior; CA3, which corresponded to Regio Inferior; CA2, which was the transition area between CA1 and CA3; and CA4, which consisted of a polymorphic area (hilus) of the dentate gyrus, and was not part of the hippocampus proper.

Figure 2

A) A schematic illustration of the exposed hippocampus *in situ*. The right and left hippocampi can be seen. In the right hippocampus the two interdigitating cell fields of the hippocampus proper and the dentate gyrus are shown. Adapted from Teyler & Discenna, (1984.)

B) A low-power photomicrograph of a frontal section of the septal one-third of the rat right hippocampus, showing the hippocampal fields and the dentate gyrus. Scale: 500 uM. (From: Swanson and Cowan, 1977.)

Abbreviations:

cc - corpus callosum

cing - cingulate bundle

df - dorsal fornix

fi - fimbria

FC - fasciola cinerea

IG - induseum griseum

LGN - lateral geniculate nucleus

LH - lateral habenula

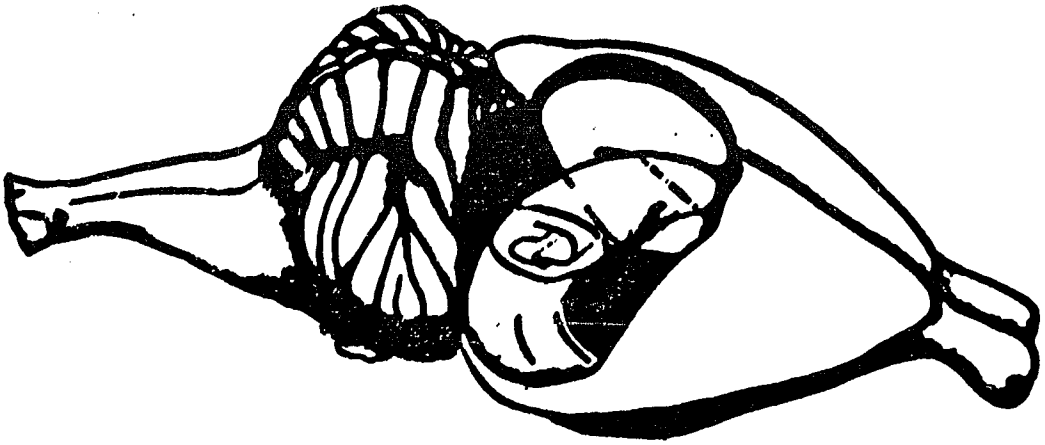
LP - lateral posterior nucleus

V3 - third ventricle

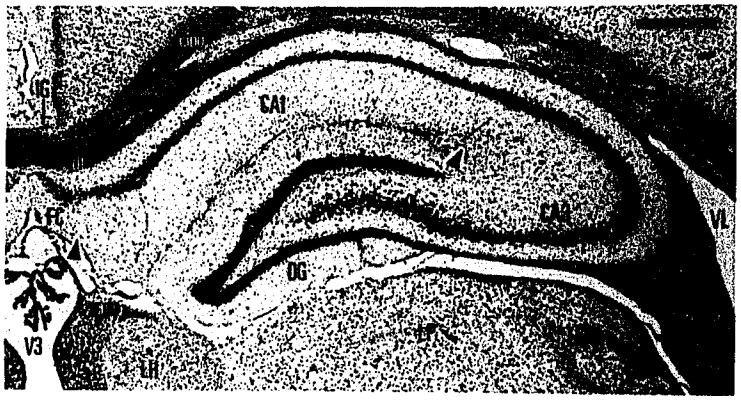
VL - lateral ventricle

Figure 2

A



B



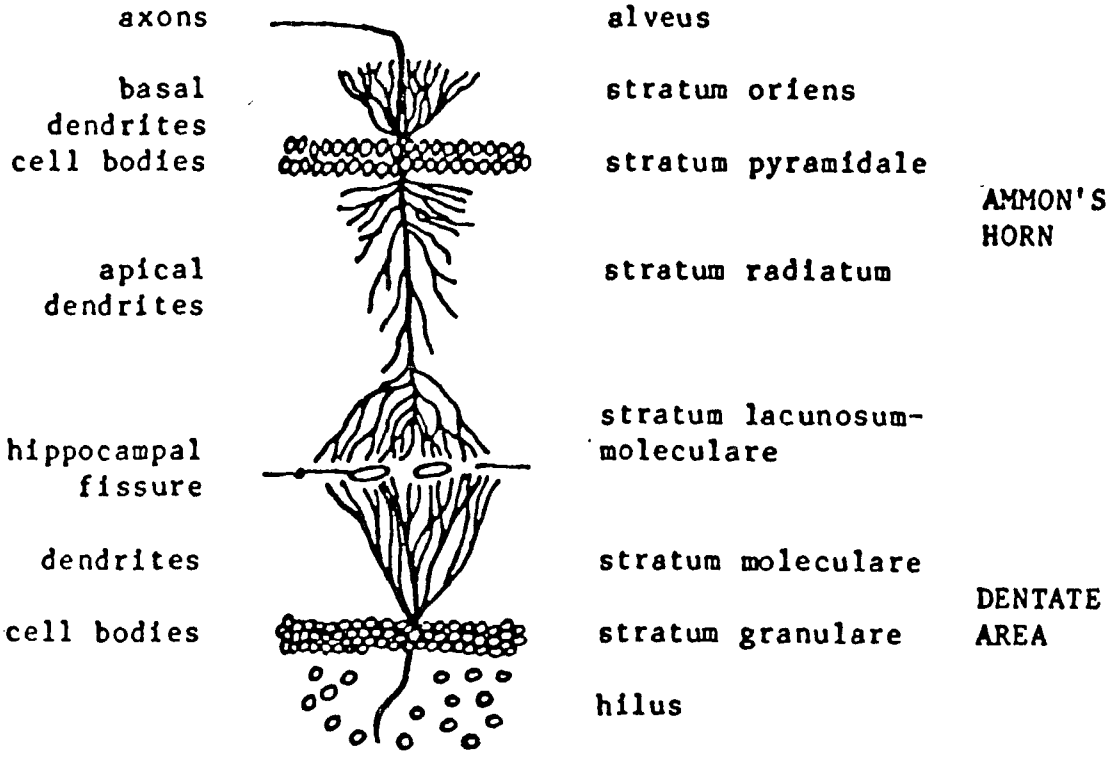
There are three principal cell layers in the hippocampus: pyramidal, molecular and polymorphic. There are also several secondary laminae within the principal layers. These laminae are layered from the ventral surface to the pia in the following order: alveus, which is located immediately below the ependyma of the lateral ventricle and consists of pyramidal axons; stratum (s.) oriens, which includes polymorphic cells and dendrites; s. pyramidale, the principal lamina, which contains the pyramidal cell bodies and sends axons to s. oriens and alveus; s. lucidum, which exists only in CA3 as mossy fibers layer; s. radiatum, which receives the apical dendrites from s. pyramidale and through which the axons of the basket cells return to s. pyramidale to synapse on the pyramidal cells somata; s. lacunosum-moleculare, which contains plexuses of fibers from neighboring laminae. The last three laminae are continuous with the molecular layer of neocortex (Carpenter and Sutin, 1983). The next two laminae are those of the dentate gyrus: s. moleculare, which contains the granule cells dendrites, and s. granulare, consisting of the granule cells bodies. Below s. granulare is found the hilus (CA4 field), which consists of polymorphic and basket cells. Figure 3 shows the organization of the laminae and their content.

CA3 Field The pyramidal cell body layer in CA3 is larger and denser than the one in the CA1 field. Inhibitory interneurons, mainly basket cells, are also found in this field (Amaral, 1987). CA3 field pyramidal cells project to the CA1 field through the Schaffer collaterals, and bilaterally to the subicular complex and the hilus (Swanson, 1982). Ipsi-lateral associational and commissural connections are also made by axonal collaterals of CA3 cells (Swanson, Sawchenko and Cowan, 1981).

Figure 3

A schematic diagram, illustrating the lamination of the hippocampus and the dentate gyrus. Note that only the dorsal blade of the dentate gyrus is shown. (from Holsheimer, 1982.)

Figure 3


axons
basal dendrites
cell bodies
apical dendrites
hippocampal fissure
dendrites
cell bodies
alveus
stratum oriens
stratum pyramidale
stratum radiatum
stratum lacunosum-moleculare
stratum moleculare
stratum granulare
hilus
AMMON'S HORN
DENTATE AREA

There is also a projection to the lateral septum through the fornix, which is the only sub-cortical target of the hippocampus proper (Teyler and Discenna, 1984). Septal parts of CA3 field project to dorsal parts of the lateral septum, whereas temporal parts in CA3 project on ventral parts of the lateral septum (Swanson and Cowan, 1977.)

CA1 Field Pyramidal and inhibitory basket cells also populate this field (Amaral, 1987). CA1 field projects through the alveus to the subiculum, lateral septum (with a topographic organization similar to the CA3 projections) and pre-frontal cortex (Teyler and Discenna, 1984). There are also projections to the medial and lateral EC, terminating in layer IV (Swanson, 1982).

Dentate Gyrus The continued growth of the cortical tissue that formed the hippocampus, also created the dentate gyrus (DG). The name of the gyrus has been derived from the shape of the surface, which is beaded and toothed (Barr, 1979). The DG is the simplest cortical field in the hippocampal formation, but its location is extremely important: for a stimulus to affect the hippocampus, it must activate the EC-DG synapse, as will be described below. The DG has superior and inferior blades, and between them the hilus is located, which is composed of polymorphic cells and interneurons (Teyler and Discenna, 1984). There are three layers in the DG: a) molecular layer that is continuous with that of the hippocampus; b) polymorphic layer which constitutes the hilus; c) granule layer, which contains the cell bodies of the principal cell: the granule cell. This layer is 5-10 cells thick, and is found 200 um below the pial surface (Shepherd, 1979). The granule cells dendrites are oriented away from the center of the "C", towards the pia (Chronister and White, 1971) and project to the molecular

layer, which receives most of the afferents (Amaral, 1987). The granule cell projects only intrinsically in the hippocampus, as described below. The other cell type in the DG is the basket cell. This is an inhibitory cell, containing GABA as its neurotransmitter. The inhibitory effect of the basket cell is mediated by increasing chloride (Cl^-) conductance (Andersen, 1982). The basket cell is activated by collaterals from the granule cells axons (Mossy fibers) and synapses on the soma and dendrites of the granule cells, forming an inhibitory feedback loop. The DG is innervated by cholinergic, noradrenergic, dopaminergic, serotonergic, and glutaminergic fibers and also has some neuroactive peptides, such as somatostatin, CCK, neuropeptide Y, substance P and opiates (Amaral and Campbell, 1986). The hilus of the DG constitutes the CA4 field and projects commissural and ipsilateral association fibers to the inner third of the molecular layer of the DG (Swanson, 1982).

The Trisynaptic Circuit Synaptic transmission in the hippocampus is unidirectional, forming a circuit that involves three major synapses. The DG is the first link in this chain of events. It receives projections from layers I and II of the EC. This massive projection, the perforant path (PP), travels through the subicular complex and synapses on the outer two-third of the granule cell dendrites in the DG. Sparser contacts are made by the EC on the distal dendrites of the pyramidal cells of fields CA1 and CA3. The PP fibers that originate from the lateral EC terminate on the most distal third of the granule cell dendrites while those fibers originating from the medial EC terminate on more proximal parts of those dendrites (Hjorth-Simonsen,

1972; Hjorth-Simonsen & Jeune, 1972). The granule cells send their axons (Mossy fibers) to synapse on proximal dendrites of CA3 pyramidal cells. This constitutes the second synapse of the circuit. The last synapse is formed by the CA3 pyramidal cells axons (Schaffer collaterals), which synapse on the CA1 pyramidal cells dendrites, located in s. radiatum. CA1 field cells send axons to the subicular complex, which projects to the EC, thereby completing the circuit. This internal flow of transmission is identical in rodents and primates (Amaral, 1987). Electrophysiological data have demonstrated the flow of information in the circuit. Simultaneous recordings from the different stations in the circuit following PP or EC stimulation showed that granule cells are the earliest neuronal population to fire. Then, the CA3 cells are excited, and these, in turn, send impulses to the CA1 pyramidal cell population. These CA1 cells activate, in turn, the subiculum. The activation latency increases with increasing distance from the CA3 field. All three synapses are excitatory, and all axons form synapses *en passage* with long sheets of neurons (Andersen, 1982). See Figure 4 for an illustration of the trisynaptic circuit.

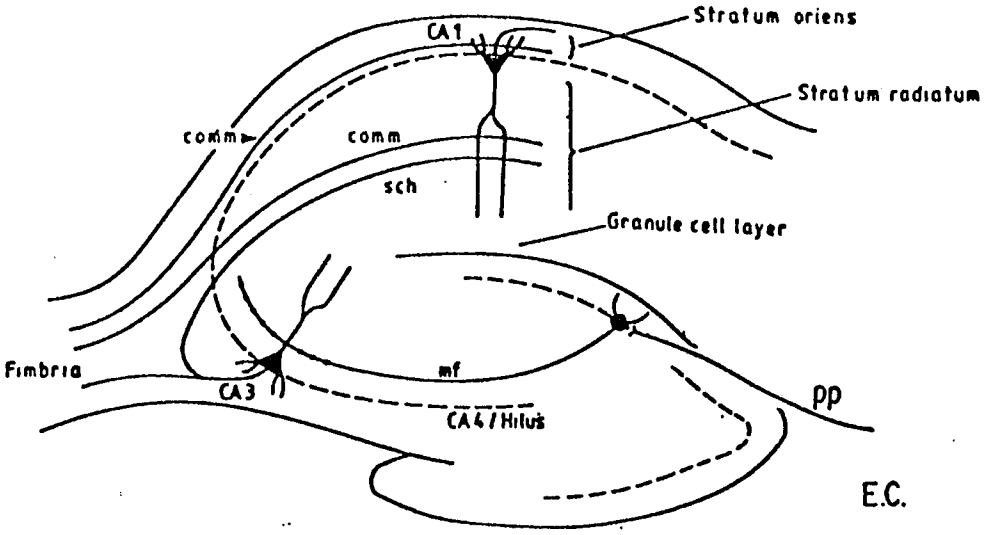
Summary of the Afferents of the Hippocampus and DG

HRP studies have shown that regions such as the ventral tegmental area, dorsal and median raphe and locus coeruleus project to the whole hippocampal formation (Segal and Landis, 1974a). The medial septum and the diagonal band of Broca project to the hippocampal formation through four distinct paths: a) the fimbria; b) the dorsal fornix; c) the supra-callosal stria; d) the amygdaloid cortex. The topography of these projections is such that medial parts of the medial septum project to the dorsal hippocampus, while more lateral parts project to the ventral

Figure 4

A schematic sagittal section through the hippocampus of the rat. The three synapses that form the trisynaptic circuit are shown. The first synapse is formed by the perforant path (pp) originating from the entorhinal cortex (EC), and terminating in the dentate. The second synapse is formed by the mossy fibers (mf), originating from the dentate gyrus and terminating in the CA3 field. Schaffer collaterals (sch) are sent from CA3 field and terminate in the CA1 field. Commissural axons (comm) also innervate the CA1 field. (Adapted from Lancaster and Wheal, 1982.)

Figure 4


CA1
Stratum oriens
Stratum radiatum
comm
comm
sch
Granule cell layer
Fimbria
CA3
mf
CA4 / Hilus
pp
E.C.

hippocampus (Segal and Landis, 1974). The septal projections are mainly cholinergic (CH1 and CH2 groups; Mesulam, Mufson, Wainer and Levey, 1983) and play an important role in the modulation of theta rhythm, as described below. The lateral and supra-mamillary areas of the hypothalamus project to the DG, CA2 and CA3 fields. The anterior, dorsal-lateral and midline nuclei of the thalamus also project to the hippocampal formation. Projections from neocortical and cingulate areas to CA1 field have been also documented. Projections from the entorhinal cortex were mentioned above.

Summary of the Efferents of the hippocampus and DG

The major efferent structure of the hippocampus is the alveus, consisting of pyramidal axons that converge on the medial surface of the hippocampus and form the fimbria. The fimbria on each side continues as the crus of the fornix, arches around the thalamus, crosses contralaterally and forms the body of the fornix. This decussation produces the hippocampal commissure (psalterium). Precommissural fibers of the fornix originate mainly from CA1-3 fields and subiculum. The CA1-3 fibers synapse on the subicular complex and it, in turn, projects to the mamillary bodies, anterior nuclei of the thalamus, pre-optic area, and lateral hypothalamus. The dorsal hippocampus projects through the medial fornix to the lateral septum and from there it synapses on the medial septum and diagonal band of Broca (Swanson, 1978). The ventral hippocampus projects through the lateral fornix to the lateral septum and nucleus accumbens (Powell and Hines, 1975). Postcommissural fibers originate mainly from the presubiculum and terminate in the mamillary bodies, anterior nuclei of the thalamus (AM, AV), lateral-dorsal nucleus of the thalamus, ventro-

medial nucleus of the hypothalamus and peri-aquedactal gray (Brodal, 1981; Shepherd, 1979, Swanson and Cowan, 1977). The hippocampus projects directly to the EC and through the subicular complex to the entorhinal, perirhinal, retrosplenial, and cingulate cortices (Swanson and Cowan, 1977). The hippocampus, through the CA1 field projects also to prefrontal association cortex (Swanson, 1982). All fields of the hippocampus in the rat are connected via commissural fibers.

Figures 5 and 6 illustrate, respectively, the cortical and subcortical connections of the hippocampal formation.

The measurement of field potentials in the hippocampus provides a measure of synaptic efficacy and is frequently used to detect the existence of LTP. Chapter 2 describes the nature and analysis of hippocampal field potentials.

Figure 5

Schematic illustration of neo-, meso- , and paleo-cortical connections of the hippocampal formation. In addition, connections to several structures of the limbic system are shown. Note the convergence of cortical afferents from association cortex on the entorhinal cortex and the projection from the entorhinal cortex to the DG (through the PP). Also, note the trisynaptic circuit, and the returning pathways, back from the hippocampus (CA1 and CA3) to subicular complex, entorhinal cortex, and finally, association cortex. The medial septal projections to the entorhinal cortex, hippocampus and dentate gyrus will be discussed below, in reference to theta rhythm.

Figure 5

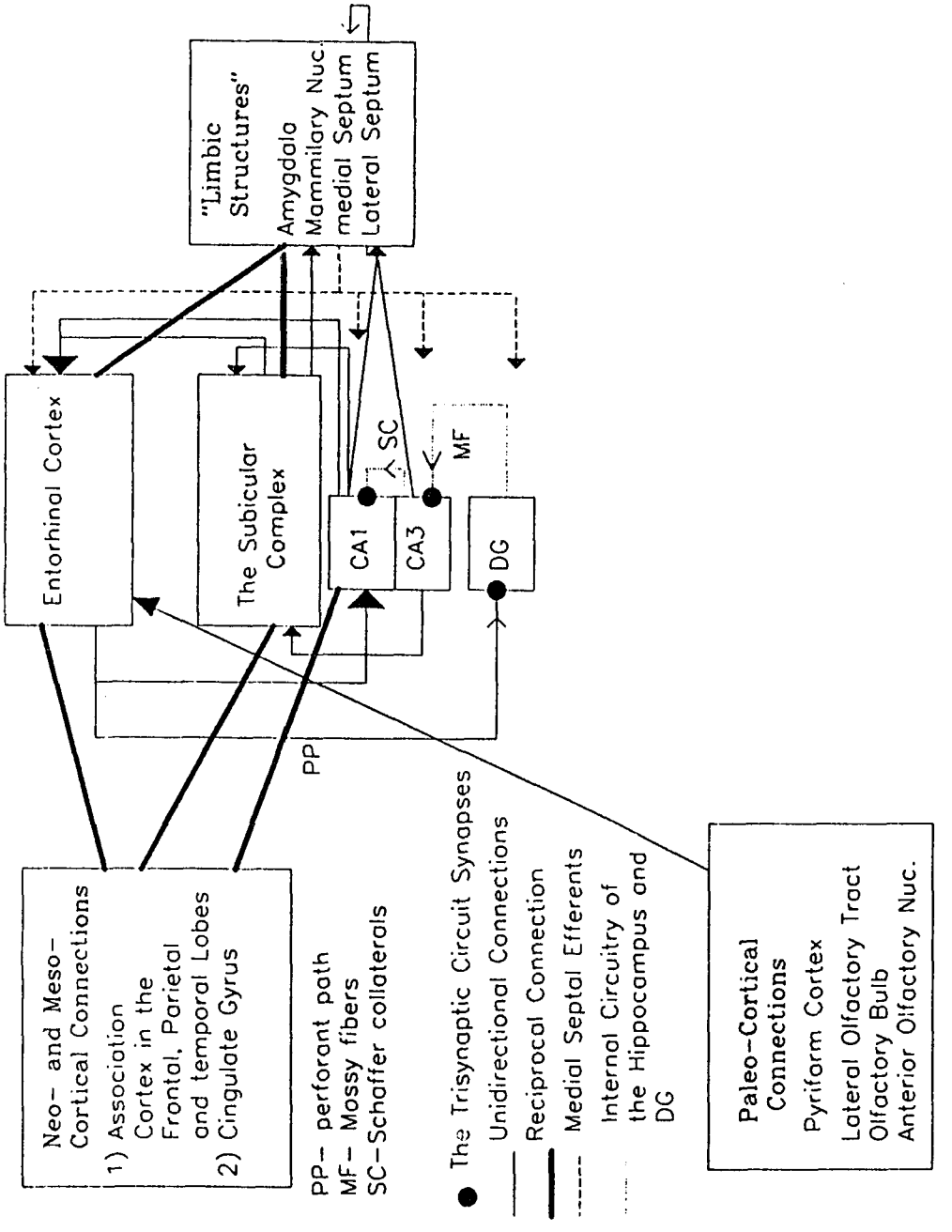

"Limbic Structures"
Amygdala
Mammilary Nuc.
medial Septum
Lateral Septum
Entorhinal Cortex
The Subicular Complex
CA1
CA3
DG
SC
MF
PP
Neo- and Meso-Cortical Connections
1) Association Cortex in the Frontal, Parietal and temporal Lobes
2) Cingulate Gyrus
PP- perforant path
MF- Mossy fibers
SC-Schaffer collaterals
The Trisynaptic Circuit Synapses
Unidirectional Connections
Reciprocal Connection
Medial Septal Efferents
Internal Circuitry of the Hippocampus and DG
Paleo-Cortical Connections
Pyriform Cortex
Lateral Olfactory Tract
Olfactory Bulb
Anterior Olfactory Nuc.

Figure 6

Schematic illustration of the connections between the hippocampal formation and sub-cortical structures (excluding "limbic" structures that are shown in Figure 5.)

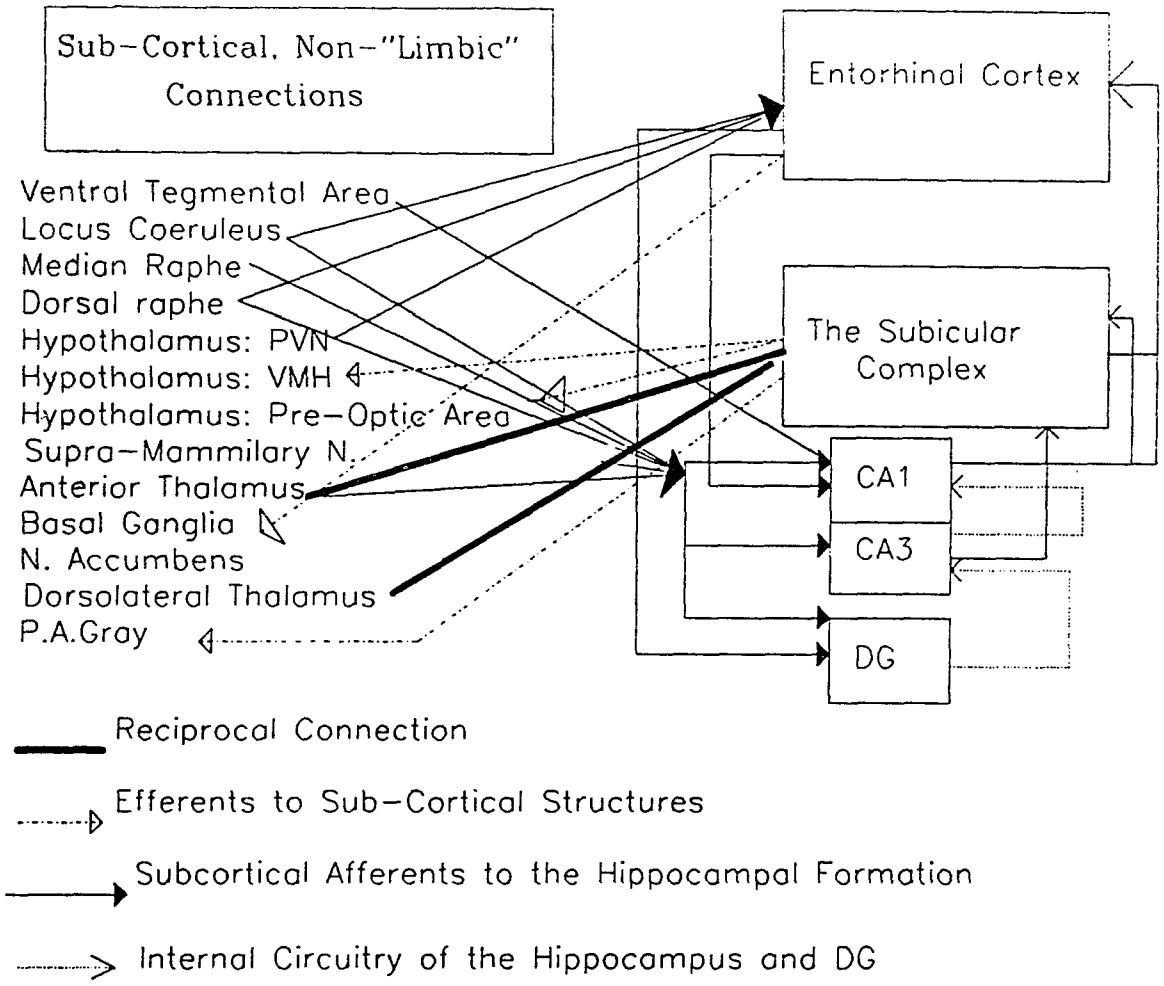

Sub-Cortical, Non-"Limbic" Connections
Entorhinal Cortex
Ventral Tegmental Area
Locus Coeruleus
Median Raphe
Dorsal raphe
Hypothalamus: PVN
Hypothalamus: VMH
Hypothalamus: Pre-Optic Area
Supra-Mammilary N.
Anterior Thalamus
Basal Ganglia
N. Accumbens
Dorsolateral Thalamus
P.A.Gray
The Subicular Complex
CA1
CA3
DG
Reciprocal Connection
Efferents to Sub-Cortical Structures
Subcortical Afferents to the Hippocampal Formation
Internal Circuitry of the Hippocampus and DG

Figure 6

2. Volume Conduction and Field Potentials in the Hippocampal Formation

Introduction

The theory of volume conduction was developed by Lorente De No (1947). According to his formulation, the neuron is surrounded by an extracellular medium with low resistance that acts as a conducting medium (a "volume conductor"). An extracellular electrode located in this medium will record any current that will flow between different regions of the cell. Current flows into the cell when the membrane potential of two adjacent areas in the neuron change, so that one part of the membrane is more depolarized than the other. For example, when the dendritic membrane is depolarized by an incoming pulse, sodium ions (Na^+) channels open, allowing an inward flow of Na^+ current. Coincident with this inward current, there is an outward current that supplies the inward current and completes the circuit. This outward, extracellular current will be detected by an extracellular electrode, and may be recorded as a field potential. If the membrane polarity is uniform, no intracellular current will be formed and, consequently, no extracellular field will be measured (Hubbard, Llinas and Quastel, 1969, Lemon, 1984). The field potential reflects the synchronous firing of a large group of neurons. All the potential differences that exist around the tip of the electrode sum algebraically and linearly (Nicholson, 1979). Since neurons can manifest different orientations, some potentials will summate while others will cancel out. Therefore, the electrode records an average of all field potentials found at its tip (Phillips, 1973).

The profile of the field potential reflects the existence of "sources" and "sinks" in the neuronal membrane (Hubbard, Llinas and Quastel, 1969). A sink is an active, depolarized area of the membrane, where an inward flow of current occurs. A source is a passive area of the membrane, from where an outward current flows which supplies the sink. When the current flows from the source to the sink, the source becomes more positive, relative to a reference electrode, positioned elsewhere. The sink, on the other hand, will be more negative, relative to that electrode.

Field potentials in the Dentate Gyrus

Figure 7 shows four records of field potentials recorded from the granule cell body layer of the dentate gyrus. Four pulses at selected intensities (16, 40, 60, and 150 uA) were delivered to the medial perforant path (MPP), that synapses on the mid-third of the granule cells dendrites. A single pulse, applied to the MPP, will depolarize the dendrites, creating an inward current into the active dendritic synaptic site (the sink) and an outward current flowing away from the soma (the source) and into the dendrites. The outward current is recorded as a positive deflection by an extracellular electrode positioned in the cell body layer. This positive outgoing potential is termed the evoked synaptic potential (ESP). The initial slope of the ESP reflects the rate of the flow of current away from the soma and into the dendrites (Figure 7a). Lomo (1971) found that the ESP correlated with an intracellular EPSP, as recorded in the granule cells, confirming that the ESP reflects synaptic depolarization.

As the MPP stimulation becomes more intense, the slope of the ESP

grows and its latency decreases. At a high enough intensity, a sharp negative deflection is now imposed on the ESP (Figure 7b). This sharp negativity is called the Population Spike, which is used as a measure of the number of granule cells firing action potentials. The relationship between the spike amplitude and the number of active cells was found by Andersen, Bliss and Skrede (1971), using extracellular and multi-unit recordings. A temporal correlation between the individual units discharge and the appearance of the population spike was also found (Lomo, 1966). With increasing intensity of stimulation, more individual spikes were observed and a higher amplitude of the population spike was recorded.

Field potentials in CA1 field

Field potentials can also be recorded in the CA1 field, in response to stimulation of the CA3 Schaffer collaterals or the commissural (psalterium) pathways that synapse on the CA1 pyramidal cells dendrites. Figure 8 shows four records of field potentials recorded in the CA1 field, after stimulating the ventral commissural fibers at different intensities. The initial slope of the CA1 field potential is not a reliable measure because of its instability. Therefore, the population spike is used more frequently as a measure of synaptic efficacy in CA1 field studies.

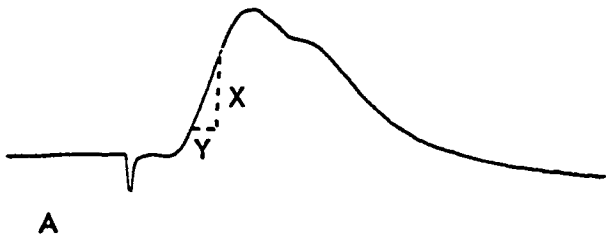
Theta rhythm is a unique physiological phenomena that characterizes the hippocampal formation. It is highly regular, almost sinusoidal in shape, and synchronizes the activity of numerous cells in the limbic system. Chapter 3 describes the nature of hippocampal theta rhythm.

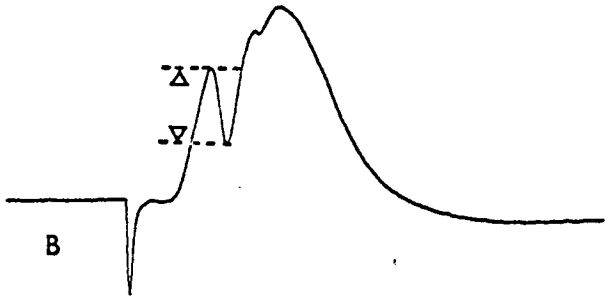
Figure 7

Four field potentials, as recorded from the cell body layer in the dorsal blade of the dentate gyrus in the anesthetized rat. A pulse (0.25 msec width) applied to the MPP, that synapses on the granule cell dendrites, evokes a positive outgoing field potential, termed the Evoked Synaptic Potential (ESP). Figure A depicts an ESP evoked by a relatively weak current (16 uA). The slope of the ESP (x/y) reflects the rate of current flow from the soma into the dendrites (see text). At a higher current (40 uA), a negative deflection is imposed on the ESP, termed the Population Spike (Figure 5B). The amplitude of the population spike (limited by the two arrowheads in B) reflects the number of granule cells firing synchronously. As the stimulation intensity increases (60 and 150 uA), the slope and spike grow, and their latency decreases (Figures C,D). In the specific animal described, intensities beyond 150 uA did not cause further change in either slope or spike. This intensity of 150 uA is termed Saturation Intensity. The recording were performed in this laboratory.

Values for these records: A: current intensity, 16 uA; slope, 3.4 mV/msec. B: current, 40 uA, slope; 6.7 mV/msec; spike, 5.7 mV. C: current 60 uA; slope, 7.6 mV/msec; spike, 9.7 mV. D: current, 150 uA; slope, 9.0 mV/msec; spike, 11.4 mV.

Figure 7





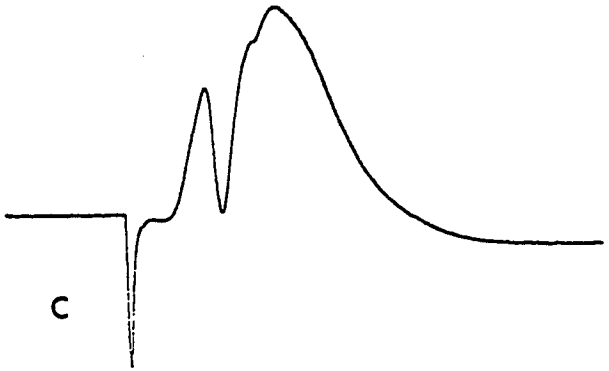


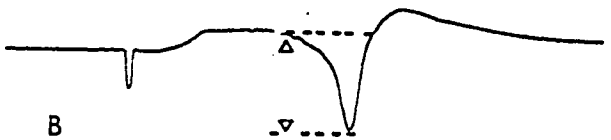
Figure 8

Four field potentials, as recorded from the pyramidal cell layer in the CA1 field in the anesthetized rat. A pulse (0.15 msec pulse width) was applied at four different intensities (40, 70, 150, and 700 uA) to the ventral commissural afferents, that synapse on the pyramidal cell dendrites. Only the population spike was used as the measure of synaptic efficacy. The amplitude of the population spike is limited by the two arrowheads in B. Saturation intensity in this experiment was 700 uA. The recordings were performed in this laboratory.

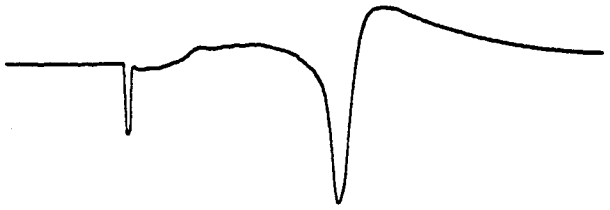
Values for these records: A: current intensity, 20 uA; spike, 2.4 mV; B: current, 40 uA, spike, 8.2 mV; C: current, 150 uA, spike, 12.77 mV; D: current 700 uA; spike, 17.8 mV.

Figure 8

A



B



C

D

3. Hippocampal Theta Rhythm

Introduction

Three basic electroencephalographic (EEG) patterns can be recorded in the non-primate archicortex: 1) a rhythmic slow activity, with frequency ranging between 4 and 12 Hz (theta), characterizing the aroused and/or locomoting animal and also REM sleep; 2) slow, irregular waves, with a frequency range of 0.2-2.5 Hz, which occurs during relaxation; 3) a desynchronous, fast, low amplitude activity, at a frequency range of 15-60 Hz, which appears during hyperexcitement and related movements (Buzsaki, 1986; Holsheimer, 1982; Leung, Lopes Da Silva and Wadman, 1982).

Theta rhythm is the most prominent feature of the hippocampal EEG in non-primate mammals. It has the highest amplitude (1-3 mV), and the most regular, almost sinusoidal shape (Bennet, 1971). Theta was initially recorded in the rabbit by Jung and Kornmuller (1938). Studies on rabbits, cats and monkeys followed (Green and Arduini, 1954). Theta was found to appear with every sensory stimulus, while simultaneous recordings in the neocortex showed desynchronization. In addition, electrolytic lesions of the medial septum and/or dorsal fornix were shown to abolish hippocampal theta.

The ease of recording theta is inversely related to the development of neocortex. It is easy to record in the rat, guinea pig and rabbit. It is more difficult to record in the cat, and very difficult in the monkey (Bennet, 1971).

There are two profiles of theta in the rat. The first profile characterizes the curarized rat. There is a maximum amplitude of theta

(of about 1 mV) in s. oriens in CA1 field. When the electrode is advanced vertically, there is a null zone of about 100 uM in width, where theta activity is absent. Then, there is a sudden phase reversal, and a second maximal amplitude (of about 2 mV) occurs in s. moleculare in the upper blade of the dentate gyrus. When the electrode is further advanced in the dentate, theta disappears in the hilus, and a rhythm of about 30-50 Hz is recorded there. Theta reappears in the lower blade with a maximum of about 1 mV and without phase reversal. The second theta profile characterizes the freely moving rat. Here the theta maxima appears in the same areas, but there is a gradual phase reversal between the CA1 and dentate regions, and no null zone is observed (Winson, 1974, 1976).

Intra-hippocampal theta generators

Two theta cellular generators have been identified in the rat: a) in the CA1- s. oriens, with an average amplitude of a 1 mV; b) in the upper blade of the dentate gyrus - s. moleculare, with an average amplitude of 2 mV. Both generators are approximately 180° out of phase (Bland and Whishaw, 1976). There is an additional maximal amplitude recorded in the lower blade of the dentate gyrus, which is at the same phase with theta activity recorded at the upper blade and 180° out of phase with the theta waves recorded at the CA1 field (Andersen, 1980). Both generators are independent: cooling or lesioning the CA1 field eliminates theta recorded from that area but leaves the dentate theta intact (Andersen, 1980). Also, differential septal lesion affected one type of theta, leaving the other active (Monmaur, 1982).

It is not clear which hippocampal cells generate theta activity.

Cells in CA1 field and dentate gyrus increase firing rate when the theta frequency increases, and also fire synchronously with the rhythm. The behavioral correlates of these cells are similar to those of theta, namely locomotion and postural changes in rats and locomotion and alert immobility in rabbits (see below), (Bland, Seto and Rowntree, 1983). It has been argued that these "theta" cells are interneurons (Fox, Wolfson and Ranck, 1983). Rose (1983; Rose, Diamond and Lynch, 1983), on the other hand, claim that "theta" cells are granule cells. In recording from the dentate gyrus, three types of granule cells have been identified by Rose (1983). The first type of granule cell increases its rate of firing during theta; the second fires synchronously with theta; and the third type of cell fires during locomotion. These cells constitute 89% of the cells in s. granulare. Recently, two types of cells have been identified in the DG and CA1 field: 1) theta-on cells, which fire rhythmically during spontaneous and hypothalamic-induced theta. These cells show a direct relationship between their rate of firing and spontaneous theta frequency or dorso-medial hypothalamus stimulation intensity; and 2) Theta-off cells which are silent when hippocampal electrical activity is above 0.5 Hz. When the frequency decreases below the 0.5-2.5 Hz range, these cells fire continuously (Colom, Ford and Bland, 1987).

In addition, theta activity can be recorded within the cell. Rhythmic fluctuations in the membrane potential in CA1 cells in rabbits during theta were reported by Fujita and Sato (1964). A burst discharge was found on the depolarizing phase of the intracellular theta, indicating the presence of an excitatory post-synaptic potential (EPSP). The positive phase of theta was correlated with an

intracellular hyperpolarization, while the negative phase was associated with depolarization. In CA1 cells of anesthetized rats, intracellular recordings during theta showed oscillations of the membrane potential that were in theta frequency and were locked in phase to the extracellularly recorded theta. A correlation of 0.976 was found between the intracellular theta amplitude and the inhibitory post-synaptic potentials (IPSPs), suggesting that theta was caused by rhythmic modulation of IPSPs impinging on the CA1 cells (Leung and Yim, 1986). Andersen (1980) recorded intracellularly in the granule cell layers, and found that during theta there were oscillations in the membrane potential and depolarization waves with single spikes, recorded extracellularly as negativity.

Extra-hippocampal theta generators

Hippocampal theta rhythmicity originates in the medial septum and the nucleus of the diagonal band of Broca. Hippocampal theta occurs only with the appearance of rhythmic firing in the medial septum (Macador, Roig, Monte and Budelli, 1970). Lesioning the septo-hippocampal border, including parts of the lateral portion of the medial septal nucleus eliminates theta in the ipsilateral hippocampus. There is a direct relationship between the amount of tissue destroyed and the degree of theta elimination. Lesioning the alveus or perforant path has no effect (Andersen, Bland, Myhrer and Schwartzkroin, 1979). The medial septum drives the three theta generators: CA1, DG and entorhinal cortex (see below). Fibers from the medial septum and the diagonal band of Broca terminate in the hilus and in the supragranular layer of the dentate. In the dentate these terminations are thought to

occur on interneurons or granule cells dendrites (Mosko, Lynch and Cotman, 1973). The medial, non-cholinergic portion of the medial septum projects to more septal parts of dentate and hippocampus. More lateral parts in the medial septum, which are cholinergic, terminate more temporally in the DG and hippocampus (Amaral and Kurz, 1985). In the CA1 field, there are medial septal synapses above and below s. pyramidale. Electrolytic lesions of the medial septum have revealed that lateral and medial parts control theta that can be recorded during walking, while anterior and rostral parts of the dorso-lateral septum and dorsal parts of the diagonal band control theta that is dominant during REM sleep (Monmaur, Houcine and Delacour, 1979).

Three types of cells were identified in the medial septum (Apostol and Creutzfeldt, 1974; Petsche, Stumpf and Gogolak, 1962): 1) type A, that fired more or less randomly at 100-600 bursts/sec, and constituted about 52% of the cells; 2) type B, found mainly in the medial part of the nucleus, and fired rhythmically in theta periodicity, phase-locked to the hippocampal theta. This cell fired irregularly when theta was absent and accounted for about 30% of the total number of cells; and 3) type C constituted 18% of the medial septal cells and fired about 5-50 spikes/sec. This cell was also locked to the hippocampal theta phase, with different cells being locked to different phases.

It is not known through which mechanism the medial septum induces hippocampal theta. It has been suggested that the medial septum activates the pyramidal and granule cells, and recurrent collaterals of these cells activate inhibitory interneurons that inhibit the principal cells in a periodic manner. The oscillations between excitation and

inhibition create rhythmic fluctuations in the membrane potential that are recorded extracellularly as theta (Buzsaki, Leung and Vanderwolf, 1983). This suggestion is supported by the finding that interneurons were found to be modulated by the septum (Buzsaki and Eidelberg, 1983).

Another putative theta generator is found in the entorhinal cortex. Cells in the EC fire rhythmically and are locked in phase to hippocampal theta (Mitchell and Ranck, 1980). Theta rhythm is recorded in the medial entorhinal cortex during REM sleep and voluntary movement. Bilateral lesioning of the entorhinal cortex produces reduction in the correlation observed between theta and voluntary movement, and the correlation between theta and alert immobility in the guinea pig (Montoya and Sainsbury, unpublished). Atropine abolishes both types of theta in the freely moving rat after lesioning or deafferentation of the entorhinal cortex, pointing to the necessity of entorhinal cortex in producing theta during locomotion (Vanderwolf, Leung, & Cooly, 1985).

The cingulate cortex may also be the site of a possible theta generator. Cells have been identified in this area that are coupled to the dentate gyrus and CA1 generators (Feenstra and Holsheimer, 1979). Periodic trains of spikes that are phase-locked to hippocampal theta have been recorded in the cingulate, and a phase reversal has also been observed (Holsheimer, 1982).

Brain stem input affects hippocampal EEG, according to the stimulation site, frequency and the intensity of the input. Desynchronization of hippocampal EEG is probably induced by an input

from raphe nuclei, specifically by the median nucleus. In anesthetized rats, stimulation of median raphe inhibits firing of medial septal cells that fire rhythmically and in phase with hippocampal theta, resulting in desynchronization of the hippocampus (Assaf and Miller, 1978). Stimulation of the raphe nuclei and nucleus pontis caudalis also induces hippocampal desynchronization (Macador, Chalupa and Lindsley, 1974).

Synchronization of hippocampal EEG can also be achieved by stimulating several brain stem and midbrain nuclei. Vertes (1980) found that stimulation of the nucleus pontis caudalis induced synchronization in the anesthetized rat. Recordings from the nuclei pontis caudalis and oralis showed that 83% of the cells there fired during "theta" behaviors and fired less during "non-theta" behaviors (Vertes, 1977). In contradiction to previous studies, it was found that stimulation of the raphe magnus induced hippocampal theta and motor cortex desynchronization, with behavioral orienting (Pole and Monnier, 1970). Therefore, a central region of the brain stem reticular formation is involved in hippocampal synchronization. Three major nuclei have been implicated in this process: 1) nucleus gigantocellularis; 2) nucleus pontis caudalis; 3) nucleus pontis oralis. In the midbrain, the nucleus cuneiformis is also involved in this process. In addition, several nuclei such as locus coeruleus, and midbrain and pontine central gray were also implicated (Vertes, 1982). Vertes concluded that the pontine reticular formation and the three ascending fibers that travel through it: the medial longitudinal fasciculus (MLF), the medial forebrain bundle (MFB), and the central tegmental tract (CTT)) were the main synchronizing centers in the brain stem and midbrain (Vertes,

1981). The supramamillary nucleus may also be involved in theta generation in that its stimulation induced hippocampal theta. The pontine reticular formation synapses on the supramamillary nucleus, that projects in turn to the MFB, septum and hippocampus (Vertes, 1986).

It has been suggested that the medial septum changes constant input current that arrives from the reticular formation, to a discrete pattern of firing that drives hippocampal theta rhythm (Petsche, Gogolak and Van Zwieta, 1965). When the reticular formation is stimulated in order to induce hippocampal theta, the septal B units increase their firing rate, while still being phase-locked to theta. As the intensity of the input from the brain stem reticular formation to medial septum increases, higher frequencies of theta are recorded in the hippocampus. This leads to the suggestion that theta activity indicates the levels of sensory stimulation that reaches the animal (Tombol and Petsche, 1969).

Theta activity and behavior Several theories have been suggested to relate theta rhythm to behavioral functions.

Arousal: Green and Arduini's (1954) original hypothesis was that since hippocampal theta was always associated with neocortical desynchronization, and appeared when the animal oriented towards a stimulus, theta activity therefore represented a "paleocortical arousal". Grastyan is the current proponent of this theory. In one experiment (Grastyan, Karmos, Ureczky and Kellenyi, 1966), cats learned to approach or avoid stimuli after the presentation of a tone. During the early stages of learning, theta was absent. After the animal

started to approach the stimulus or avoid it, theta appeared, and after the establishment of the CR, it disappeared. Theta appeared only when the animal oriented towards the source of the stimulus. After repeated presentations, there was a decrease in the orienting response toward the CS+, and theta was replaced by faster activity. Theta activity also increased during presentation of the signal in an aversive conditioning task (Buzsaki, Grastyan, Haubenreisen, Czopf and Kellenyi, 1982). By stimulating the hippocampus, it was shown that the orienting response disappeared. Therefore, Grastyan and his associates concluded that the hippocampus functions to inhibit the orientation response to irrelevant stimuli.

Memory and information processing: Ross Adey, in numerous studies, suggested that theta is involved in memory. In Adey's experiments, chronically implanted cats were trained on a discrimination task. The cats ran down an alley, where a choice point with a two-sided box was placed. A food pellet was hidden in one compartment, signaled by a lighted rectangle (S+). The S+ was shifted randomly from side to side across trials. During exposure to the learning situation, there was a 4-7 Hz synchronous pattern in hippocampus and entorhinal cortex. When the animal approached the box and also during the acquisition stage, theta frequency changed to 5-6 Hz and was limited to the dorsal hippocampus. Theta was present also after acquisition. The rhythm disappeared during extinction and reappeared during retraining. Between the trials, theta activity was absent (Adey, Dunlop and Hendrix, 1960). The closer the animal approached the goal, the higher was the theta frequency (ranging from 4 to 6 Hz). It was therefore suggested that theta plays a role in acquisition and storage of information, and that

the role of the hippocampus in learning is to form functional connections with subcortical structures, this function being achieved through theta rhythm (Adey, 1967). Other evidence has implicated theta in memory functions. Abolishing theta activity by lesioning the medial septum interferes with spatial learning (Winson, 1978). Those animals that continue to show theta activity also show retention of the task. In rabbits, trained in the nictitating membrane conditioning paradigm, EEG was measured before training, and a positive correlation was found between the time the hippocampus showed a 8-22 Hz activity and the number of trials to criterion. A negative correlation was found between the time there was a 2-8 Hz activity pattern in the hippocampus and trials to criterion, indicating that animals that learned faster manifested EEG in the theta range. Any frequency below 8 Hz was negatively correlated with trials to criterion, and any record above 7 Hz was positively correlated with the learning rate (Berry and Thompson, 1978).

Voluntary locomotion: Vanderwolf is the leading proponent of the idea that since theta appears during voluntary locomotion, it may have some function related to locomotion. In one study, (Vanderwolf, Kramis, Gillespie and Bland, 1975) theta appeared during walking, head movements and postural changes. During eating or grooming theta changed to irregular, small amplitude activity (Fox, Wolfson and Ranck, 1983). Bland (1986) suggested that theta rhythm affects the motor system, either by signaling it that the animal is ready to move (when the animal is immobile), or changing the amplitude of the movement while the animal is in motion.

A more detailed analysis of the relationship between theta and behavior has revealed 2 types of theta: a) Theta Type 1, (6-12 Hz), which was prominent during movement, head movements, postural shifts, swimming, rearing, jumping and digging. This type of theta was insensitive to atropine, but was sensitive to anesthetics; b) Theta Type 2 (4-9 Hz), which appeared during alert immobility, was sensitive to anticholinergics such as scopolamine and atropine, and was insensitive to anesthetics. A third EEG pattern, large amplitude-irregular activity (LIA)- appeared in "automatic" motor responses, such as grooming, shivering, chewing, scratching, and sneezing and was not considered to be within the theta range (Bland, Seto and Rowntree, 1983). The two theta types were independent; administration of physostigmine induced theta type 2 and atropine abolished it, while theta type 1 continued to exist during locomotion (Bland, Seto, Sinclair and Fraser, 1984). Theta cells doubled their rate of firing and also fired rhythmically during theta type 1; during theta type 2 they also fired rhythmically but with no change in the rate of firing; and during LIA, the pattern of firing was arrhythmic (Sinclair, Seto and Bland, 1982). After isolation of the entorhinal cortex from the neocortex and cingulate gyrus, a 6-12 Hz theta existed during locomotion, and this theta was abolished by atropine, indicating that the entorhinal cortex isolation destroyed Type 1 theta. It was therefore suggested that the pathways which regulate theta type 1 travel through the hypothalamus, cingulate and neocortex before reaching the hippocampal formation through the entorhinal cortex. As an additional support for this theory, it was found that a lesion in the cingulum partially abolished theta, and atropine reduced its amplitude,

indicating that theta type 1 was affected by the lesion (Vanderwolf, Leung and Cooley, 1985).

There are certain species differences in the behavioral correlates of theta. As previously mentioned, theta is easy to record in the rat, guinea pig and rabbit, while it is more difficult to record in the cat, and is very difficult to record in the monkey (Bennet, 1971). In primates, the EEG is usually irregular with high or low amplitude (Crowne and Radcliffe, 1975). Rarely does theta appear in the EEG spectrum of the monkey, but its behavioral correlates are not clear. In humans, during "Theta behaviors", such as performance on a memory task, a marked desynchronization was seen in the hippocampus (Halgren, Babb & Crandall, 1978). In rats, theta was recorded in the dorsal hippocampus during voluntary motor behavior (Robinson, 1980). It has been argued that there is no theta in the rat during alert immobility. However, when a cat or ferret were presented to a restrained rat, theta was produced, and sensory stimuli that normally do not produce theta in the immobile rat did produce this rhythm in the presence of the cat. It was therefore suggested that theta type 2 will appear in the immobile rat EEG only during high level of arousal (Sainsbury, Heynen and Montoya, 1987). Theta in the rat also appears during sniffing behavior, but only if the head moves. The same pattern of theta characterized the mouse, gerbil and guinea pig EEG (Winson, 1972). In cats, theta appears as a correlate of attention, alertness and orientation (Bennet, 1975), and also during walking and head movements (Robinson, 1980). Theta reaches its peak amplitude during alert visual searching (Winson, 1972). In rabbits, theta appears during voluntary locomotion and also

during alert immobility, when a sensory stimulus is presented (Robinson, 1980). Theta habituates after repeated presentations of the stimulus (Winson, 1972). In dogs, theta is prominent during walking and head movements, and the amplitude grows with an increase in the speed of walking.

In all the species mentioned above, theta appears during REM sleep, and the frequency of theta grows during the muscle twitches and decreases during immobility (Robinson, 1980).

Figure 9 presents several EEG records of a rat during different behaviors.

Long-term potentiation (LTP) is a form of synaptic plasticity that has a long duration, and hence is considered a candidate mnemonic mechanism. Chapter 4 describes the nature and mechanisms of long-term potentiation.

Figure 9

EEG recordings that were obtained from a rat dentate gyrus during the following behaviors:

1 - Voluntary movement

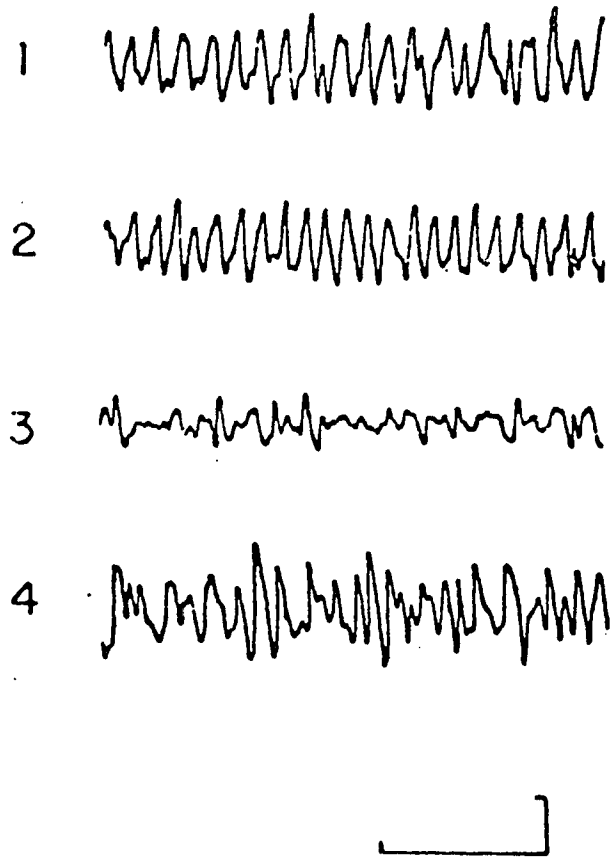
2 - REM sleep

3 - Still-alert state

4 - Slow-wave sleep

Note the prominence of theta rhythm during voluntary movement and REM sleep. Scale: 1 sec and 500 uV. (From: Winson, 1978).

Figure 9



4. Long-Term Potentiation

Introduction

Four types of stimulation-induced synaptic plasticity exist in the nervous system: a) Facilitation, which is caused by an increased transmitter release, possibly due to enhanced potassium (K^+) conductance, and decays exponentially with a time constant of about 100 msec.; b) Augumentation, which can be observed after a short tetanization and decays with a time constant of about 5 sec.; c) Post-Tetanic Potentiation (PTP), which is evident after longer tetanic trains and decays after a few seconds or minutes; d) Long-Term Potentiation (LTP), which follows the time course of decay of PTP but does not return to base line and lasts longer. LTP lasts in hippocampal slices up to about 4 hours, several hours in the anesthetized animal (depending on the level of the anesthesia), and up to eight weeks in the freely walking animal (Andersen and Hvalby, 1986).

The immediate consequence of a strong and prolonged tetanic stimulation is a short PTP, that decays after about 1-2 minutes. Following PTP, there is LTP, a long-term enhancement of the field responses. Both population spike and the ESP's slope increase their magnitude and decrease their latency (Abraham, Bliss and Goddard, 1985). There is also an increase in discharge probability of individual units because a smaller volley is sufficient to discharge them. The latency of the discharge also decreases (Andersen, 1978). There is no change in cell excitability in CA1 slices, based on measurements of membrane resistance and membrane depolarization. Typical changes found in LTP consist of an increase of 50% of the slope, with a range of 0-

100%; and an average increase of the population spike of 250%, with a range of 0-1200% (Teyler and Discenna, 1987). LTP is specific to the synapses that had been potentiated. No transfer of potentiation has been found between the potentiated apical and the non-potentiated basal dendrites in CA1 pyramidal cells or between the potentiated medial PP and the non potentiated lateral PP and vice versa (Bliss, 1979). All fields of the hippocampus, including the different interneurons and the dentate gyrus cells show evidence of LTP. Different structures in the limbic forebrain such as amygdala, pyriform cortex and septum also show the capacity to produce LTP. The hippocampus, however, has the lowest threshold, largest magnitude and longest decay constant of LTP (Racine, Milgram and Hafner, 1983). The neocortex (Lee, 1983); superior colliculi; striatum; sympathetic ganglia (Brown and McAfee, 1982); medial geniculate body in cats; and the neuromuscular junction (Baxter, Bittener and Brown, 1985) also show evidence of LTP.

The early reports on LTP were published in 1970 by Bliss and Lomo, and in 1971 by Lomo, and Bliss and Gardner-Medwin. In anesthetized rabbits, a recording electrode was placed in the dentate area, and a stimulating electrode was placed in the medial perforant path. Three to four tetanic trains at 100 Hz were administered to the medial perforant path while ESPs were recorded from the dentate gyrus. As a result of the tetanic stimulation there were significant increases in the population spike amplitude, EPS's slope, and a reduction in the spike latency that lasted for 10 hours (Bliss and Lomo, 1973). In freely walking rabbits, the effect persisted for up to 3 days (Bliss and Gardner-Medwin, 1973). Douglas and Goddard (1975) replicated these

studies in chronically implanted rats and found that the enhancement persisted in some rats for two months after tetanization.

Successful induction of LTP Several requirements must be met for a successful LTP induction to take place:

Frequency: The optimal stimulation parameters that produce LTP consist of 10 trains of 8-10 bursts, at a frequency of 400 Hz, with an inter-train interval of 10 seconds (Eccles, 1983). If the stimulation frequency goes beyond 500 Hz, less potentiation is produced. The frequency of 500 Hz is close to the refractory period of the perforant path axons (McNaughton, 1983). A frequency below 2 Hz will not produce LTP either (McNaughton, 1983). At such low frequencies (10 stimuli at 1 Hz) there was an inhibition in the DG (Alger and Teyler, 1976), in the Schaffer-commissural fibers (Barrionuevo, Schottler and Lynch, 1980), and also in the CA1 field in slices, where heterosynaptic depression was observed (Dunwiddie and Lynch, 1978). Several blocks of tetanic stimulation are required to produce LTP. The same number of stimuli distributed throughout the same period will not produce LTP.

Intensity: As the stimulation intensity rises, more LTP is produced (McNaughton, Douglas and Goddard, 1978). This has been found in the DG, CA1 and CA3 fields. Intensities that do not produce a population spike will usually not produce LTP. Above the threshold of a spike production, increase in intensity will facilitate LTP production and will increase its magnitude even if the stimulation frequency is reduced (McNaughton, 1983). This finding has led to the idea of cooperativity: that the strong intensity of stimulation is needed to stimulate synchronously a large number of afferent fibers in the dentate in order to induce LTP. Some evidence supports the

cooperativity idea: it was found that concurrent stimulation of the medial and lateral PP created LTP in intensities that in each pathway alone did not produce LTP (McNaughton, Douglas and Goddard, 1978). A larger LTP was also produced in CA1 field if adjacent afferents were also activated (Lee, 1983). LTP is a physiological phenomenon in the sense that there are cells that fire at high frequencies, but the synchronization of large groups of fibers is not within the physiological range (Andersen, 1978; Racine and Kairiss, 1987).

Calcium (Ca^{2+}) ions: Removal of these ions blocks LTP induction (Lynch, Larson, Kelso, Barrionuevo and Schottler, 1983), and increasing their extracellular concentration induces LTP (Higashima and Yamamoto, 1985; Turner, Baimbridge and Miller, 1982). There are no changes in the fiber volley or anti-dromically evoked spikes, indicating that no overall increase in excitability due to the presence of Ca^{2+} occurs. Also, increasing the intracellular concentration of Ca^{2+} ions by blocking potassium ions (K^{+}) current results in an enhancement of LTP in the CA1 slice (Lee, Anwyl and Rowan, 1986).

Monoamines: The presence of serotonin and norepinephrine (NE) is required for LTP induction. Depleting norepinephrine reduces LTP by 50%, with no effect on LTP threshold. Serotonin depletion reduces LTP by 70% and raises its threshold. LTP duration is not affected by the absence of any of these monoamines (Bliss, Goddard and Riives, 1983). Adding norepinephrine to CA3 slices during 100 Hz tetanization increases LTP magnitude, duration and probability of occurrence. During low-frequency stimulation (0.2 Hz) this effect is not observed (Hopkins and Johnston, 1984).

Active NMDA receptor: Blockade of this receptors inhibits LTP formation (Collingridge et al, 1983). (see "Synaptic mechanisms of LTP", below).

Morphological changes associated with LTP

Rall (as cited in Bliss, 1979) suggested that synaptic efficacy is affected by changes in the width of the dendritic spine. If the spine base widens, more current will flow into the dendrite, and hence, the probability of discharge will grow. LTP has indeed been found to be associated with such changes. Fifkova and Van Harreveld (1977) studied the dendritic spines in the proximal third in s. moleculare in the dentate after LTP and found that after 2-6 min there was an increase in the spines size by 15%, and after 10-60 min there was a further increase of 38%. Four to eight hours later an additional increase of 35% was detected and after 23 hours there was a further increase of 23%. The authors suggested that the increase in the spine size reduced the spine resistance, increased the length constant and eventually led to increased probability that the cell will be depolarized. Schuster, Krug, Plaschke et al. (1986) studied the effects of LTP in the mid-third of s. moleculare. No change in vesicle density was observed eight hours after tetanization, but an increase of 70% in pre-synaptic invaginations was noted. Forty-eight hours later an additional increase of 30% in the pre-synaptic invaginations took place, and the vesicle density decreased by 20% in the area of transmission. At that time an increase of 40% in axo-dendritic contacts in the enhanced synapse in the dentate was also noted (Wenzel and Matthies, 1985). Desmond and Levy (1986a,b) administered 24 trains of 8 pulses each at 400 Hz. and analyzed only those animals that showed LTP of at least 50% or more.

The size of the post-synaptic density, created by the entorhinal cortex axons on the granule dendrites, increased only in the activated area. The overall area of the post synaptic density that is associated with concave synapses increased, with a parallel decrease in non-concave synapses. The head of the spine grew and became concave, accounting perhaps for the enlargement the receptive area of the spine. In CA3-CA1 synapses, Lee, Schottler, Oliver and Lynch (1980) observed that after LTP there was an increase of 33% in the number of shaft synapses. Furthermore, there was an increase in the number of vesicles attached to the active area in the membrane, an enlargement of the area of the spine and a decrease in the vesicle density.

The synaptic mechanisms of LTP

Excitatory amino acids, especially glutamate, are putative neurotransmitters in the hippocampal formation. Three subtypes of glutamate receptors have been observed in the brain, based on the agonist that binds to them with the highest affinity: kainate, quisqualate, and N-methyl-D-aspartate (NMDA). The NMDA subtype receptor is involved with large conductances while the kainate and quisqualate subtypes allow smaller conductances (Jahr and Stevens, 1987). The chemical D-2-amino-5-phosphonovalerate (APV) is considered an NMDA receptor antagonist.

There is evidence that LTP is mediated by the NMDA receptor subtype. Administration of NMDA produced LTP (Collingridge, 1985). APV reversibly blocks LTP in CA1 field in slices with no effect on normal transmission, as is evident by the presence of normal evoked potentials (Harris, Ganong and Cotman, 1984). Magnesium ions (Mg^{2+}) block NMDA

receptors and inhibit LTP production in CA1 slices (Pockett and Lippold, 1986). Magnesium ions block the NMDA receptors during low frequency stimulation but not during tetanic stimulation. It is therefore suggested that during tetanization there is enough depolarization to overcome the Mg^{2+} blockade (Herron, Lester, Coan and Collingridge, 1985).

There is a controversy in the literature whether LTP is caused by pre- or post-synaptic mechanisms, or both. The evidence supporting each position will be described below.

Presynaptic involvement: The evidence for presynaptic mechanisms of LTP comes from studies showing an increase in transmitter release following LTP formation. Tetanic stimulation in CA3 field causes a long-term increase in tritiated aspartate and glutamate release during resting conditions and in response to stimulation (Skrede and Malthe-Sorenssen, 1981). Using push-pull cannula and tritiated glutamine while also monitoring evoked potentials, Dolphin, Errington and Bliss (1982) demonstrated a long-term increase in glutamate synthesis and release after LTP. The increased glutamate release continued up to 3 hours, whereas aspartate release was maintained up to 2 hours. In animals where LTP was not produced, no increase in transmitter release was observed. *In vitro* studies also showed an increase in glutamate release after LTP with no change in its uptake or in the number of its binding sites (Lynch, Errington and Bliss, 1985). Blocking LTP by a train to a contralateral commissural input just before the tetanus causes blockade of LTP and also inhibits the increases in glutamate and aspartate release. Administering the glutamate receptor antagonist gamma-glutamylglycine inhibits LTP formation in walking rats, but when the

standard medium was returned to the cannula, LTP appears, indicating that LTP is merely masked. This argues against involvement of the postsynaptic glutamate receptors, which were blocked during the tetanization (Dolphin, 1983). Also, after LTP there was no change in response to glutamate, indicating that the receptors sensitivity or their number do not change (Turner, Baimbridge and Miller, 1982).

Postsynaptic involvement: Several studies have shown that some post-synaptic changes may be involved with LTP induction. Using voltage clamp in the CA3 field, Barrionuevo, Kelso, Johnston and Brown (1986), showed that after LTP there was an increase of 44% in the post-synaptic membrane conductance. Also, injecting hyperpolarizing current to the post-synaptic cell during tetanization blocks LTP formation, indicating that there is a post-synaptic site that is voltage sensitive and is essential for LTP production (Malinow and Miller, 1986). Such a site may be the NMDA receptor. As was described above, blocking this receptor interferes with LTP induction. The NMDA receptor is voltage sensitive; depolarization of the cell opens the NMDA channel, allowing the influx of Ca^{2+} and Cl^{+} ions. The importance of Ca^{2+} presence for LTP induction was described previously.

A theory of LTP that involves post-synaptic receptors was offered by Eccles (1983). He emphasized the importance of Ca^{2+} ions for LTP induction and suggested that the dendritic spine is the functional unit in LTP. According to his theory, strong and continuous depolarization of the membrane causes opening of voltage sensitive calcium channels. Calcium ions enter the cell and enhance the depolarization by forming self-regenerating calcium spikes. Calcium

ions also bind to calmodulin and the Ca^{2+}-calmodulin complex activates adenylate cyclase, inducing the conversion of ATP to cyclic AMP. Cyclic-AMP, in turn, induces phosphorylation of membrane proteins and activates or exposes glutamate receptors found in the post-synaptic density. Cyclic AMP activity also leads to swelling of the dendritic spine (Eccles 1983).

A comprehensive theory of post-synaptic involvement of glutamate receptors in LTP was proposed by Lynch and Baudry. Glutamate is perhaps the major excitatory transmitter of the hippocampus, and has been identified in terminal areas of the perforant paths, mossy fibers and pyramidal axons. An uptake system of glutamate has also been described in the hippocampus. After tetanization, the intracellular concentration of Ca^{2+} ions grows, which causes an increase of 25-30% in the uptake of tritiated glutamate, with no change in glutamate affinity (Baudry, Oliver, Gregor, Wieraszto and Lynch, 1980). In low frequency tetanus no LTP is found and no increase of glutamate uptake takes place. These and other findings have led to the suggestion that the increased intracellular concentration of calcium ions after tetanization induces proteolysis of the membrane protein fodrin by the proteolytic agent calpain. This causes an uncovering of glutamate receptors and also changes them from a labile state (in which they are inaccessible to the transmitter) to a stable state (that allows interaction between the transmitter and the receptor) (Baudry and Lynch, 1982; 1984a,b). Leupeptine, which blocks the effects of calpain, was found to block LTP and also interfered with acquisition in a learning task (Lynch, Kessler and Baudry, 1984). Some evidence that contradicts this theory has been also obtained: Excitability of CA1

cells, in response to iontophoretic applications of glutamate, does not rise after LTP, but is found to be depressed (Lynch, Gribkoff and Deadwyler, 1976).

A different postsynaptic theory was offered by Herron, Lester, Coan and Collingridge (1986), this time involving the NMDA receptor. As was described before, during low frequency stimulation, the NMDA receptor is blocked by magnesium ions. Under high frequency stimulation, magnesium blockade is removed, and Na^{2+} and Ca^{2+} enter through the NMDA channel. (It is possible that the depolarization of the membrane is responsible for blocking Mg^{2+} ions, because depolarization has been shown to reduce magnesium inhibition.) It was therefore suggested that a single neurotransmitter is acting on both NMDA and non-NMDA receptors, but since the former are blocked by Mg^{2+}, the frequency of the input will determine which type of receptor will be activated and, hence, what kind of information is delivered to the cell. In one experiment, CA1 slices were stimulated by low frequency (0.1 Hz) or high frequency (200 Hz) shocks. When magnesium ions were removed from the medium LTP was observed in the high frequency condition. It was suggested that during the high frequency stimulation magnesium ions were probably blocked, allowing Na^{2+} and Ca^{2+} to enter the NMDA channel and induce LTP. The Malinow and Miller (1986) finding that hyperpolarization of the post synaptic membrane blocks LTP is explained as follows: hyperpolarization blocks the depolarization that is essential to remove Mg^{2+} inhibition, allowing the blockade of the NMDA receptor. APV has also been found to block depolarization of the dendrites (Wigstrom and Gustafsson, 1984), accounting perhaps for the

blocking effect of APV on LTP, as described previously.

Smith (1987) suggested that a coupling of pre- and post-synaptic mechanisms might account for the phenomenon of LTP. It has been described previously that the NMDA channels are voltage sensitive, are very permeable to calcium, and open when the membrane is depolarized and a transmitter is bound to it. Smith suggested that the post-synaptic NMDA channel detects a temporal contiguity of pre- and post-synaptic activity. This contiguity is signaled by a postsynaptic calcium influx through the NMDA channels that were activated by the transmitter. These suggestions explain why post-synaptic depolarization without synaptic input does not induce LTP and also why post-synaptic hyperpolarization during synaptic input will inhibit LTP induction. However, post-synaptic depolarization coupled with a weak synaptic input will induce LTP. This theory also explains the synaptic specifity of LTP and the cooperativity phenomenon- that concurrent activity of many afferents will sufficiently depolarize the post-synaptic membrane and induce LTP.

LTP and Behavior

Several characteristics of LTP make it suitable as a candidate for a mnemonic mechanism: its onset is fast, it is long in duration, it increases with repetition (Barnes, 1979), seizures interfere with its development (Hess and Teyler, 1976), and antibiotics block it (Stanton and Sarvey, 1984). Some empirical evidence exists that relates LTP and behavioral changes in learning tasks. Berger (1984) produced LTP in rabbits and then trained them in a classical conditioning task of the nictitating membrane. Animals that showed LTP also had a higher rate of acquisition and showed more CR to the CS+, with no differences in the

number of responses to the CS-. A correlation between the rate of development of LTP and acquisition rate was also found in young and old rats, trained on a spatial task by Barnes and McNaughton (1985). The old rats learned more slowly and forgot more rapidly, and their LTP showed a similar pattern. APV, which blocks NMDA receptors and interferes with LTP induction, was infused chronically to rats, and was found to impair performance on a water maze task, which is sensitive to hippocampal damage, but had no effect on a visual discrimination task, which is preserved in hippocampal animals (Morris, Anderson, Lynch and Baudry, 1986).

Evidence showing no association between LTP and learning and memory has also been obtained. Brace, Jeffreys and Mellanby (1985) created an epileptic focus using a tetanus toxin and recorded evoked potentials in the CA3 field during a radial arm maze task. Even though LTP was preserved, the animals showed learning deficits.

Figure 10 illustrates typical changes found in the dentate gyrus after LTP, using modest intensities (as used in this laboratory). Figure 11 illustrates such changes as they occur in the CA1 field.

Both LTP and theta rhythm were implicated in memory processes. If a relationship exists between theta rhythm and LTP, a normal mechanism for the induction of LTP in the hippocampus might be revealed. This thesis investigates whether stimulating hippocampal afferents at theta rhythm periodicity, or concurrently with a certain theta phase, will facilitate the induction of LTP. It is hypothesized that stimulating hippocampal afferents in a way that mimics the normal firing pattern recorded in the hippocampus, might facilitate the induction of LTP. In

all experiments that follow, low intensity stimulation is used in order to delineate the effect of theta rhythm phase and periodicity on the induction of LTP. The common theme in all experiments is to evaluate whether theta periodicity or phase might reduce the threshold for LTP formation.

Figure 10

Records obtained before and after LTP in the dentate gyrus. Figure 10A (before LTP) describes the field potential, as recorded in the granule cell layer after administering a single pulse (100 uA, 0.25 msec width) to the perforant path (PP). Figure 10B (after LTP) shows a field potential recorded from the same animal after tetanization of the PP (with 6 trains at an inter-train interval of 1 sec; each train consisted of 5 pulses applied at 400 Hz, pulse width 0.15 ms, and intensity of 150 uA). Note that after LTP a population spike is apparent. The slope was potentiated by 36%, the potentiation lasting for 30 minutes (the longest interval tested). In Figure 10C, both records are imposed on each other to illustrate the reduction in the latency and the increase in the size of the slope after LTP. The record obtained before LTP is traced with a darker line. The recordings were performed in this laboratory.

Figure 10

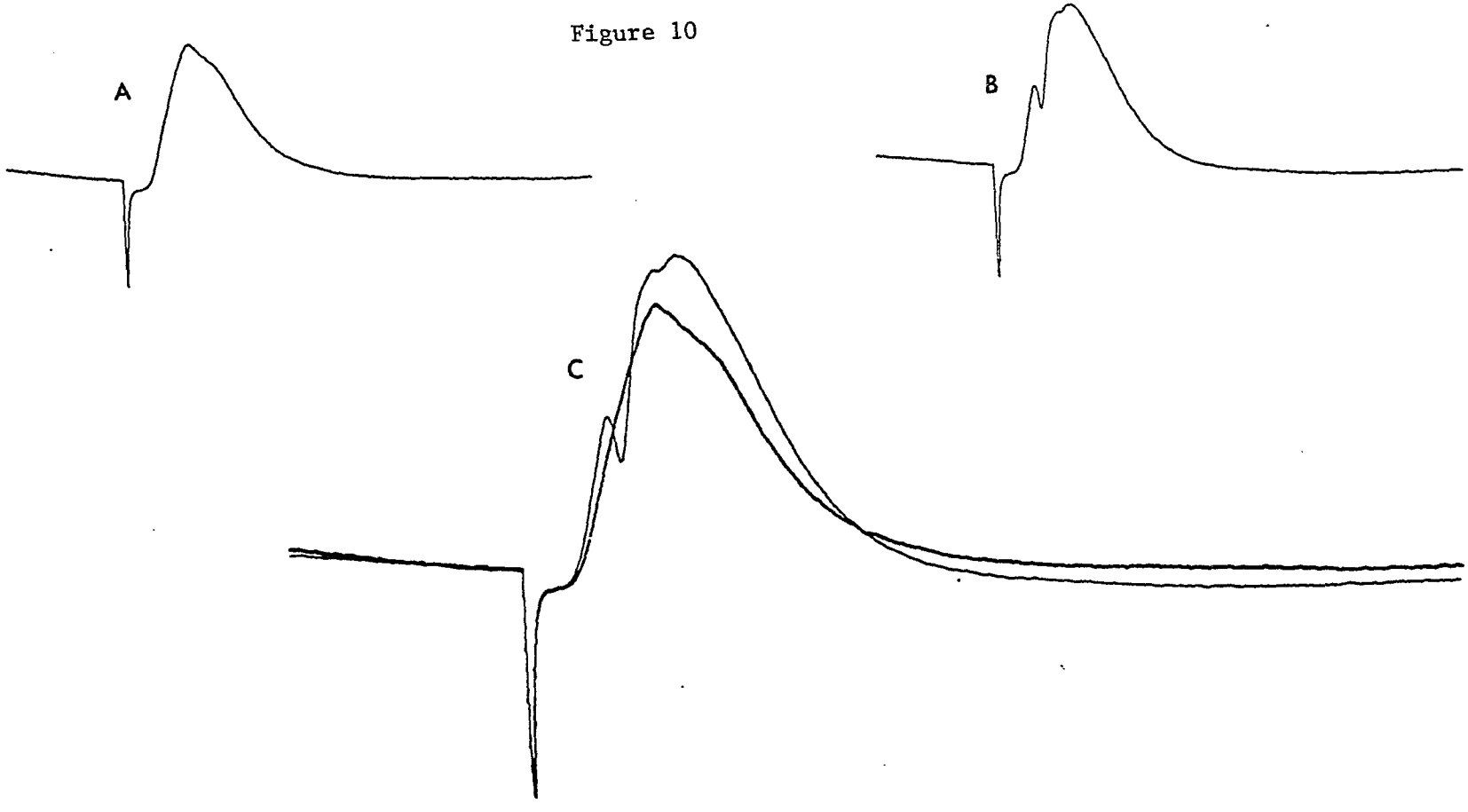
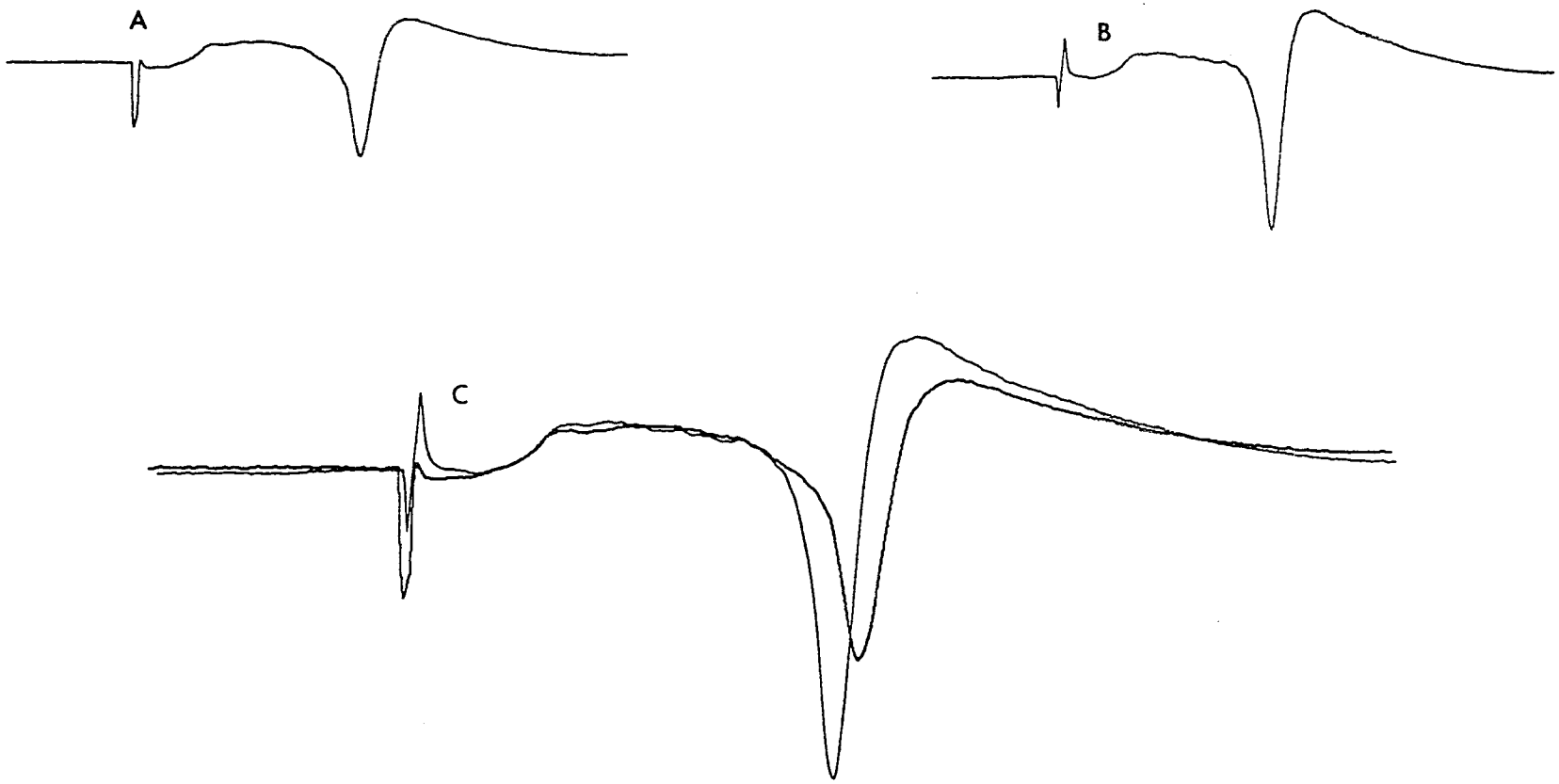


Figure 11

Records obtained in this laboratory before and after LTP in the CA1 field. Figure 11A (before LTP) illustrates the field potential, as recorded in the pyramidal cell layer in the CA1 field after administering a single pulse (150 uA, 0.25 msec width) to the commissural pathways that terminate in that area. Figure 11B (after LTP) shows a field potential recorded from the same animal after tetanization of the commissural afferents (LTP stimuli: 12 trains at an inter-train interval of 150 msec; each train consisted of 5 pulses applied at 400 Hz, pulse width 0.15 us, and intensity of 150 uA). The spike was potentiated by 51%, the potentiation lasting for 20 minutes (the longest interval tested). The records are imposed on each other in Figure 11C. Note the decrease in the latency and increase in the amplitude of the spike after LTP (the record before LTP is traced in a darker line).

Figure 11



5. Experiment 1 - LTP Induction by Priming in the Dentate Gyrus*

Introduction

LTP induction requires long, intense tetanic trains of stimulation, which are outside the physiological range. In the search for more physiologically relevant patterns of stimulation and for ways to reduce LTP threshold, different researchers have been trying to imitate the normal pattern of firing of cells in the hippocampus. Such an approach has revealed that tetanic stimuli, administered at time intervals close to the periodicity of theta rhythm, preferentially induce LTP. Working in the CA1 field in the hippocampal slice, Larson and Lynch (1986) administered tetanic trains (4 pulses at 100 Hz), with inter-train interval of 2, 1, 0.2 or 0.1 sec. Each slice was given a single series of 5, 10, or 20 bursts at one of these frequencies. LTP was induced at 1, 0.2 and 0.1 sec intervals, (corresponding to 1, 5 and 10 Hz, respectively). However, the largest amount of potentiation occurred at the 0.2 sec interval, which corresponds to 5 Hz. Also, at this frequency, the probability of occurrence of LTP was the highest. In another study, Two stimulating electrodes were placed in the CA1 field in the slice, to stimulate two separate sets of afferents, and intracellular recordings revealed the existence of LTP only if the stimulation was administered at a 200 msec interval (Larson, Wong and Lynch, 1986). Recently, such stimulation was administered to freely walking rats and was found to induce LTP that persisted for 10-21 days (Staubli and Lynch, 1987).

---

* The data presented in this chapter was published in Greenstein, Pavlides & Winson (1988).

In another study, Rose and Dunwiddie (1986) reported that administering a tetanic train, preceded by a single priming pulse at an interval of 170 msec., successfully induced LTP. Administering the train alone, or reversing the order of the stimuli, so that the train preceded the single, priming pulse, failed to induce LTP. These studies were carried out in the CA1 field in the slice, and were recently replicated in the CA1 field *in vivo* (Diamond and Rose, 1987). Priming intervals of 170 msec (6 Hz) or 140 msec (7 Hz) preferentially induced LTP. Intervals below 70 msec (14 Hz) or above 500 msec (2 Hz) had no effect on synaptic efficacy. The above mentioned intervals (170-200 msec) correspond to the naturally occurring hippocampal theta rhythm (approximately 5-7 Hz), suggesting a possible link between LTP and this rhythm.

Within the rat hippocampus, two independent theta generators have been found, one in the CA1 field and a second one in the dentate gyrus (see Chapter 3.) If the preferential induction of LTP at a 170-200 Hz msec interval in the CA1 field is associated with theta rhythm, a similar result might be obtained in the dentate gyrus. Furthermore, the EC-DG synapse is the first synapse in the trisynaptic circuit, through which most information flows into the hippocampus. If priming at theta rhythm periodicity will induce LTP at this synapse, this might offer even stronger support to the idea that the rhythm affects information processing in the hippocampus. The first experiment will test this hypothesis using the priming paradigm of Rose, Dunwiddie and Diamond, and will further investigate the temporal range of stimuli that are effective in inducing LTP in the dentate gyrus.

Materials and Method

Subjects:

The experiment was performed on ten male Sprague-Dawley rats (Charles River), ranging in weight between 250-350 g. The rats were housed in a colony room (three per cage) for at least a week before the experiment, on a 12-12 hours light-dark cycle. Food and drink were given ad lib.

Surgery:

The animals were anesthetized with urethane (1.7 g/kg, i.p.) and put on a stereotaxic instrument (David Kopf) with the plane of the skull oriented horizontally. The skull was exposed and burr holes of approximately 1-2 mm in diameter were drilled in the left hemisphere at coordinates (relative to Bregma) of AP -3.9, ML +2.3 (for recording from the dentate gyrus), and AP -7.9 and ML +4.1 (over the medial entorhinal cortex). The dura was slit with a sharp needle and bone residues were removed. A glass micropipette for recording and a monopolar stimulating electrode were lowered to the cell body layer of the dentate and the medial perforant path, respectively, while stimulating currents were being administered every 15 sec. The glass micropipette had a tip diameter of 5-10 uM and was filled with 3M NaCl and fast green dye (resistance approximately 2-3 Mn). The stimulating electrode was made of stainless steel insect pin (size 00) coated with 5 layers of epoxylite resin (Epoxylite Corp.) and cleared for distance of 500 uM from the tip, (resistance approximately 250 Kn). The final depths of the recording and the stimulating electrodes were adjusted to give the maximum field potential recorded in s. granulare. Two

additional holes were made in the right and left frontal bones for the placement of a ground screw and a second screw which served as the reference electrode for recording. In the right parietal bone, one hole was made for a ground screw for the return path of the stimulation currents of the perforant path electrode.

Stimulation and Recording:

The signals were amplified by an AC differential pre-amplifier with high and low frequency filters set at 3 Hz and 3 KHz, and at a gain of 100. All recordings were done in an electrically shielded chamber (120 x 118 x 70 cm.)

Extracellular field potentials were evoked in the dentate gyrus by stimulation of the perforant path by square pulses at a pulse duration of 0.25 msec ("Test Stimulus"), delivered via a photo cell stimulus isolator (Grass). The Test Stimulus was chosen to elicit a small population spike (1-2 mV, approximately 50% of saturation current). In each animal the magnitude of the Test Stimulus was maintained for the duration of the experiment. LTP was induced by one train consisting of 6 pulses with an interpulse interval of 10 msec (100 Hz), at a pulse duration of 0.15 msec ("LTP Train"). The train was preceded by a single pulse ("Prime") at different time intervals (see Experimental Design below). The Prime intensity and duration were equal to those of the train pulses. The signals from the animal were fed into an oscilloscope which displayed the field potentials. Figure 12 shows schematically the position of the stimulation and recording electrodes. Field potentials were fed to and displayed on an IBM PC AT microcomputer, where analysis of slope and spike was performed on-line using three movable cursors. The data was also stored on magnetic disks.

Figure 12 Schematic diagram showing the approximate position of the stimulating and recording electrodes in the dentate gyrus.

a) The schematic configuration of the experiment.

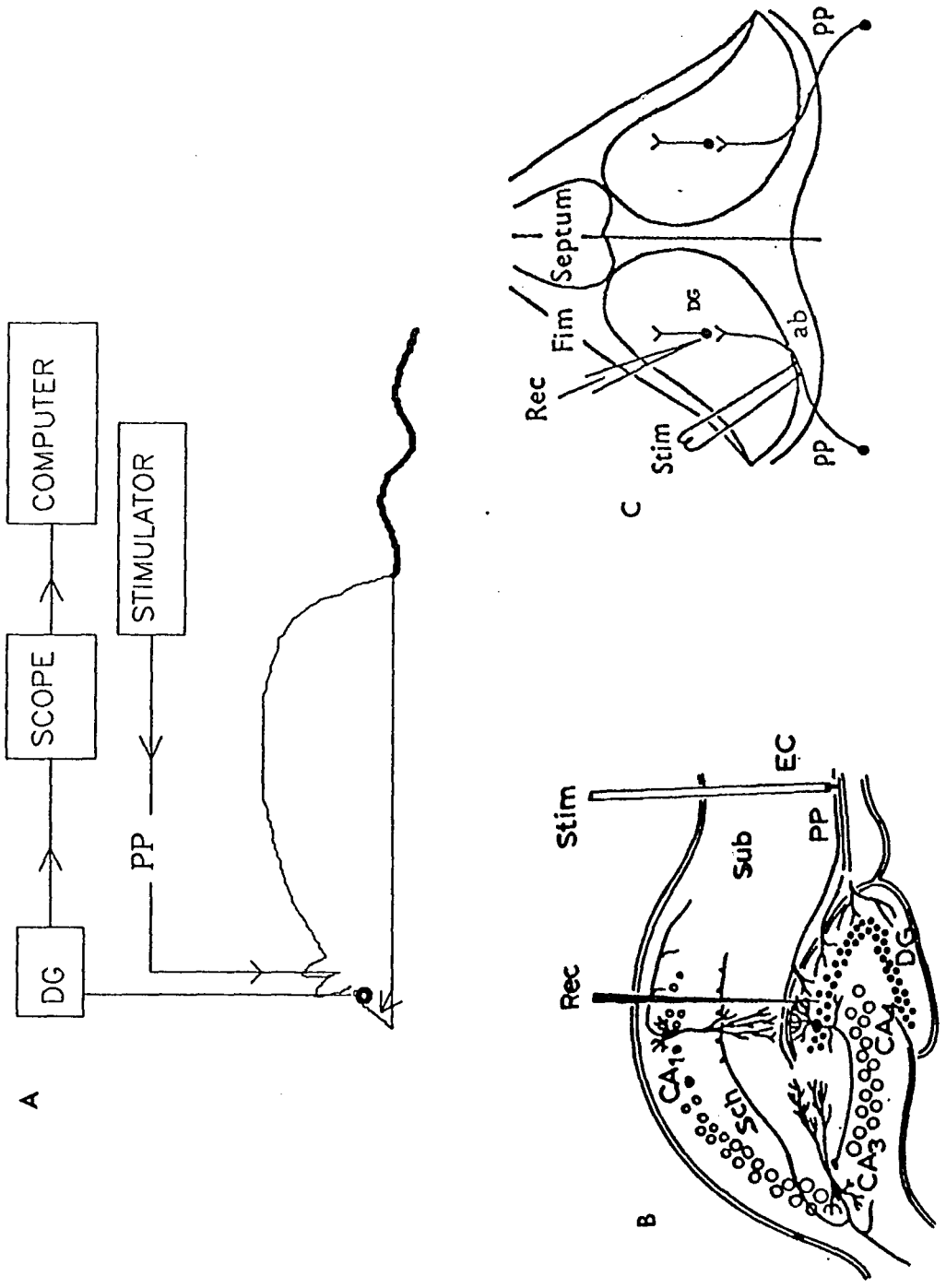
b) A transverse view of the hippocampus, with the recording electrode in s. granulare and the stimulating electrode in the perforant path.

c) A dorsal view of the hippocampal formation with the stimulating and recording electrodes in place. (Adapted from Bliss and Lomo, 1973.)

Abbreviations:

ab	-	angular bundle
DG	-	dentate gyrus
EC	-	entorhinal cortex
Fim	-	fimbria
PP	-	perforant path
REC	-	recording electrode
Sch	-	Schaffer collaterals
Stim	-	stimulating electrode
Sub	-	subiculum

Figure 12


A
DG
SCOPE
COMPUTER
STIMULATOR
PP
B
Stim
EC
Sub
PP
Rec
DG
CA1
Sch
CA3
CA4
C
PP
Septum
Fim
Rec
DG
ab
Stim
PP

Design and Procedure

The experiment was begun by determining the minimum current required to elicit a field response and a population spike, and a saturation current above which there was no further change in either slope or spike. An intensity of the Test Stimulus was selected that would elicit a medium size spike (approximately 50% of saturation current), and an input/output (I/O) curve (slope of the field response versus stimulus current) was obtained by administering pulses every 15 seconds at a series of stimulation intensities. The intensities of the I/O curve pulses ranged from the minimal intensity required to elicit a response to the saturation current.

After obtaining an I/O curve, baseline measurements of the field response were taken for a period of twenty minutes by applying the Test Current to the medial PP every thirty seconds. Then, the Prime was administered, followed by the LTP Train. The intensity of both Prime and LTP Train were chosen to be subthreshold for LTP induction (approximately 40 - 50% of saturation current). The intervals between the Prime and the LTP Train were 50, 100, 200, 350 and 500 msec., corresponding to 20, 10, 5, 2.9 and 2 Hz, respectively. Tetanic stimuli at all priming intervals were administered to each animal in a quasi-random order, so that each animal was exposed to a different sequence. After each tetanization, testing the slope and spike took place for twenty minutes (at thirty second intervals) using the Test Current, as was done in the baseline session. If no LTP was observed, the current intensity was raised by a small amount (50-75 uA), and stimulation at the chosen series of priming intervals was repeated until LTP was induced in one of the intervals. When LTP was detected, tetanization at

the remaining intervals was also performed. The slope and spike were then tested for thirty minutes, final I/O curves were obtained and the experiment was terminated. The animal was then killed by an overdose of Nembutal.

Results

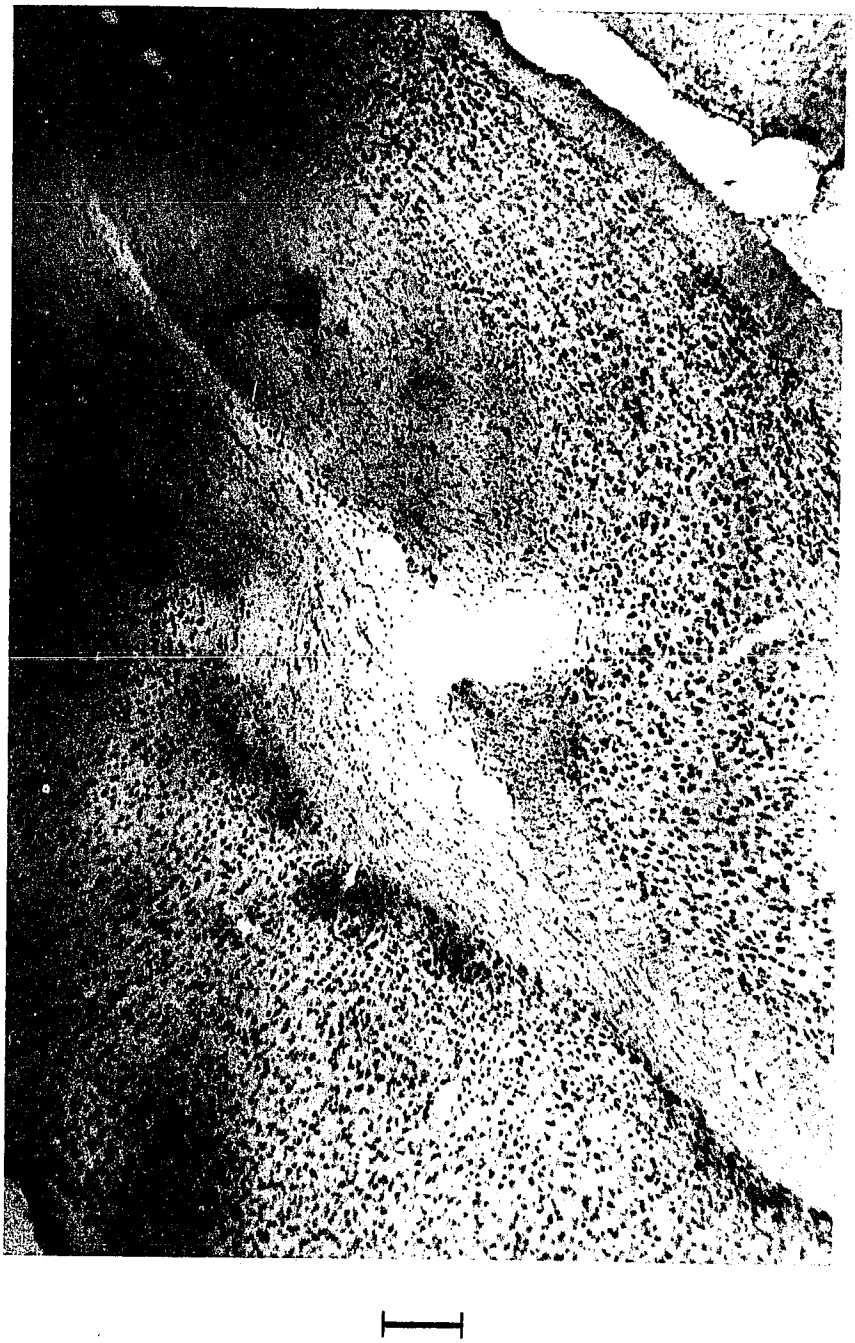
Histological Analysis At the termination of the experiment, a dye mark and a lesion were left in the brain by passing a 10 uA current at a 1 Hz frequency (500 msec pulse duration) through the recording electrode (electrode tip negative relative to the preparation) for 10 minutes. The animal was then perfused with a 0.9% saline solution mixed with 10% formaldehyde, 4% potassium ferrocyanide and 4% acetic acid and the brain was removed and kept for a week in a solution containing 40% sucrose and 10% formaldehyde. A week later, frozen sections at 40 uM thickness were cut, and then stained by the Kluver-Barrera (1953) method. Figures 13 and 14 show a characteristic histological slide of the recording electrode in the dentate gyrus and of the stimulating electrode in the perforant path.

All animals eventually showed LTP in this experiment. LTP occurred only at the priming interval of 200 msec., which corresponds to 5 Hz. Table 1 describes the mean percent potentiation of both slope and spike of all the animals for each of the priming intervals. The average percent potentiation of the slope in the 200 msec priming interval was 11.6% ± 6.2, $F(1,9)=11.1$, $p< .01$. The average percent potentiation of the spike in this condition was 31.7% ± 10.4, $F(1,9)=9.01$, $p<.01$. Potentiation at the other priming intervals were all less than 1.58% for the slope and 4.65% for the spike and no change reached the 0.05

Figure 13 a) A photomicrograph showing the track of the stimulating electrode in the perforant path. Note that the tip of the electrode penetrates the perforant path as it passes near the subicular complex. Magnification x 40. Scale: 260 uM. b) A schematic illustration of the location of the electrode, taken from Paxinos & Watson (1982). The vertical line indicates the location of the electrode. Abbreviations:

4- trochlear nucleus
Aq - cerebral aqueduct
bas - basilar artery
bic - brachium inf colliculus
bp - brachium pontis
CG - central gray
CGD - central gray, dorsalis
CGM - central gray, medialis
CLi - caudal nucleus raphe
DpG - deep gray layer sup colli
DpWh - deep white sup colliculus
DR - dorsal raphe
Dsc - lamina dissecans EC
Ent - entorhinal cortex
InG - intermed gray sup colli
InWh - intermed white sup colli
lfp - longitudinal fasciculus
ll - lateral lemniscus
m5 - motor root trigeminalis
mcp - middle cerebral peduncle
Me5 - nuc mesenceph trigeminal
ml - medial lemniscus
mlf - med longitudinal fasciculus
MnR - median raphe
Op - optic nerve layer sup colli
Pas - parasubiculum
PBg - parabigheminal nucleus
Pn - pontine nuclei
PnO - pontine reticular nuc oral
Prs - presubiculum
RF - rhinal fissure
RR - retrorubral nucleus
rs - rubrospinal tract
RSpl - retrosplenial cortex
S - subiculum
s5 - sensory root trigeminalis
Str17 - striate cortex 17
Str18(a) - striate cortex 18(a)
SuG - superficial gray sup colli
tfp- transverse fibers pons
xscp - decussation sup cere peduncle
Zo - zonal layer sup colliculus

Figure 13a



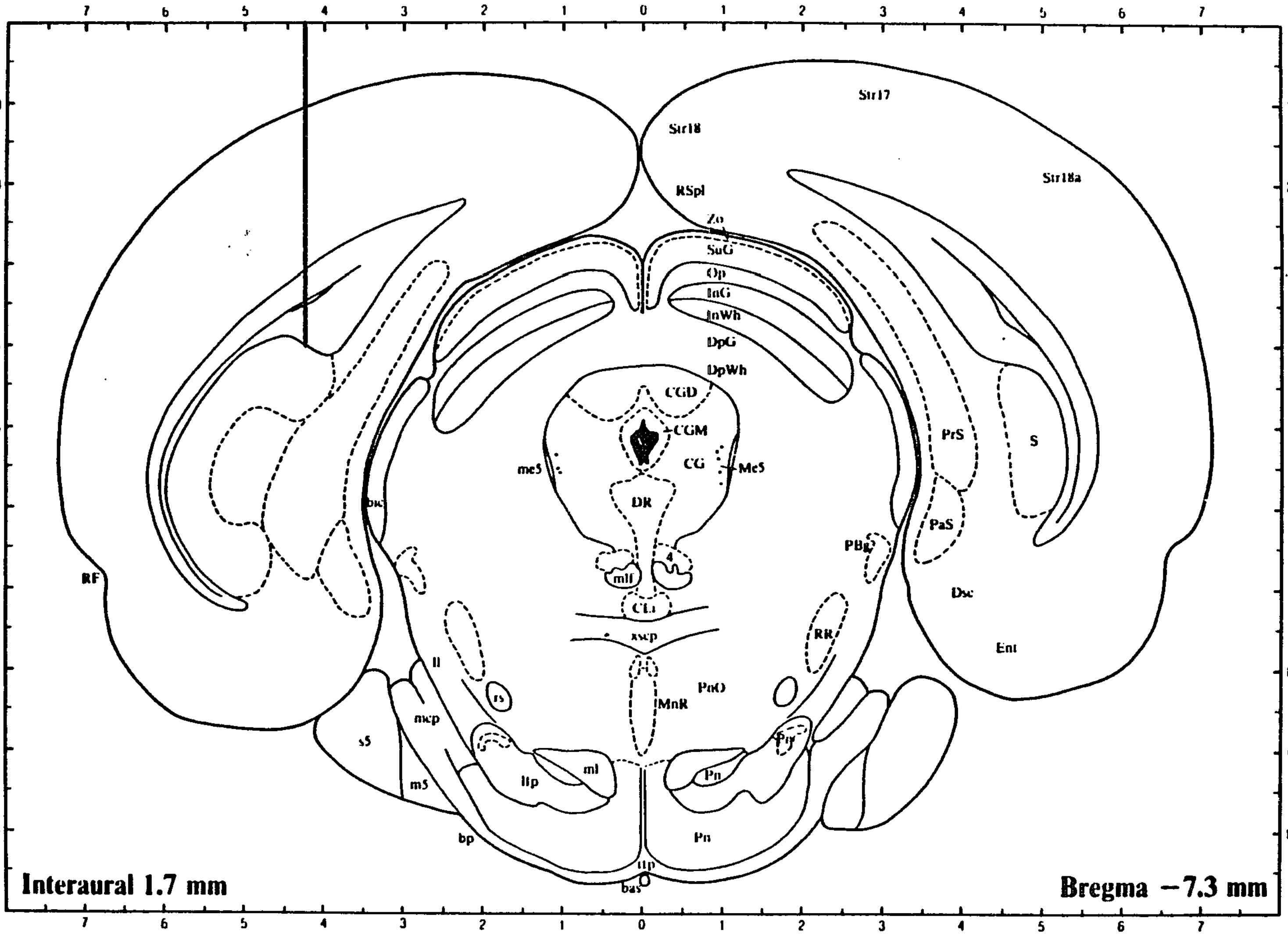

Str17
Str18
Str18a
RSpl
Zo
SuG
Op
InG
InWh
DpG
DpWh
CGD
CGM
CG
Me5
me5
DR
PrS
S
PaS
PBg
Dsc
Ent
RF
bic
mlf
4
CLi
xscp
RR
ll
PnO
MnR
rs
ncp
s5
m5
ml
tfp
Pn
bp
bas
Interaural 1.7 mm
Bregma −7.3 mm

Figure 13b

Figure 14 a) A photomicrograph showing a green spot left at the tip of the recording electrode in the dorsal blade of the dentate gyrus. Magnification: x 40. Scale: 260 uM. b) A schematic illustration of the location of the recording electrode, taken from Paxinos & Watson (1982). The vertical line indicates the location of the electrode.

Abbreviations:

3V - third ventricle
AF- amygdaloid fissure
AHi - amygdalohippocampal area
alv - alveus
APTD - ant pretectal area, dorsal
APTV - ant pretectal area, ventral
BL - basolateral amygdala nucleus
bsc - brachium superior colliculus
cg - cingulum
cp - cerebral peduncle, basal
df - dorsal fornix
dhc - dorsal psalterium
Dk - nucleus of Darkschewitsch
DLG - dorsal lateral geniculate
Ent - entorhinal cortex nucleus
fr - fasciculus retroflexus
FrPaSS - frontoparietal SS cortex
GEM - gemini nuclei
hbc - habenular commissure
Hif - hippocampal fissure
IMCPC - interstitial nuc post comm
InC - interstitial nuc Cajal
IntG - intermediate geniculate
LM - lateral mamillary nuc
MG - medial geniculate
ML - med mamillary nuc, lat
ml - medial lemniscus
MM - med mamillary nuc, med
MP - med mamillary nuc, post
mp - mamillary peduncle
mt - mamillothalamic tract
mtg - mamillotegmental tract
OPT - olivary pretectal nuc
opt - optic tract
OT - nuc optic tract
pc - posterior commissure
PCg - post cingulate cortex
PMCo - posteromedial amygdala
Po - posterior thalamus
PP - peripeduncular nucleus
PR - prerubral field
RF - rhinal fissure

Abbreviations (Continued from previous page)

S - subiculum

scc - splenium corpus callosum

SCO - subcommissural organ

scp - sup cerebellar peduncle

SG - supragenículate thalamus

SNC - substantia nigra, compacta

SNR - substantia nigra, reticulada

str - superior thalamus radiation

SuM - supramamillary nucleus

sumx - supramamillary decussation

VLGMC - vent lat geniculate nuc, magnocellular

VLGPC - vent lat geniculate nuc, parvocellular

VTA - ventral tegmental area

ZI - zona incerta

Figure 14a

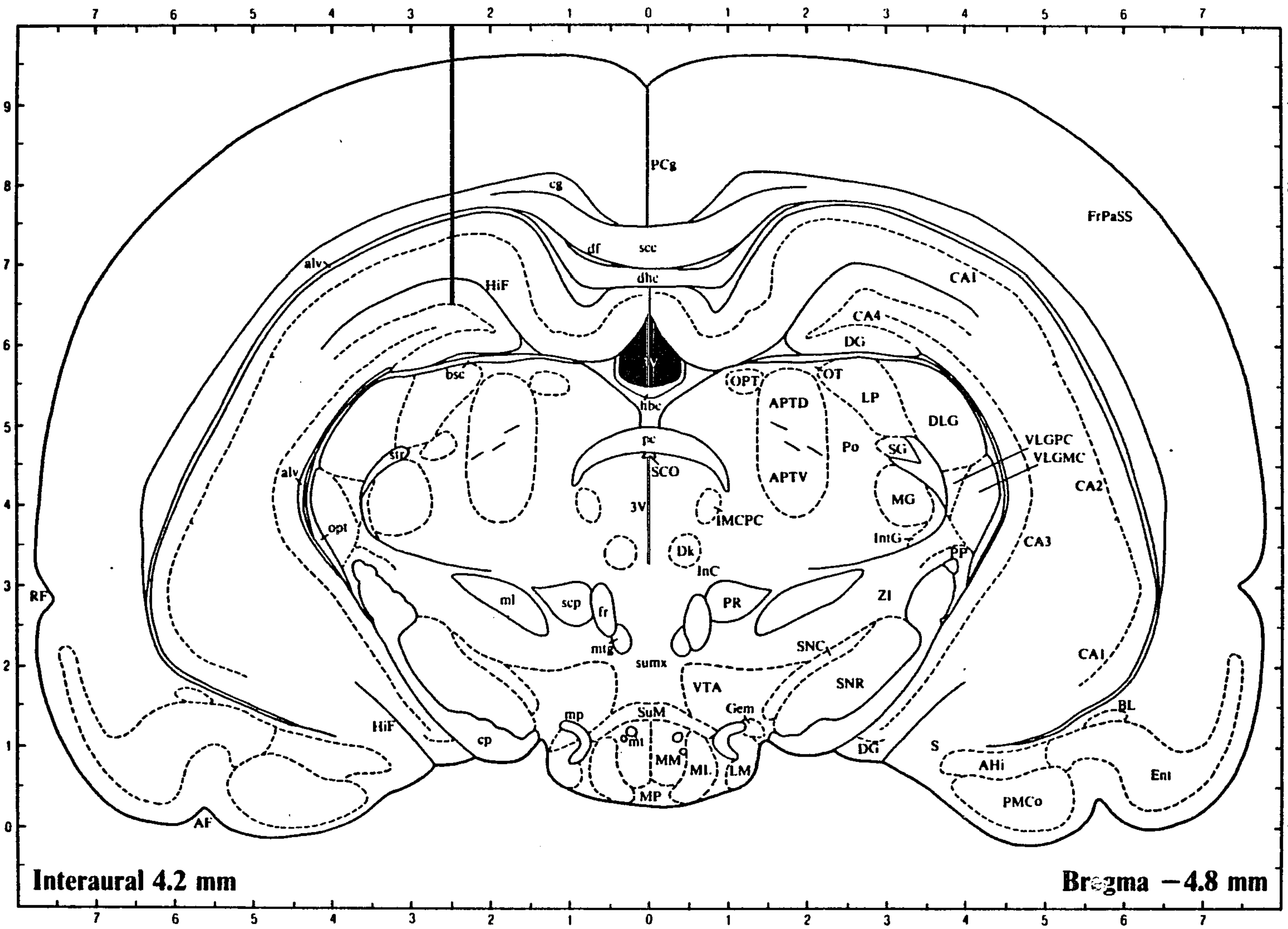

PCg
cg
FrPaSS
df
scc
dhc
alv
HiF
CA1
CA4
DG
bsc
hbc
OPT
OT
APTD
LP
DLG
Po
SG
VLGPC
VLGMC
pc
SCO
APTV
CA2
str
alv
3V
IMCPC
MG
opt
Dk
IntG
CA3
InC
PP
RF
ml
scp
fr
PR
ZI
mtg
SNC
sumx
CA1
VTA
SNR
BL
HiF
mp
SuM
Gem
cp
mt
MM
ML
LM
DG
S
AHi
Ent
MP
PMCo
AF
Interaural 4.2 mm
Bregma −4.8 mm

Figure 14b

significance level. Figure 15 illustrates the results of the experiment. Figures 16 and 17 present data from a characteristic subject.

Discussion

The present results, in conjunction with those reported previously in the CA1 field, indicate that a temporal component exists in the induction of LTP in both CA1 and the DG, the two hippocampal fields in which theta rhythm is generated. For priming to be effective, the tetanic stimuli must follow the priming stimulus by approximately 200 msec (5 Hz). The effect obtained in this experiment is more striking than Larson and Lynch's (1986) finding. In their study, frequencies of 1000 and 100 msec also induced LTP, while in our study, a priming interval of 100 msec had no effect. One possible reason for the discrepancy in the results, in addition to the differences in the hippocampal region (CA1 vs. DG) and type of preparation (slice vs anesthetized animal) is that the stimulation intensity in their study was kept constant, while in the present experiment, the initial intensity was very weak, and was incremented by small steps, until LTP was evident. Such an incremental approach may be more suitable for finding the LTP threshold.

The cellular mechanisms underlying the priming effect are largely unknown. However, several hypotheses have been proposed in an attempt to explain this phenomenon. Most hypotheses have concentrated on the possible effect of the priming pulse on the removal of inhibition from the NMDA channel. These approaches have originated from the finding that the removal of GABA inhibition, using picrotoxin, has greatly facilitated LTP induction. It has been suggested that the post synaptic

Table 1

Mean Percent Potentiation That Took Place in Each of the Priming Intervals

Priming Interval	% Potentiation of the Slope	% Potentiation of the Spike
50 msec		
M	0.77	4.65
SEM	0.52	2.00
100 msec		
M	0.47	0.99
SEM	0.32	0.80
200 msec		
M	11.6*	31.7*
SEM	6.2	10.4
350 msec		
M	0.91	1.80
SEM	0.65	1.33
500 msec		
M	1.58	2.47
SEM	1.27	1.63

* - $p < .01$

Figure 15 A histogram illustrating the percent potentiation of both slope and spike of all the animals in the different priming intervals. Note that significant potentiation of both slope and spike took place only at the 200 msec. interval, which corresponds to 5 Hz. The arrowheads indicate the application of tetanic stimulation, preceded by the Prime.

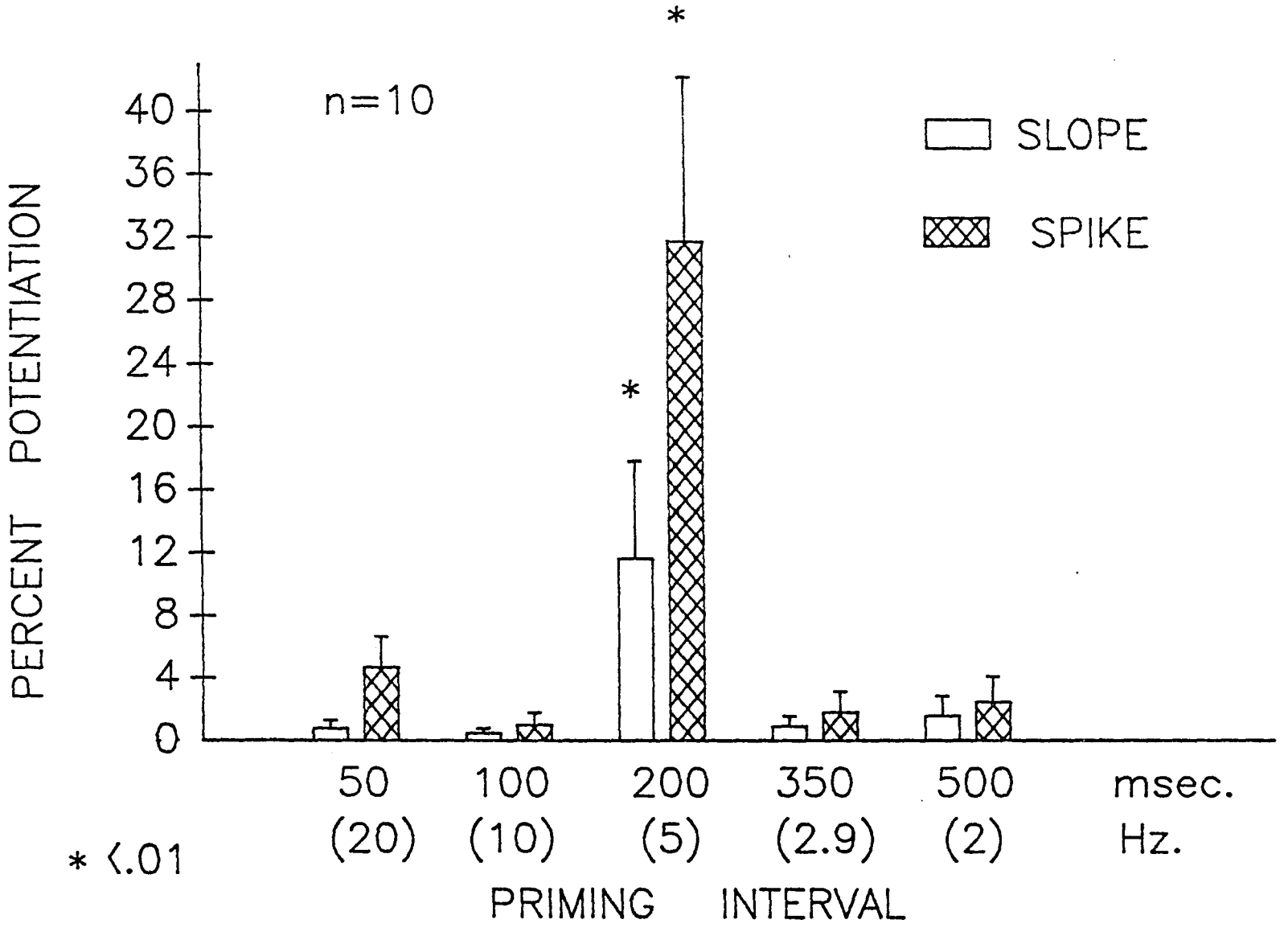

n=10
SLOPE
SPIKE
PERCENT POTENTIATION
40
36
32
28
24
20
16
12
8
4
0
*
*
50
(20)
100
(10)
200
(5)
350
(2.9)
500
(2)
msec.
Hz.
* <.01
PRIMING INTERVAL

Figure 15

Figure 16 Illustration of the slope data from a characteristic subject.

A significant potentiation of slope of the ESP (63%) took place only at the priming interval of 200 msec, which corresponds to 5 Hz. The arrow heads indicate the application of tetanic stimulation, preceded by the Prime.

Figure 16

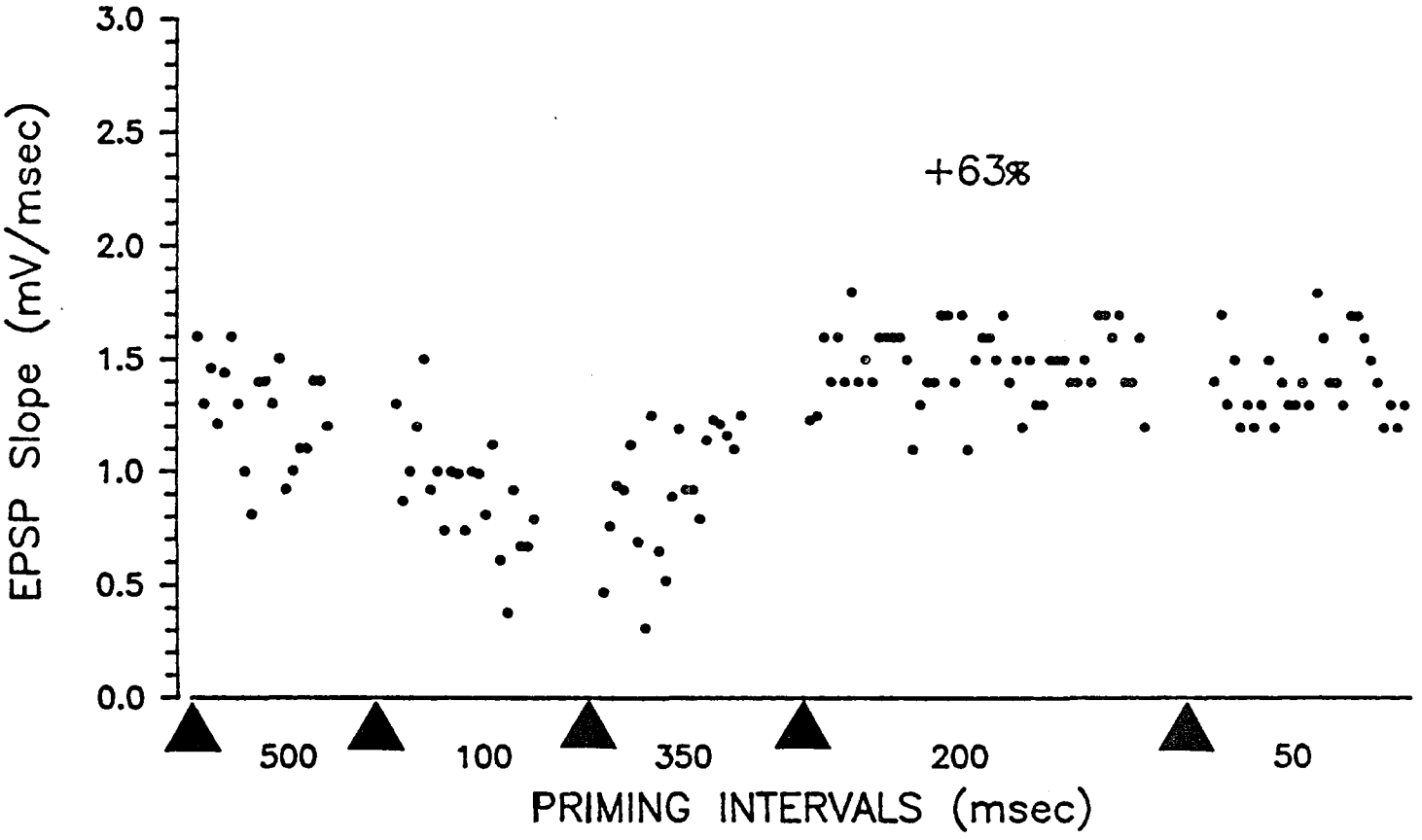

3.0
2.5
2.0
1.5
1.0
0.5
0.0
EPSP Slope (mV/msec)
+63%
500
100
350
200
50
PRIMING INTERVALS (msec)

Figure 17 Illustration of the population spike amplitude data from the same subject depicted in Figure 16.

A significant potentiation of the amplitude of the population spike (105%) took place only at the priming interval of 200 msec, which correspond to 5 Hz. The arrow heads indicate the application of tetanic stimulation, preceded by the Prime.

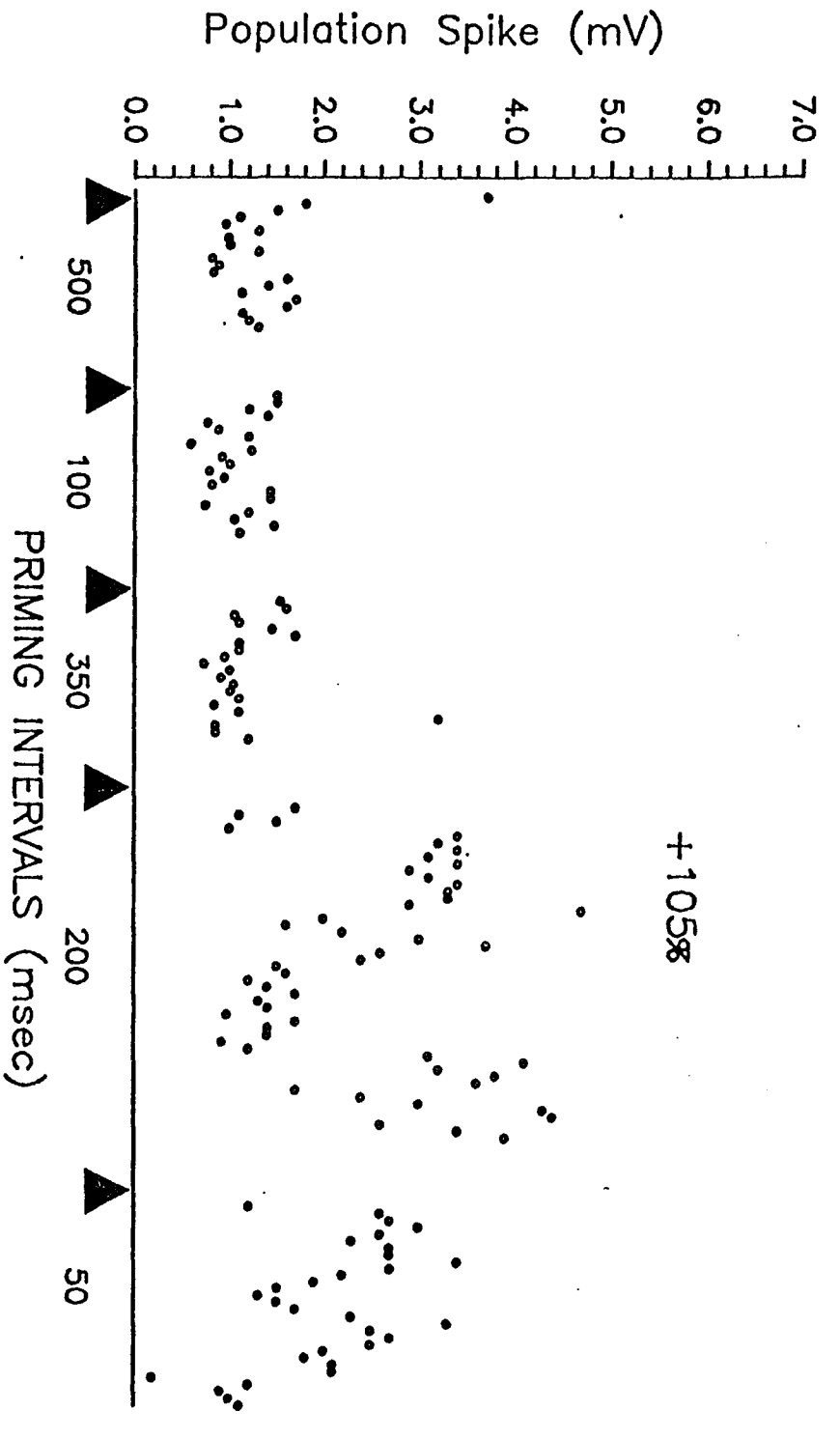

Population Spike (mV)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
500
100
350
200
50
PRIMING INTERVALS (msec)
+105%

Figure 17

depolarization induced by the Prime, acts to remove inhibitory influences from the post synaptic cell, thereby facilitating LTP induction by the arriving tetanic train. This form of inhibition may be provided by Mg^{2+} ions that exist in the vicinity of the post synaptic NMDA channel. As was mentioned previously, removal of these ions facilitates the activation of the NMDA channel, and increasing their concentration blocks LTP induction (Huang, Wigstrom and Gustafsson, 1987). The involvement of NMDA channels in the priming effect in the CA1 field was suggested by the fact that the priming effect could be blocked by APV, which is an NMDA channel blocker (Larson and Lynch, 1987; Rose and Diamond, 1987). The importance of post synaptic depolarization for LTP development was highlighted in several studies. LTP could be induced by pairing a single, depolarizing pulse with a short tetanic train. In CA1 slices washed with the GABA antagonist picrotoxin (to lower the effect of the inhibitory interneurons), pairing a depolarizing pulse with a short tetanic train (2-15 pulses at 50 Hz) administered to a different set of afferents that converge on the same cell population in s. radiatum, induced potentiation of the field potential elicited by the pulse. The pulse had to appear in conjuction with the train or before it, but no more than 40 msec earlier. The maximal LTP appeared if the pulse came at the beginning of the train (Gustafsson and Wigstrom, 1986). In a second experiment, single afferent pulses were administered alternately (at 3.5 sec intervals) to two separate sets of afferents that project to the same cell population in s. radiatum. A depolarizing current pulse (5-8 nA, 100 msec width) was administered periodically during the experiment. If the depolarizing current was given 400 msec after the afferent

stimulus, no effect on the field potentials was observed. If the depolarizing pulse was applied just after the peak of the intracellular EPSP, LTP developed (Wigstrom, Gustafsson, Huang and Abraham, 1986). It was thus concluded that a tetanic train is not needed to induce LTP if the post synaptic membrane is sufficiently depolarized. This LTP was blocked by APV, indicating that NMDA receptors were involved (Gustafsson, Wigstrom, Abraham and Huang, 1987). These experiments led to the conclusion that local, post-synaptic depolarization of the dendrites is important for the production of LTP (Wigstrom, Gustafsson and Huang, 1985). According to these studies, LTP is produced if there is a transmitter release coupled to post synaptic depolarization. If post synaptic depolarization is absent, LTP will not be produced. Wigstrom and Gustafsson (1986) therefore suggested that the NMDA receptor is an associative device- it must decide whether simultaneous appearance of pre synaptic transmitter release and post depolarization is taking place. The NMDA channels will open only if these two events occur concurrently or within a narrow time window, and when the channels open Ca^{2+} ions enter and contribute to LTP development.

How do these findings and ideas account for the priming effect? Post synaptic depolarization is required to remove the inhibitory Mg^{2+} ions from the NMDA channel (Collingridge and Bliss, 1987). It is possible that the priming pulse causes depolarization that acts to remove the Mg^{2+} inhibition from the NMDA channel, allowing Ca^{2+} to enter (Jahr and Stevens, 1987). This results in the reduction of the threshold for LTP development by the tetanic train that was arriving later. But why is the 200 msec the optimal priming interval? The time course of

development of an EPSP in the NMDA channel is slower and more prolonged than a non-NMDA channel. While an EPSP in a non-NMDA channel rises within 1-3 msec and decays after 10-50 msec, the NMDA EPSP rises within 20-25 msec and decays after 200-300 msec (MacDermott and Dale, 1987). During the opening of the channel, both Ca^{2+} and Na^{+} ions enter the cell, while only Na^{+} ions enter the non-NMDA glutamate channel (Cotman and Iversen, 1987). The slower time course of the NMDA channel might account for the fact that LTP occurred in the present experiment only at the 200 msec interval. Administering the train at 50 or 100 msec after the prime may not allow enough build-up of Ca^{2+} ions within the cell. Administering the train 350 or 500 msec after the prime probably requires higher intensities to induce LTP, as compared to the threshold for LTP induction, because the NMDA channels are partially or totally closed at that time. NMDA receptors have been documented in the DG (Cotman, Monaghan, Otterson and Storm-Mathisen, 1987), and it remains to be shown whether priming in the DG is mediated by these receptors.

Central to the understanding of the possible functional role of priming is the question whether priming conditions, shown in this experiment and other studies to induce LTP, are present in the freely moving animal. Theta rhythm is generated in the entorhinal cortex, the site of origin of the PP. The firing of cells in the EC has been found to be phase locked to theta rhythm (Quirk and Ranck, 1986). It is not known, however, whether these cells project to the DG. Further study is required to determine if the PP transmits action potentials at theta rhythm periodicity, thus providing an input to the DG corresponding to priming stimuli used in our experiment. In the DG itself, repetitive bursts of granule cell action potentials uniformly accompany and are

phase locked to theta rhythm (Rose, 1983) during voluntary movement and REM sleep. From the DG, bursts of granule cell action potentials at theta rhythm periodicity may then be communicated to the CA3 and CA1 fields of the trisynaptic circuit. This could allow further synaptic modification such as has been demonstrated in response to priming stimuli in the CA1 field (Larson and Lynch, 1986; Larson, Wong & Lynch, 1986; Rose and Dunwiddie, 1986). The successful induction of LTP using patterned stimuli lends further support for the physiological relevance of LTP, and suggests that theta rhythm is intimately involved in this form of plasticity.

6. Experiment 2 - The Relationship Between Theta Phase and LTP*

Introduction

The first experiment showed that tetanic stimulation, administered to the dentate gyrus afferents at theta rhythm periodicity, preferentially induced LTP. This finding suggests the oscillatory post-synaptic potentials, that are believed to change the membrane potential in a sinusoidal way, and consequently to generate the extracellularly recorded theta rhythm (see "Hippocampal Theta Rhythm"), may serve to modulate cell firing so that it occurs at theta rhythm periodicity, either in the entorhinal cortex or the dentate gyrus itself. Firing at such a periodicity preferentially induces LTP in both the dentate gyrus and CA1 field.

Theta rhythm may also contribute to LTP in a different way. As discussed previously, theta periodicity might be related to the time course of activation of the NMDA channel, which is involved in LTP in CA1 field. The NMDA channel might serve as a time-dependent cellular mechanism that facilitates LTP induction if activated 200 msec after an intracellular depolarization. Also discussed previously is the contribution of intracellular depolarization, preceding or given concurrently with a tetanus, to the threshold reduction of LTP (see the Introduction.) This raises the possibility that favorable conditions for LTP formation exist if the membrane is depolarized periodically, at intervals of 200 msec, and a tetanic stimulation follows the depolarization within the 200 msec time window. Such rhythmic depolarization might be supplied by the naturally occurring theta

---

* The data in this study was collected in collaboration with C. Pavlides and appeared in Pavlides, Greenstein, Grudman & Winson (1988).

rhythm. It is also possible that the phase of theta rhythm, with the intra-cellular events that are associated with it, contributes to the formation of LTP. The present experiment will investigate whether tetanic stimulation, administered in conjuction with theta rhythm, will induce LTP in the dentate gyrus.

Materials and Method

Subjects:

The experiment was performed on twenty six male Sprague-Dawley rats (Charles River), ranging in weight between 250-280 g. The rats were housed in a colony room (three per cage) for at least a week before the experiment, on a 12-12 hours light-dark cycle. Food and drink were given ad lib.

Surgery:

The animals were anesthetized with urethane (1.7 g/kg). The recording and stimulating electrodes were placed in the dentate gyrus and perforant path, respectively, as described in Experiment 1. As suggested by Vertes (1986), theta rhythm was induced by stimulating the midbrain and a 4-5 mm burr hole was drilled in the right hemisphere at coordinates AP 5.5 and ML 1.5 to introduce the midbrain stimulating electrode. A monopolar stimulating electrode, similar to the one used to stimulate the PP, was used to stimulate the midbrain. The final depth was adjusted to induce stable theta rhythm. The peak-to-peak interval of the rhythm was measured. Two additional holes were made in the right and left frontal bones, for the placement of a ground screw and an indifferent electrode for recording. In the right parietal bone two holes were made for ground screws for the return paths for the

stimulation currents of the midbrain and the perforant path.

Stimulation and Recording:

The signals were amplified by an AC differential pre-amplifier (gain of 100). Cut-off frequencies for the field potentials were set at 3 Hz and 3 KHz, and at 3-20 Hz for EEG. All recordings were made in an electrically shielded chamber (120 x 118 x 70 cm).

Extracellular field potentials were evoked in the dentate gyrus by stimulation of the perforant path by squared pulses of 0.25 msec duration ("Test Stimulus"), delivered via a photo cell stimulus isolator (Grass). The intensity of the Test Stimulus was chosen to be approximately 50% of saturation current. LTP was induced by 10 bursts of tetanic stimuli each consisting of 5 pulses with an interpulse interval of 2.5 msec (400 Hz), and a pulse duration of 0.15 msec ("LTP Train"). The inter-train interval was 200 msec when they were delivered alone, or approximately 200 msec when delivered concurrently with theta rhythm (see below). Midbrain stimulation was obtained by administering a train of pulses at a pulse duration of 0.20 msec and interpulse interval of 10 msec (100 Hz) ("Theta Stimulus"). Train duration was selected that would elicit ten successive cycles of theta rhythm.

The field potential and EEG signals from the animals were each fed into separate storage oscilloscopes. In addition the EEG was fed into a theta control device (Electronics Dept., Rockefeller University, TCD), consisting of a filter (3-20 Hz), adjustable voltage discriminator and an output trigger used to activate the perforant path stimulator (Grass S11). The filter reduced frequencies in the EEG that were outside of the theta rhythm range, allowing for maximum stability and clarity of

the theta rhythm signal generated by midbrain stimulation. The voltage window could be adjusted to any desired height on the theta rhythm signal allowing the output trigger to be displayed at that voltage level on the theta signal (See Figure 18). As will be seen below, the trigger was adjusted to either the rising or falling phase of the theta wave. Activation of the trigger sent a TTL signal (± 5V) to the stimulator and a tetanic train was administered to the perforant path. Both the EEG signal and the tetanic train were monitored via the two oscilloscopes. Figure 18 shows the hippocampal EEG and the trigger imposed on the positive phase of the theta rhythm by the TCD. Field potentials were displayed on a microprocessor display (Rockefeller University, Electronics dept.), allowing the measurement of the slope of the ESP, and were stored on magnetic disks for later analysis of the population spike. Figure 19 illustrates the experimental configuration schematically.

Design and Procedure

The experiment began by determining the minimum current required to elicit a field response and a population spike, and a saturation current, above which no further change in either the slope or spike was induced. Test current was chosen to elicit a medium size spike (1-2 mV; approximately 50% of saturation current), at a pulse width of 0.25 msec. Input / Output (I/O) curve was obtained by pulses administered every 15 sec at selected intensities (pulse width of 0.25 msec). The amplitudes of the I/O curve pulses were chosen to range from the minimal intensity required to elicit a response up to saturation current.

After establishing an I/O curve, baseline measurements of the field

response were taken for a period of twenty minutes by applying the Test Stimulus every thirty seconds. After obtaining a baseline, two successive control conditions followed (Conditions 1 and 2).

Condition 1 began by administering the LTP Stimulus without inducing theta rhythm. Then, following the administration of Condition 1, testing for changes of the ESP slope and population spike was continued, using the Test Stimulus, as was done during the baseline session. The objective in each experiment was to select an initial intensity of LTP Stimulus below the threshold for the induction of LTP in Condition 1. Condition 2 consisted of midbrain stimulation by the Theta Stimulus in order to induce 10 cycles of theta rhythm, but without tetanic stimulation applied to the perforant path. Again, the Test Stimulus was administered, as described previously, to evoke the field responses and to test for the existence of LTP. Condition 3 was administered to half of the animals, while Condition 4 was administered to the other half in a random order. Condition 3 consisted of administering Theta Stimulus for producing theta rhythm and LTP Stimulus imposed on the positive peak of ten successive cycles of stimulus-generated theta rhythm. Condition 4 consisted of a similar LTP Stimulus, imposed on the negative peak of theta rhythm. Testing the field responses using the Test Stimulus after tetanization was performed as described before. If LTP was induced at this stage or at any previous stage, testing continued for 30 min. If no change was detected, the LTP Current was increased and the entire procedure was repeated. The experiment was considered complete when LTP was induced at either Condition 3 or 4, and then final I/O curves were obtained, as

Figure 18 Theta rhythm induced by midbrain stimulation. Shown is the triggering pulse generated by the TCD, in this case at the positive peak of theta rhythm. When duplicate pulses appear on a single theta wave, only the first is operative. As shown, on occasion the TCD was not triggered by a low amplitude theta wave. In this case an additional theta wave was automatically triggered at the end of the train. Calibrations, 1 box 0.5 mV vertical, 0.2 sec horizontal, positivity upward. The arrowhead points to the beginning of the midbrain stimulation. Note that the irregular EEG is synchronized to produce 5-6 Hz theta rhythm.

Theta rhythm was successfully induced by stimulation of the midbrain in the majority of the animals. The rhythm produced was strictly concurrent with the application of the stimulating current. It occurred at approximately 5-6 Hz, and appeared in all respects to be similar to the theta rhythm generated in the freely moving rat.

Figure 18

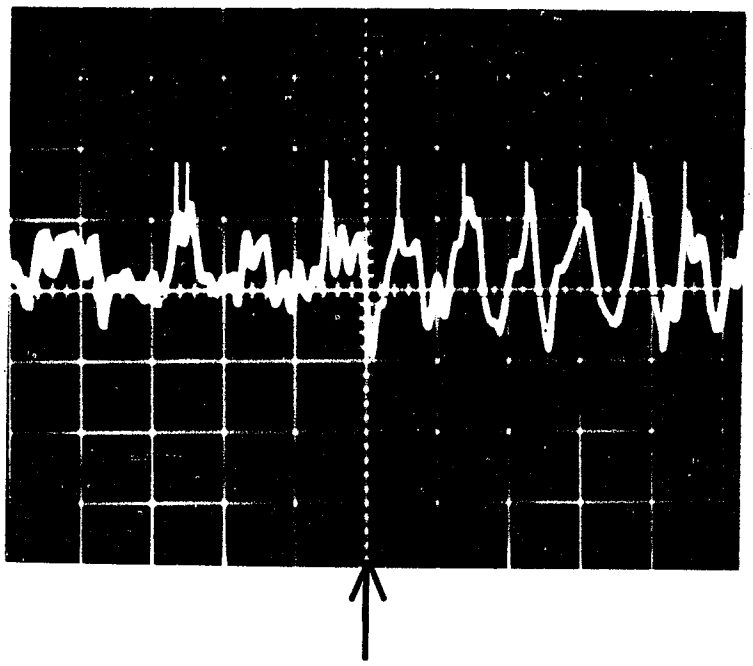


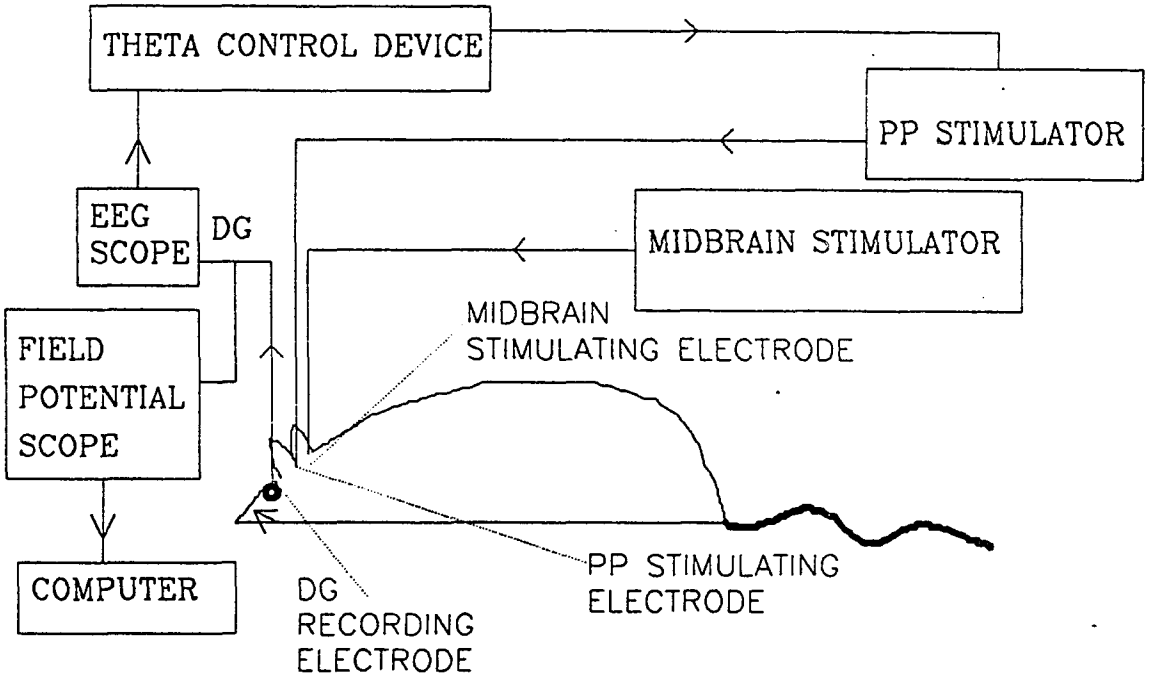
Figure 19 Schematic drawing illustrating the configuration of experiment 2. As described in the text, theta rhythm was induced by midbrain stimulation and the EEG signal was fed into the Theta Control Device (TCD). The TCD activated the perforant path stimulator, which applied tetanic stimulation in order to induce LTP. Field potentials were recorded from the dentate gyrus and displayed on a scope and a computer display. Analysis of the slope of the ESP was done on-line. See Figure 12C for the position of the recording and PP stimulation electrodes in the brain.

Abbreviations:

DG - dentate gyrus

PP - perforant path

Figure 19


THETA CONTROL DEVICE
PP STIMULATOR
EEG SCOPE
DG
MIDBRAIN STIMULATOR
FIELD POTENTIAL SCOPE
MIDBRAIN STIMULATING ELECTRODE
COMPUTER
DG RECORDING ELECTRODE
PP STIMULATING ELECTRODE

described previously. Figure 20 describes the experimental design. At the conclusion of the experiment, the animal was killed by an overdose of Nembutal.

Results

Similarly to Experiment 1, histological analysis was carried out by the end of the experiment. The same coordinates that were used in Experiment 1 to stimulate the perforant path and record from the dentate gyrus were also used in the present experiment. See Figures 13 and 14 for the approximate location of these electrodes. Figure 21 shows a characteristic slide of the midbrain electrode that was used to induce theta rhythm.

A total of twenty-six rats were run in the experiment. In all, twelve animals were discarded due to instability, unreliable theta and due to an initial selection of LTP Stimuli that induced LTP during Condition 1, preventing the evaluation of the effect of tetanic stimulation applied on either the positive (Condition 3) or negative peak (Condition 4) of theta rhythm. Of the remaining fourteen animals, seven were run in Condition 3 and seven were run in Condition 4 with definitive results.

In the rats where the LTP Stimulus was applied at the positive peak of theta rhythm (Condition 3), successful potentiation of both ESP slope and population spike was induced only in Condition 3 in five animals. The slope was potentiated in two more rats, without accompanying significant changes in the spike (due to excessive scatter). The potentiation of the slope averaged 8.7% ± 4.2 s.e.m.

Figure 20 The design of Experiment 2. Baseline measurements were taken at the beginning of the experiment, followed by the application of tetanic stimulation to the perforant path (Condition 1). Then, midbrain stimulation was performed in order to induce theta rhythm (Condition 2). Conditions 3 and 4 followed, where tetanic trains were applied either at the positive (Condition 3) or negative peak (Condition 4) of theta rhythm. Half of the animals were run in Condition 3 and the other half were run in Condition 4. The Test Stimulus was administered to establish baseline, and following each of the conditions 1 through 4, to measure the induction of LTP. I/O curves were obtained at the beginning and end of the experiment.

Figure 20

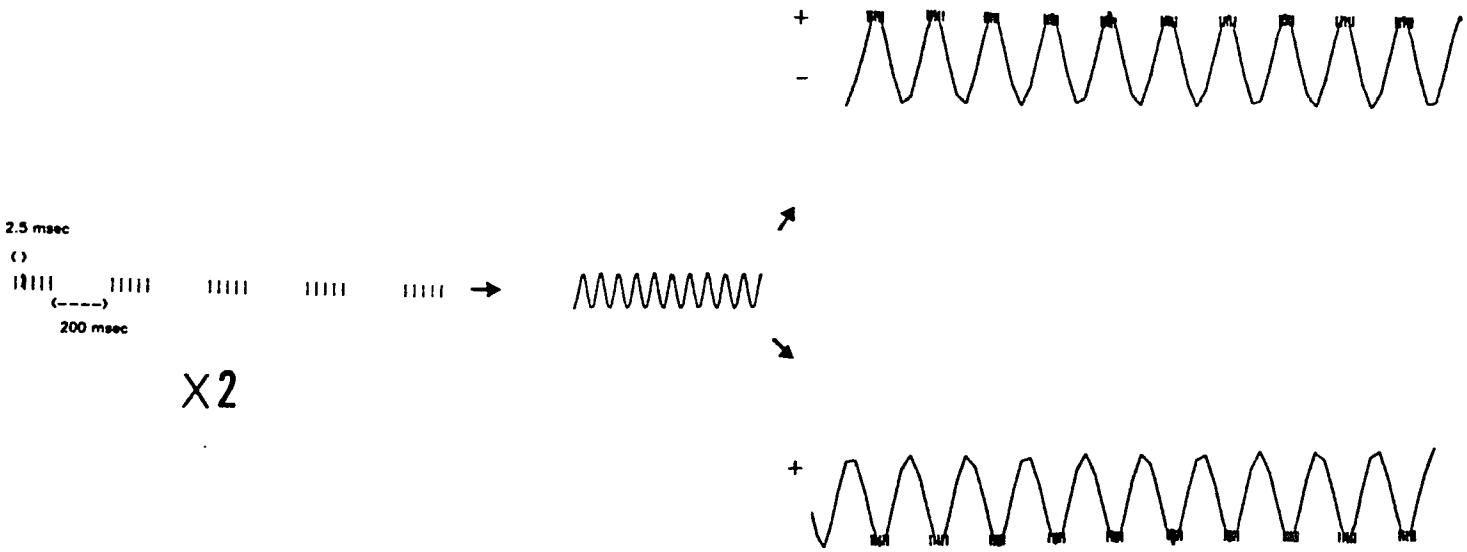


Figure 21 a) A photomicrograph showing the track of the electrode that was positioned in the midbrain to induce theta rhythm. Magnification: x 40. Scale: 260 uM. b) A schematic illustration showing the approximate location of the theta electrode (From Paxinos & Watson, 1982). The vertical line indicates location of the electrode. Abbreviations:

4- trochlear nucleus
Aq - cerebral aqueduct
bas - basilar artery
bic - brachium inf colliculus
bp - brachium pontis
CG - central gray
CGD - central gray, dorsalis
CGM - central gray, medialis
CLi - caudal nucleus raphe
DpG - deep gray layer sup colli
DpWh - deep white sup colli
DR - dorsal raphe
Dsc - lamina dissecans EC
Ent - entorhinal cortex
InG - intermed gray sup colli
InWh - intermed white sup colli
lfp - longitudinal fasciculus
ll - lateral lemniscus
m5 - motor root trigeminalis
mcp - middle cerebral peduncle
Me5 - nuc mesenceph trigeminal
ml - medial lemniscus
mlf - med longitudinal fasciculus
MnR - median raphe
Op - optic nerve layer sup colli
Pas - parasubiculum
PBg - parabigheminal nucleus
Pn - pontine nuclei
PnO - pontine reticular nuc oral
Prs - presubiculum
RF - rhinal fissure
RR - retrorubral nucleus
rs - rubrospinal tract
RSpl - retrosplenial cortex
S - subiculum
s5 - sensory root trigeminalis
Str17 - striate cortex 17
Str18(a) - striate cortex 18(a)
SuG - superficial gray sup colli
tfp- transverse fibers pons
xscp - decuss sup cerebell peduncle
Zo - zonal layer sup colliculus

Figure 21a

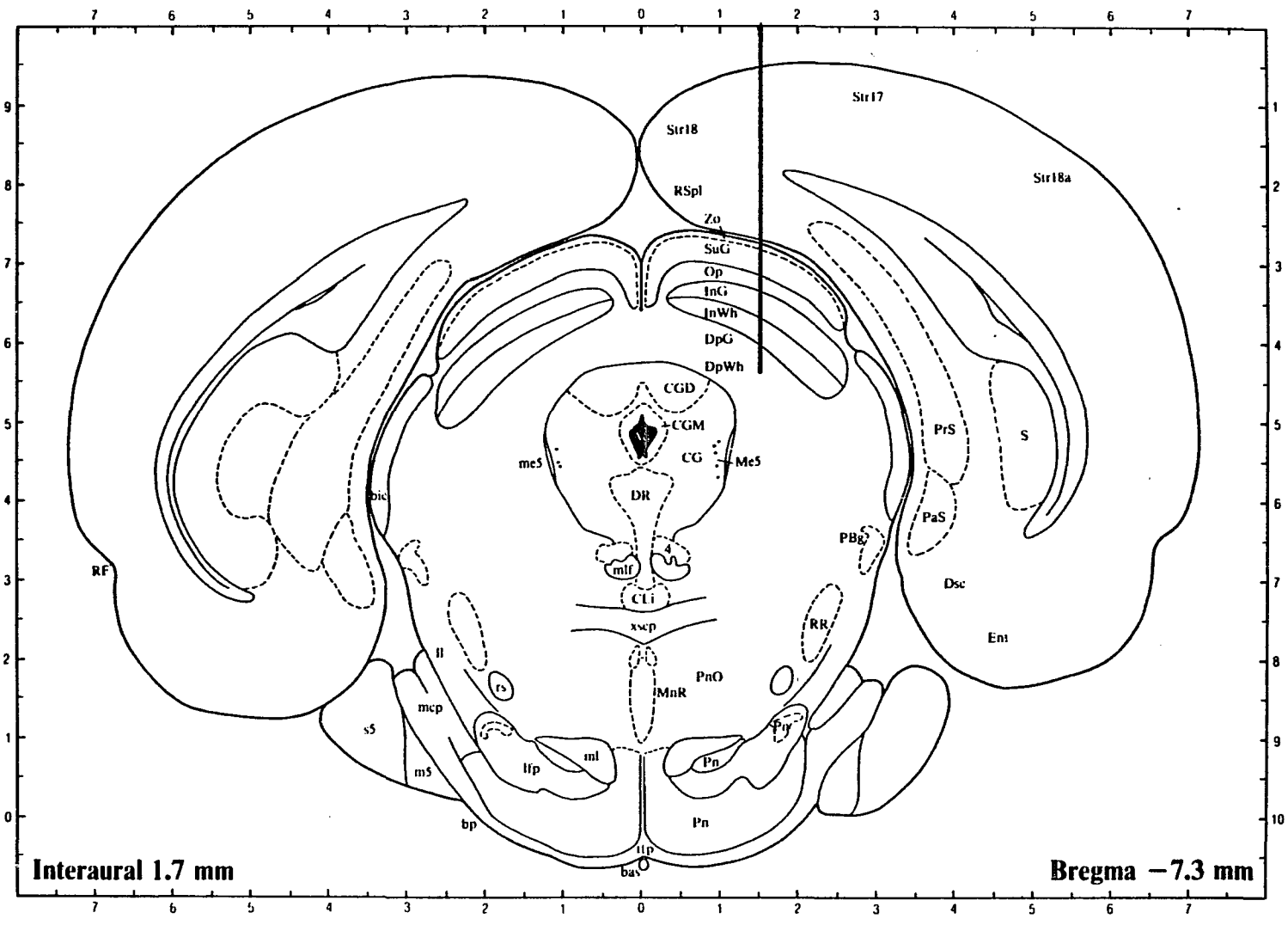

Str17
Str18
Str18a
RSpl
Zo
SuG
Op
InG
InWh
DpG
DpWh
CGD
CGM
CG
Me5
me5
DR
PrS
S
PaS
PBg
RF
mlf
CLi
xscp
RR
Dsc
Ent
ll
PnO
MnR
mcp
s5
m5
lfp
ml
Pn
bp
Interaural 1.7 mm
Bregma −7.3 mm

Figure 21b

relative to the previous Conditions 1 and 2, *T*-test= 4.9, df=6, p< .003, two-tailed. Figures 22a and b demonstrate typical records of the field responses of an animal that was run in Condition 3. In this animal, there were no significant changes in either slope or spike in Condition 1 and 2 as compared to the baseline. A significant potentiation of both slope of the ESP and amplitude of the population spike took place only in Condition 3, (p< .001). In the particular experiment illustrated, the procedure was repeated at a higher current (280 uA) and there was further significant increase only in Condition 3. The population spike was also potentiated in five rats, average potentiation being 29.7% ± 22.9 s.e.m., *T*-test= 2.9, df=6, p< 0.03, two tailed. Figures 23a and b describe typical I/O curves of the slope and spike before and after potentiation.

Considering the rats in which the tetanic stimulation was applied at the negative phase of theta (Condition 4), the LTP Stimulus intensity was increased in successive runs due to an absence of a significant change in Condition 4, as compared to the preceding baseline and conditions 1 and 2, until all rats have shown LTP of both slope and spike in Condition 1. Applying the LTP Stimulus again at the negative phase of theta (Condition 4) had caused depression of both slope and spike in two rats. The average depression of the slope was -8.7% ± 3.8 and of the population spike it was -12% ± 8.5. In the 4 remaining rats in this group there was no further change in Condition 4 despite the fact that the tetanic stimulation was reapplied as a normal component of Condition 4. The seven rats taken as whole showed no significant change in Condition 4 from the previous conditions 1 and 2 in either slope or spike. To be certain that saturation of LTP was not reached

Figure 22 A characteristic record of field potential data of an animal that was run in Condition 3 (tetanic stimulation applied on the positive peak of theta rhythm). There were no significant changes in either slope of the ESP or amplitude of the population spike in either Condition 1 (the application of tetanic stimulation to the perforant path without theta rhythm) or Condition 2 (the induction of theta rhythm in the hippocampus by midbrain stimulation) as compared to baseline. The application of tetanic stimuli at the positive peak of theta rhythm (Condition 3) produced reliable LTP of both slope and spike of the field response. In this animal, the LTP Stimulus was further increased, and potentiation was evident again only in Condition 3.

a) The slope of the ESP.

b) Amplitude of the population spike.

Abbreviations: PRE - baseline C-1 - Condition 1

C-2 - Condition 2 C-3 - Condition 3

Filled arrowhead: LTP Stimulus

Empty arrowhead: Theta Stimulus

Figure 22a

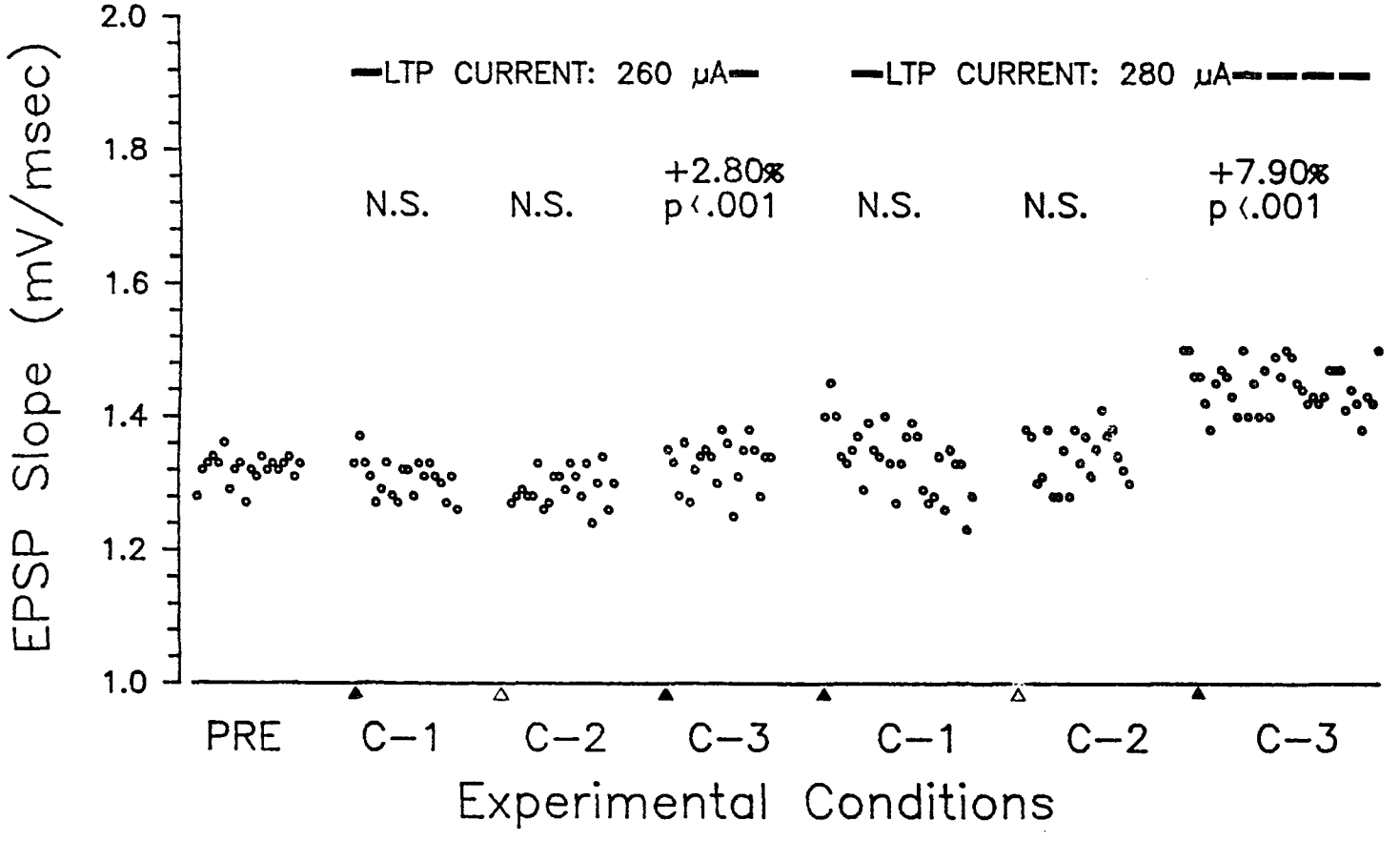

EPSP Slope (mV/msec)
2.0
1.8
1.6
1.4
1.2
1.0
LTP CURRENT: 260 µA
LTP CURRENT: 280 µA
N.S.
N.S.
+2.80%
p <.001
N.S.
N.S.
+7.90%
p <.001
PRE
C-1
C-2
C-3
C-1
C-2
C-3
Experimental Conditions

Figure 22b

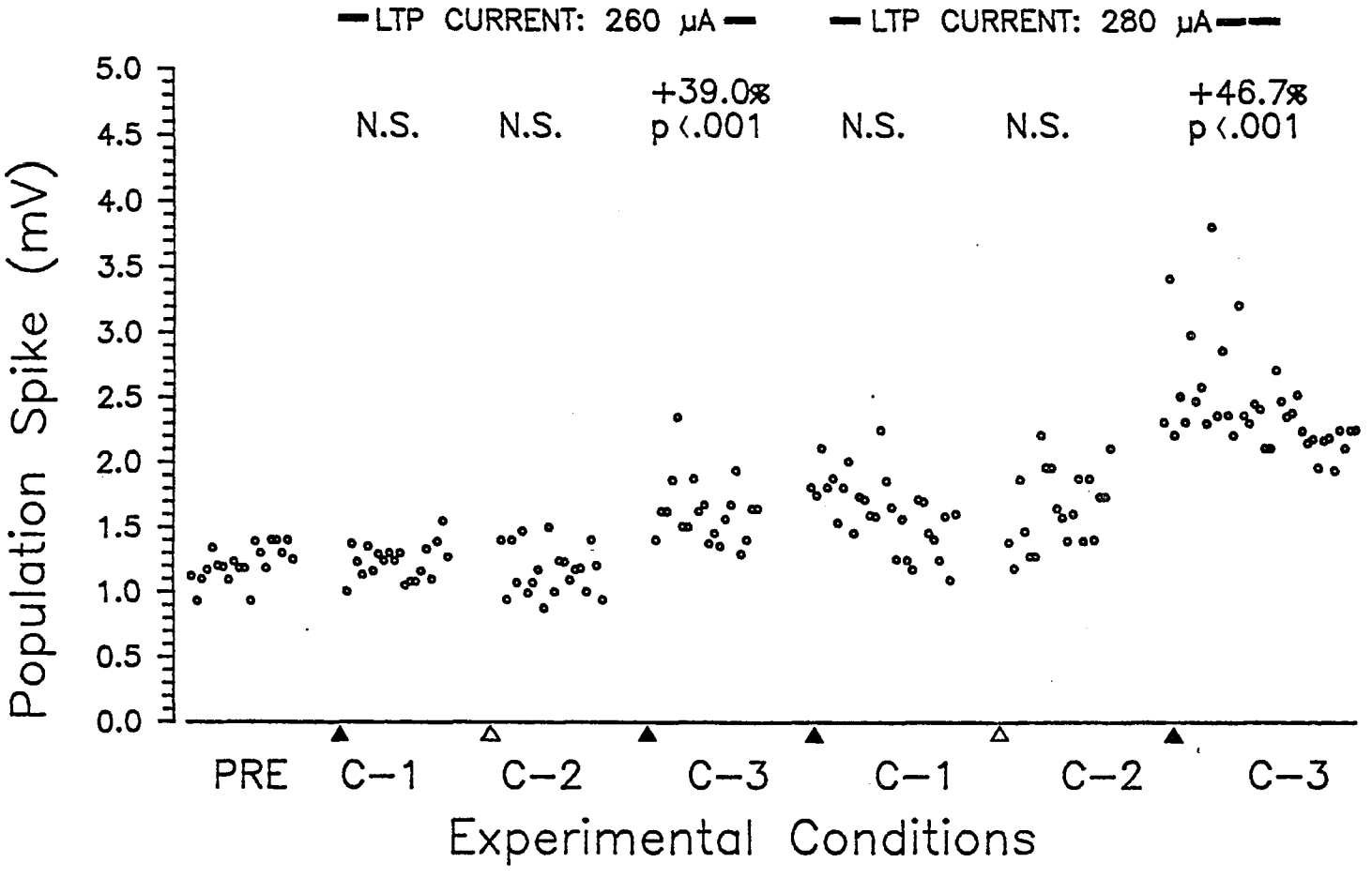

LTP CURRENT: 260 µA
LTP CURRENT: 280 µA
N.S.
N.S.
+39.0%
p <.001
N.S.
N.S.
+46.7%
p <.001
Population Spike (mV)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PRE
C-1
C-2
C-3
C-1
C-2
C-3
Experimental Conditions

Figure 23 a) I/O curve taken before and after potentiation in an animal that was run in Condition 3. As can be seen, both slope of the ESP and the amplitude of the population spike were enhanced at all current levels tested.

a) the slope of the ESP.

b) amplitude of the population spike.

Figure 23a

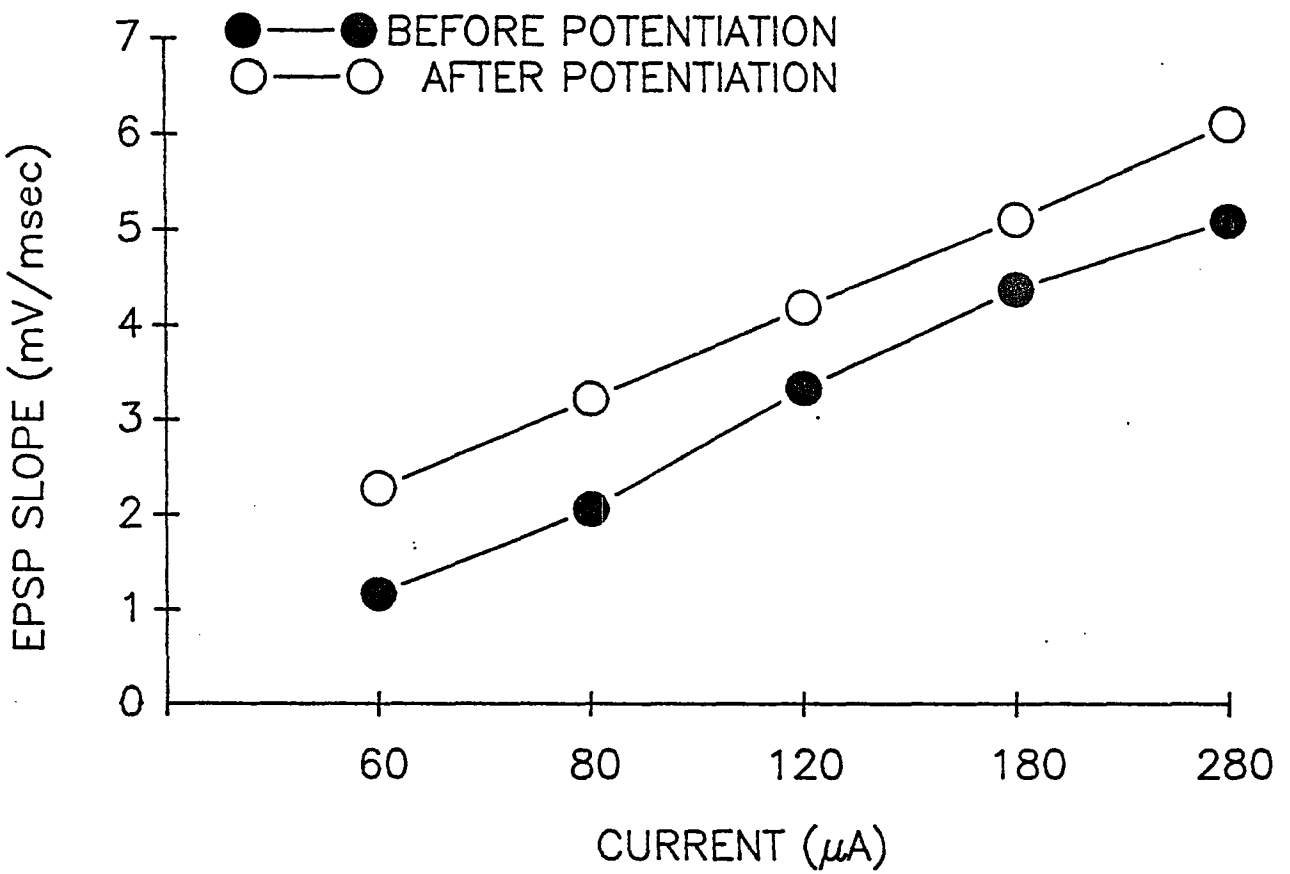
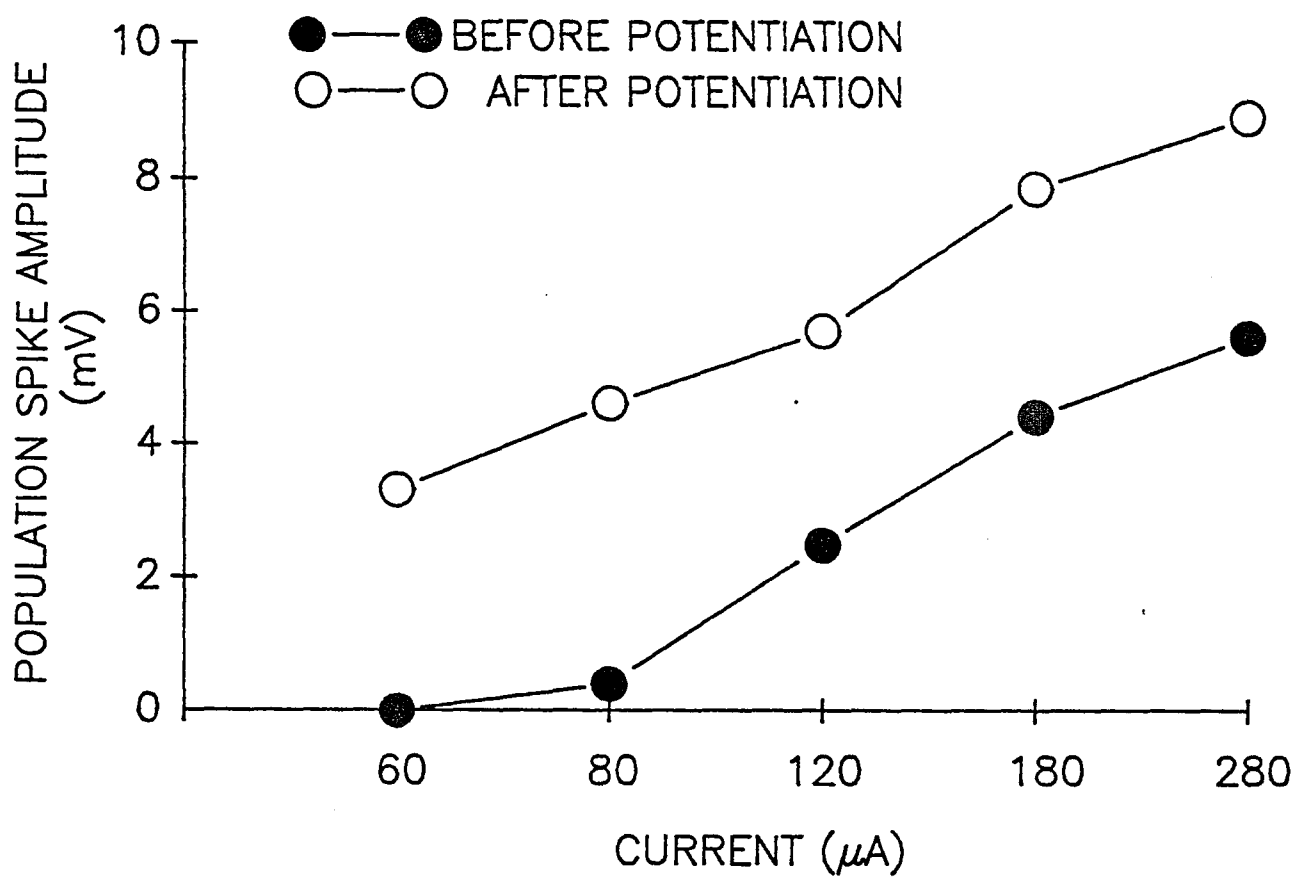

BEFORE POTENTIATION
AFTER POTENTIATION
EPSP SLOPE (mV/msec)
0
1
2
3
4
5
6
7
60
80
120
180
280
CURRENT (μA)

Figure 23b


BEFORE POTENTIATION
AFTER POTENTIATION
POPULATION SPIKE AMPLITUDE (mV)
10
8
6
4
2
0
60
80
120
180
280
CURRENT (μA)

with additional increment in the LTP Stimulus amplitude, there were further increases in both slope and spike in Condition 1 in all seven rats of the group. Figure2 24a and b depict a characteristic response in Condition 4 that shows depression of both slope and spike.

All seven animals that were run in Condition 4 showed LTP in Condition 1 prior to any significant changes taking place in Condition 4. Therefore, the initial LTP current at which LTP occurred in Condition 1 in those animals can be considered as the minimal current that was necessary for LTP induction without the contribution of theta rhythm. The rationale for this statement is as follows: the tetanic stimulation that was applied in Condition 1 was not phase-locked to theta rhythm, and activated the PP at different phases of the ongoing hippocampal EEG. During the gradual current increments in the experiment (due to the lack of any change in Condition 4) LTP threshold was reached and the tetanic stimulation, administered in Condition 1, has induced LTP. Since Condition 1 is equivalent to the priming paradigm that was used in Experiment 1 and was also employed by Larson and Lynch (1986a, b), in which tetanic trains were administered at 200 msec intervals without any relation to the ongoing theta, we have the unique opportunity to examine the effects of theta rhythm on the threshold of LTP, as compared to "Priming" stimulation. Table 2 compares the currents that induced LTP, to the currents that evoked a minimum population spike, for the different conditions of the experiment. Figure 25 shows the individual data that is averaged in Table 2. As can be seen in both the table and the figure, the presence of theta has dramatically affected the ability to induce LTP. When the tetanic trains were applied at the peak of the rhythm (Condition 3),

Figure 24 A characteristic record of an animal that was run in Condition 4. Significant potentiation of both slope and spike took place in Condition 1 (tetanic stimulation of the perforant path without theta rhythm). There were no changes in Condition 2 that followed (theta rhythm induced by midbrain stimulation), and depression of both measures took place when tetanic stimuli were applied to the perforant path on the negative peak of theta rhythm (Condition 4).

a) Slope

b) spike

Abbreviations:

PRE - baseline

Filled arrowhead: LTP Stimulus

Empty arrowhead: Theta Stimulus

Figure 24A

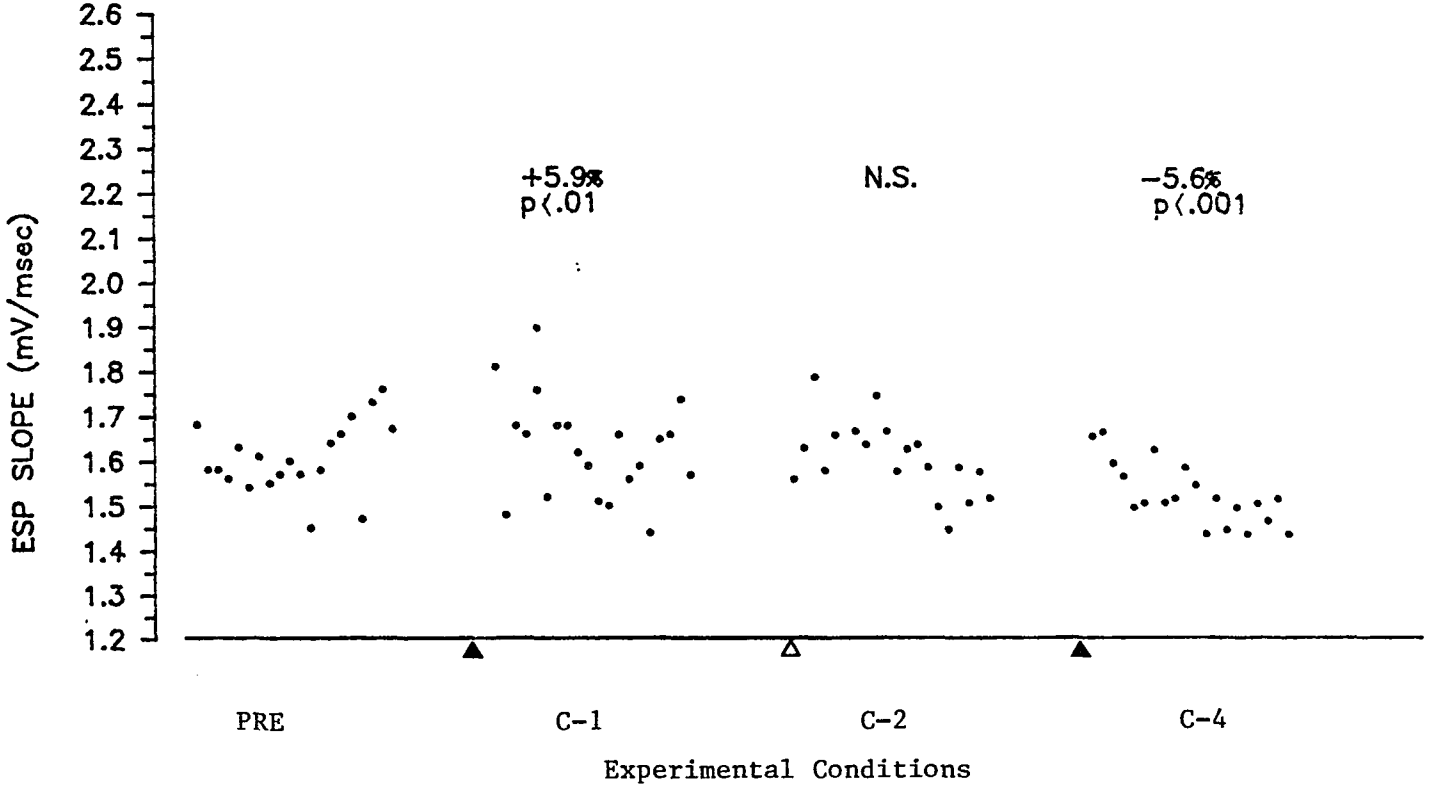

2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
ESP SLOPE (mV/msec)
+5.9%
p<.01
N.S.
-5.6%
p<.001
PRE
C-1
C-2
C-4
Experimental Conditions

Figure 24B

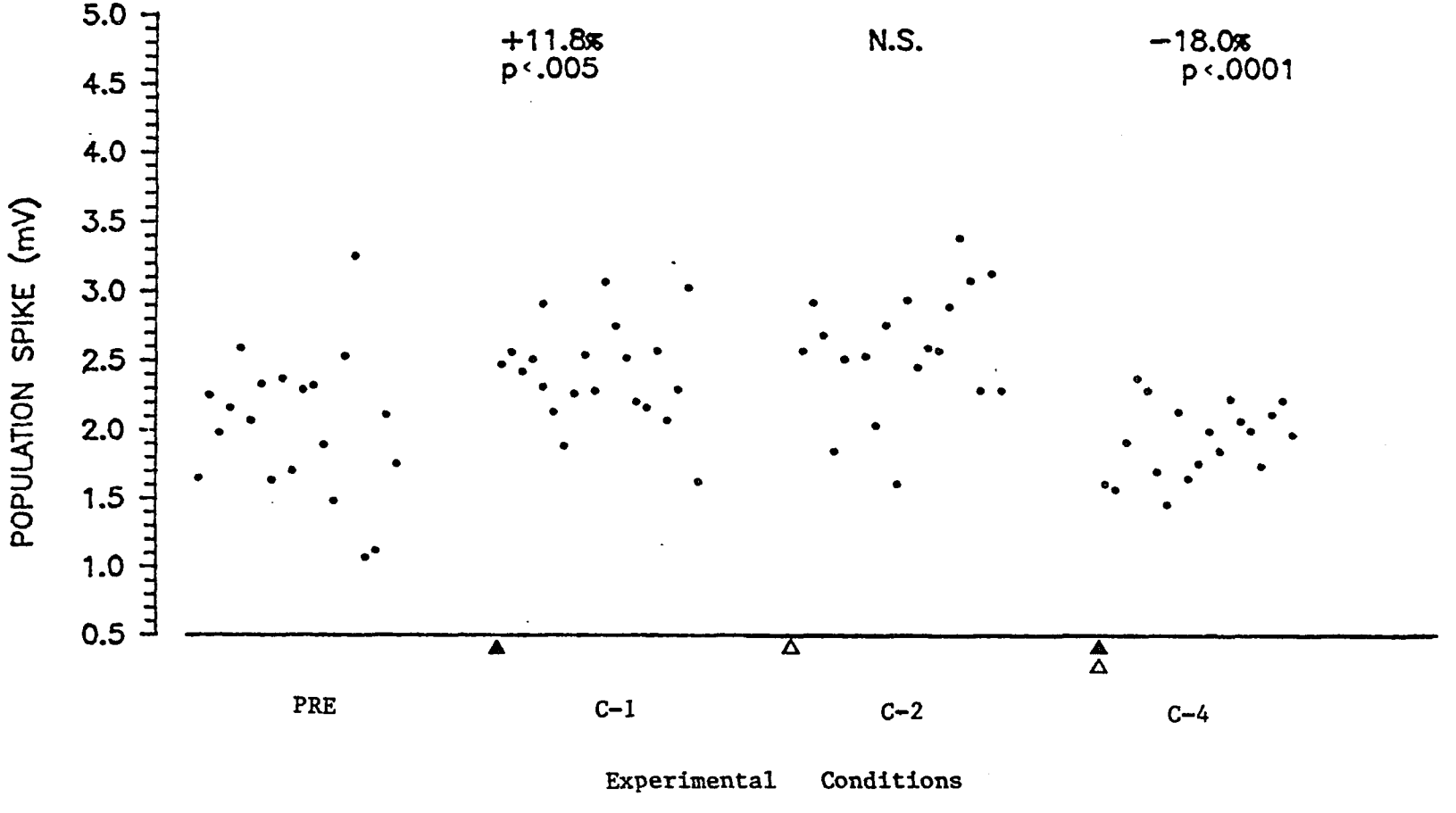

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
POPULATION SPIKE (mV)
+11.8%
p<.005
N.S.
−18.0%
p<.0001
PRE
C-1
C-2
C-4
Experimental Conditions

Table 2

Condition	Average LTP current vs. min. spike current (percent difference, n=7)
1 (tetanus is not phase-locked to theta rhythm- a priming stimulation)	28.2 (s.e.m.=9.5)
3 (tetanus locked to positive phase of theta rhythm)	3.4 (s.e.m.=7.3)
4 (tetanus locked to negative phase of theta rhythm)	70.1 (s.e.m.=20.4)

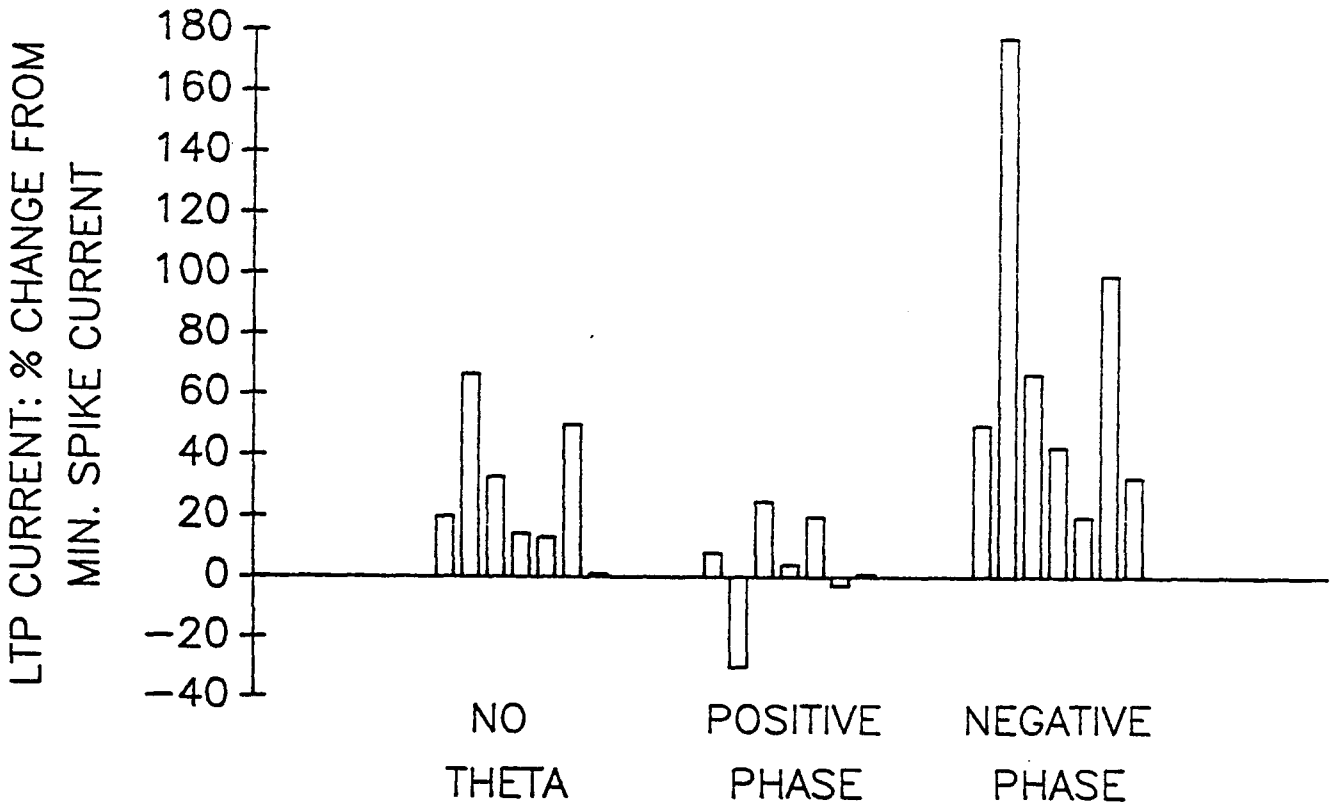
Comparing Condition 1 vs. Condition 3: *T*-test= 5.5, df=12, $p<.0001$, two-tailed.

Comparing Condition 3 vs. Condition 4: *T*-test=-2.23, df=12, $p<.05$, two-tailed.

Figure 25

A histogram describing, for each animal that was run in Experiment 2, the minimal currents that were necessary to induce LTP (as compared to the current that were used to produce a minimal population spike). Note that when the LTP currents were applied at the positive phase of theta, less current was necessary to induce LTP, as compared to the absence of theta. The application of the tetanic stimuli on the negative phase of theta necessitated a higher current in order to induce LTP.

Figure 25


LTP CURRENT: % CHANGE FROM MIN. SPIKE CURRENT
180
160
140
120
100
80
60
40
20
0
−20
−40
NO THETA
POSITIVE PHASE
NEGATIVE PHASE

less current was needed to induce LTP, as compared to tetanizing the PP in the absence of theta or when the tetanus had no systematic relation to the theta phase (Condition 1). When the tetanus was applied at the trough of theta rhythm (Condition 4), more current was needed to induce LTP, as compared to applying these stimuli at the positive phase of the rhythm or in the absence of theta.

Discussion

The association of LTP with theta rhythm is suggested by recent reports that stimuli applied to both CA1 (Larson and Lynch, 1986; Larson, Wong & Lynch, 1986; Rose and Dunwiddie, 1986) and dentate gyrus cells (Experiment 1) preferentially induce LTP if applied at theta rhythm periodicity as compared to other inter-stimulus intervals. The findings reported in Experiment 2 indicate that the presence of theta rhythm itself, and in particular its phase, affect the ability of a repetitive firing of the medial perforant path to induce LTP in dentate gyrus granule cells. Tetanizing the PP at the positive phase of the dentate gyrus theta caused LTP, while the same tetanic stimuli, applied at the negative phase caused no change or depressed synaptic efficacy.

The comparison of the minimal currents that were necessary to induce LTP clearly shows that theta rhythm has reduced the threshold of LTP when the tetanus was applied at the positive phase. The negative phase has increased the threshold for LTP and also reduced synaptic efficacy at lower currents.

The mechanism by which LTP is modulated by theta rhythm is unknown. However, the oscillatory post-synaptic potentials that are believed to generate the extracellularly recorded rhythm may play a significant

role. Thus it has recently been demonstrated that depolarization of CA1 cells by current injection facilitated the induction of LTP (Wigstrom and Gustafsson, 1986) while hyperpolarization acted to block it (Malinow and Miller, 1986). In another experiment, a single afferent volley (0.1 Hz) was paired with a depolarizing current pulse (5-8 nA, 100 msec) and the paired stimulation facilitated LTP induction. Also, if the current was injected just after the peak of the intracellular EPSP, the conjuctive stimulation caused LTP (Wigstrom, Gustafsson, Huang and Abraham, 1986). These and other studies reviewed in the Introduction suggest that LTP is produced if there is a conjuction of a presynaptic transmitter release and post synaptic depolarization. Wigstrom and Gustafsson (1986) suggested that the NMDA channels will open only if these two events occur concurrently or within a narrow time window, and then contribute to LTP development. It is possible that during the positive phase of theta, an intracellular depolarization takes place that facilitates LTP induction. The negative phase, on the other hand, might be associated with intracellular hyperpolarization that can interfere with LTP formation and also reduce synaptic transmission in response to single afferent pulses.

Ionic currents and concentrations can also be affected by the phase of theta rhythm. A larger number of Ca^{2+} ions may enter during the positive phase, facilitating the induction of LTP, while the negative phase might be associated with reduction of Ca^{2+} influx, thereby interfering with the tetanus ability to induce LTP. The extracellular concentration of Mg^{2+} might be affected by the different phases of theta as well, leading to changes in the state of the NMDA channel and, consequently, facilitating or inhibiting LTP induction. The precise

effect of theta phase on the ionic balance inside and outside the cell remains to be elucidated.

If the positive phase is associated with intracellular depolarization, then theta might function as a natural "priming" mechanism. The post synaptic depolarization might function to remove the inhibitory Mg^{2+} ions from the NMDA channel, allowing the opening of the NMDA channel, enabling an influx of Ca^{2+} ions. The time course of activation of this channel is slow and decays after about 200-300 msec (MacDermott and Dale, 1987), so that in order for LTP to develop, repetitive intracellular depolarizations should take place at intervals of approximately 200 msec, a function that might be fulfilled by the naturally occurring theta rhythm. If, concurrently with these repetitive depolarizations, information will arrive from association cortex to the entorhinal cortex, and from there to the dentate gyrus, the probability for LTP induction is the highest. NMDA receptors were documented in the dentate gyrus (Cotman, Monaghan, Otterson and Storm-Mathisen, 1987), and it remains to be shown whether LTP, as induced in this experiment in the dentate gyrus, is mediated by these receptors.

In the dentate gyrus of the freely moving rat, repetitive bursts of granule cell action potentials do accompany theta rhythm in the two behaviors in which the rhythm appears, locomotor activity and REM sleep. Fox, Wolfson and Ranck (1986), reported that the maximal probability of discharge of granule cells was found to be on the positive phase of the locally recorded theta rhythm in freely locomoting rats. This finding was also supported by Buzsaki, Leung and Vanderwolf (1983), in walking rats. They found that granule cells and

dentate interneurons tended to fire maximally on the positive phase of the local theta. These findings might suggest that the positive phase of the rhythm is associated with increased cellular excitability. Unfortunately, little evidence exists that relates the dentate gyrus cells excitability to theta phase. Rudell, Fox and Ranck (1980) studied rats that were running on a treadmill, and reported that the largest spike amplitudes in dentate and CA1 cells were obtained 110° after the positive phase of the dentate theta. Urethane has been reported to reverse the above mentioned relationship between theta phase on one hand, and cell firing and excitability on the other. What complicates the interpretation of our findings is that the type of theta that was induced in Experiment 2 is not clear. Colom, Ford and Bland (1987) reported that in the rabbit, CA1 and dentate cells were found to fire on the negative phase of the locally recorded type 2 theta (which is urethane-resistant, and atropine-sensitive). No data exists relating type 1 theta in the rabbit and neuronal excitability. Future pharmacological studies should establish the nature of the theta activity that was induced in the present experiment. Recently, it has been found in freely walking rats that medial entorhinal cells fire on the negative phase of the CA1 theta (Quirk and Ranck, 1986). If the CA1 theta is 180° out of phase with the dentate theta, this might indicate that entorhinal cortex cells are phase-locked to the positive phase of the dentate theta. If this turns out to be the case, then the source of input to the dentate gyrus, the entorhinal cells, activate the dentate cells on the theta phase that is the most conducive for LTP induction, suggesting that a natural mechanism evolved that is acting to increase the probability of LTP formation during specific behaviors

and circumstances. These points will be further elaborated in the General Discussion.

The present results, taken in conjuction with observations in experiment 1 and studies in freely moving rats therefore suggest that the naturally occurring theta rhythm is associated with the induction of LTP. Varied trains of stimuli in the hippocampus, which may or may not be of physiological relevance, can trigger LTP. The induction of LTP during theta rhythm and the possible association of LTP with memory suggest a new function of the rhythm, namely a mnemonic function during the behaviors in which it occurs.

7. Experiment 3 - The Relationship Between Theta Phase and LTP in CA1 field

Introduction

Two theta generators exist in the rodent's brain. One generator is found in the dentate gyrus (s. moleculare) with a maximal amplitude of about 2 mV. The other generator is found in the CA1 field- s. oriens. CA1 Theta rhythm has a maximal amplitude of 1 mV, and is 180° out of phase with the dentate theta. LTP has been reported in the CA1 field, and so have the "Priming" effect and the existence of the NMDA channel. The present experiment will investigate whether the relationship between the dentate theta rhythm and LTP that was reported in Experiment 2, will be found in the CA1 field.

Materials and Method

Subjects:

The experiment was performed on twenty one male Sprague-Dawley rats (Charles River), ranging in weight between 250-280 g. The rats were housed in a colony room (three per cage) for at least a week before the experiment, on a 12-12 hours light-dark cycle. Food and drink were given ad lib.

Surgery:

The animals were anesthetized with urethane (1.7 g/kg, i.p., with additional anesthetic administered if necessary during the experiment in case any slight vibrissae movement was noted) and mounted in a stereotaxic instrument (David Kopf) with the plane of the skull oriented horizontally. The skull was exposed and burr holes of

approximately 1-2 mm in diameter were drilled in the right hemisphere at coordinates (relative to Bregma) of AP -4.2, ML +2.5 (for recording from the pyramidal cell layer in the CA1 field), and in the left hemisphere at coordinates AP -1.8 and ML +1.0 (for stimulating the ventral commissural pathways, that project from CA3 field to the contralateral CA1 field). As suggested by Vertes (1986), theta rhythm was induced by stimulating the midbrain and a 4-5 mm burr hole was drilled in the right hemisphere at coordinates AP 5.5 and ML 1.0 to introduce the midbrain stimulating electrode. The dura was slit with a sharp needle and bone residues were removed. A recording glass micropipette and a monopolar stimulating electrode were lowered to the cell body layer of CA1 field and the ventral hippocampal commissure, the final depths of the recording and the stimulating electrodes being adjusted to give the maximum field potential recorded in CA1 s. pyramidale. The glass micropipette had a tip diameter of 20-30 uM and was filled with 3M NaCl and fast green dye. The tip resistance was approximately 2-3 Mn. The stimulating electrode was made of stainless steel insect pin (size 00) coated with five layers of epoxylite resin (Epoxylite Corp.) and cleared for distance of 500 uM from the tip (resistance approximately 250 Kn). The recording electrode was also connected to an amplifier and a speaker, in order to detect the characteristic pattern of firing of CA1 complex spike cells (a compound action potential, consisting of several spikes of decreasing amplitude). A monopolar stimulating electrode was lowered to stimulate the midbrain. The final depth was adjusted to induce stable theta rhythm. The peak-to-peak interval of the rhythm was measured. Two additional holes were made in the right and left frontal bones, for

the placement of a ground screw and an indifferent electrode for recording. In the left parietal and occipital bones two holes were made for ground screws for the return paths for the stimulation currents of the midbrain and the ventral commissural pathway.

Stimulation and Recording:

The signals were amplified by an AC differential pre-amplifier (gain of 100). Cut-off frequencies for the field potentials were set at 1 Hz and 3 KHz, and at 3-30 Hz for EEG. For recordings of the complex-spike cells in the CA1 field, the filter was set at a 300 Hz-10 KHz range, at a gain of 1000. All recordings were made in an electrically shielded chamber (120 x 118 x 70 cm).

Extracellular field potentials were evoked in the CA1 field by stimulation of the ventral commissural projections from CA3 field by squared pulses of 0.25 msec duration ("Test Stimulus"), delivered via a photo cell stimulus isolator (Grass). The intensity of the Test Stimulus was chosen to be approximately 50% of saturation current. LTP was induced by 20 trains of tetanic stimuli each consisting of 5 pulses with an interpulse interval of 2.5 msec (400 Hz), and a pulse duration of 0.15 msec ("LTP Train"). The inter-train interval was 1000 msec when they were delivered alone and when delivered concurrently with theta rhythm (see below). Each five trains were grouped together, and delivered at inter-group interval of 10 sec. Midbrain stimulation was obtained by administering a train of pulses at a pulse duration of 0.20 msec and interpulse interval of 10 msec (100 Hz) ("Theta Stimulus"). Train duration was selected that would elicit approximately 80 successive cycles of theta rhythm, each 20 cycles separated by 10 sec.

See Figure 28 for description of the design.

The field potential and EEG signals from the animals were each fed into separate storage oscilloscopes. In addition the EEG was fed into a theta control device (TCD) as described in Experiment 2. The TCD trigger was adjusted to either the rising or falling phase of the theta wave. Activation of the trigger sent a TTL signal to the stimulator and a tetanic train was administered to the commissural fibers. Both the EEG signal and the tetanic train were monitored via the two oscilloscopes. Field potentials were displayed on a an IBM PC AT display, allowing the measurement of the population spike using movable cursors, and were stored on magnetic disks for later analysis, if deemed necessary. The experimental configuration and approximate locations of the electrodes are described in Figure 26.

As was described in experiment 2, theta rhythm was successfully induced by stimulation of the midbrain in the majority of the animals. The rhythm produced was strictly concurrent with the application of the stimulating current. It occurred at approximately 5-6 Hz, and appeared in all respects to be similar to the theta rhythm generated in the freely moving rat. See Figure 27 for an example of midbrain stimulation-induced theta in the CA1 field.

Design and Procedure

The experiment began by determining the minimum currents required to elicit a field response and a population spike, and a saturation current. Test current was chosen to elicit a medium size spike (1-2 mV), at a pulse width of 0.25 msec. Baseline measurements of the field response were taken for a period of twenty minutes by applying the Test Stimulus every 15 seconds. In order to reduce the variability of the

Figure 26 A) Schematic drawing illustrating the configuration of experiment 3. To optimally place the recording electrode in CA1 field, a speaker was connected to the electrode in order to identify the characteristic bursts of the CA1 complex spike cells. Theta rhythm was induced by midbrain stimulation and the EEG signal was fed into the Theta Control Device (TCD). The TCD activated the commissural fibers stimulator, which applied tetanic stimulation in order to induce LTP. Field potentials were recorded from the CA1 field and displayed on a scope and a computer display. Analysis of the amplitude of the population spike was done on-line.

Abbreviations:

CA1 - CA1 field

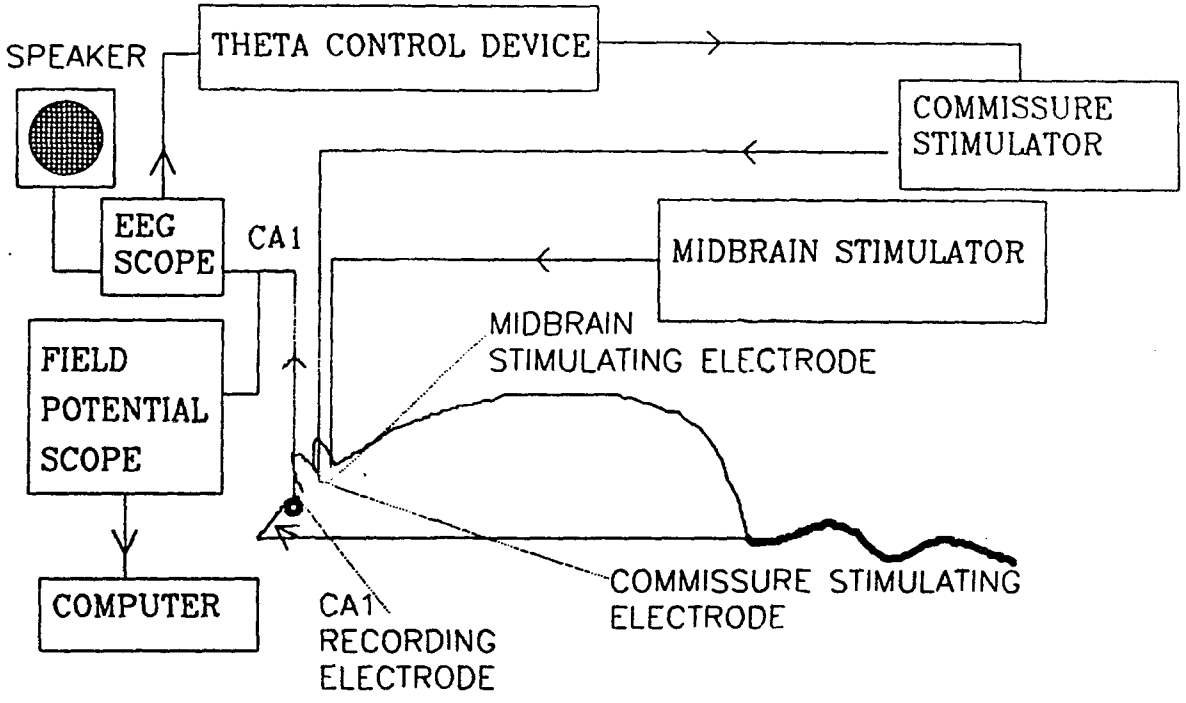
CF - Commissural fibers

B) A dorsal view of the hippocampus, illustrating the approximate location of the recording (REC) in the CA1 field and stimulating (STIM) electrodes in the ventral psalterium (COM). (Adapted from Andersen, Holmqvist & Voorhoeve, 1966).

Abbreviations:

ENT- entorhinal cortex. DG- dentate gyrus.

A


SPEAKER
THETA CONTROL DEVICE
COMMISSURE STIMULATOR
EEG SCOPE
CA1
MIDBRAIN STIMULATOR
MIDBRAIN STIMULATING ELECTRODE
FIELD POTENTIAL SCOPE
COMPUTER
CA1 RECORDING ELECTRODE
COMMISSURE STIMULATING ELECTRODE

B

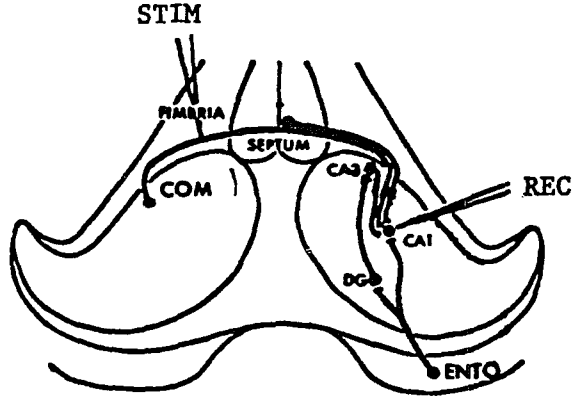
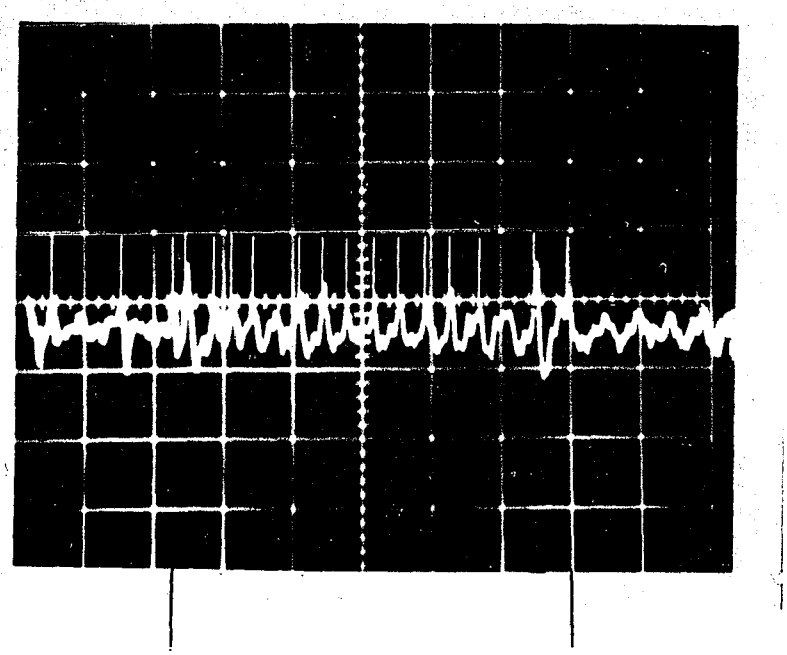

STIM
FIMBRIA
SEPTUM
COM
CA3
REC
CA1
DG
ENTO

Figure 26

Figure 27 CA1 theta as induced by midbrain stimulation. The arrowheads indicate the beginning and the end of the stimulation. One division: 0.5 mV vertically, 500 msec horizontally, positivity upward. Note that the irregular EEG was synchronized by midbrain stimulation to yield a 5-6 Hz theta rhythm.

Figure 27



data, every four responses were averaged on-line by the computer. After obtaining a baseline, two successive control conditions followed (Conditions 1 and 2). The objective in each experiment was to select an initial intensity of LTP Stimulus below the threshold for the induction of LTP in Condition 1. Condition 3 and Condition 4 followed, administered randomly, as described in Experiment 2. If LTP was induced at any stage, testing continued for 30 min. If no change was detected, the LTP Current was increased and the entire procedure was repeated. At the conclusion of the experiment, the animal was killed by an overdose of Nembutal. See Figure 28 for the Experimental design.

Results

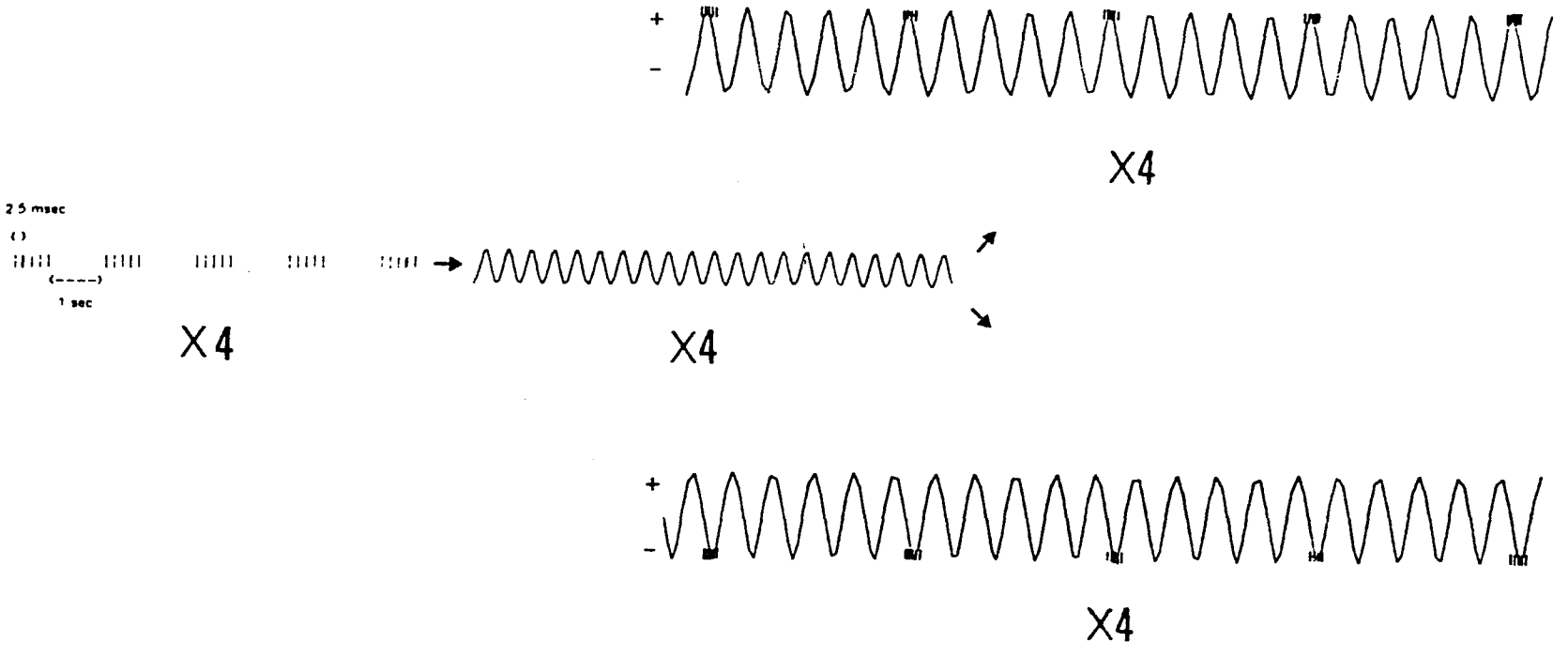
As described in Experiments 1 and 2, histological analysis was performed on several animals at the conclusion of the experiment. Figures 29 through 31 describe the stimulating and recording electrodes, in the hippocampal commissure and CA1 field, respectively, and the midbrain theta electrode.

A total of 21 rats were run in the experiment. In all, ten animals were discarded due to instability, technical difficulties and an initial selection of LTP Stimuli that induced LTP during Condition 1, preventing the evaluation of the effect of tetanic stimulation applied on either the positive (Condition 3) or negative peak (Condition 4) of theta rhythm. Of the remaining eleven animals, six were run in Condition 3 and five were run in Condition 4 with definitive results.

In the rats where the LTP Stimulus was applied at the positive peak of theta rhythm (Condition 3), successful potentiation of the population spike was induced only in Condition 3 in six animals. The

Figure 28 The design of experiment 3. Baseline measurements were taken at the beginning of the experiment, followed by the application of tetanic stimulation to the commissural pathways (Condition 1). Then, midbrain stimulation was performed in order to induce theta rhythm (Condition 2). Conditions 3 and 4 followed, where tetanic trains were applied either at the positive (Condition 3) or negative peak (Condition 4) of theta rhythm. Half of the animals were run in Condition 3 and the other half were run in Condition 4. The Test Stimulus was administered to establish baseline, and following each of the conditions 1 through 4, to measure the induction of LTP.

Figure 28



potentiation of the spike averaged 35.47% ± 6.9 s.e.m. relative to the previous Conditions 1 and 2, *T*-test= 4.24, df=5, p< .01, two-tailed. Figure 32 demonstrates a typical record of the population spike of an animal that was run in Condition 3. In this animal there were no significant changes in the spike in Conditions 1 and 2 as compared to the baseline. A significant potentiation of the amplitude of the population spike took place only in Condition 3.

Considering the rats in which the tetanic stimulation was applied at the negative phase of theta (Condition 4), the LTP Stimulus intensity was increased in successive runs due to an absence of a significant change in Condition 4, as compared to the preceding baseline and conditions 1 and 2, until all rats have shown LTP of the spike in Condition 1. Applying the LTP Stimulus again at the negative phase of theta (Condition 4) had caused depression of the spike in four rats. The average depression of the spike was -17.8% ± 6.69, *T*-test= 3.87, df=3, p <.03, two-tailed. Figure 33 depicts a characteristic response in Condition 4 that shows depression of the spike. In the specific animal shown, the LTP stimulus was applied, and LTP was induced in Condition 1, and reapplying the LTP stimulus on the negative phase of theta caused a depression of the spike. The fifth rat showed potentiation of the spike when the tetanic stimulation was reapplied in Condition 4, the potentiation was 11.3% as compared to the previous Condition 2, *T*-test= 2.9, df=58, p< .01.

Figure 29 a) A photomicrograph showing the track left by the tip of the recording electrode in the cell body layer of the CA1 field. Magnification: x 40. Scale: 260 uM. b) A schematic illustration of the location of the recording electrode, taken from Paxinos & Watson (1982). The vertical line indicates the location of the electrode.

Abbreviations:

3V - third ventricle
AF- amygdaloid fissure
AHi - amygdalohippocampal area
alv - alveus
APTD - ant pretectal area, dorsal
APTV - ant pretectal area, ventral
BL - basolateral amygdala nucleus
bsc - brachium superior colliculus
cg - cingulum
cp - cerbral peduncle, basal
df - dorsal fornix
dhc - dorsal psalterium
Dk - nucleus of Darkschewitsch
DLG - dorsal lateral geniculate
Ent - entorhinal cortex nucleus
fr - fasciculus retroflexus
FrPaSS - frontoparietal SS cortex
GEM - gemini nuclei
hbc - habenular commissure
Hif - hippocampal fissure
IMCPC - interstitial nuc post comm
InC - interstitial nuc Cajal
IntG - intermediate geniculate
LM - lateral mamillary nuc
MG - medial geniculate
ML - med mamillary nuc, lat
ml - medial lemniscus
MM - med mamillary nuc, med
MP - med mamillary nuc, post
mp - mamillary peduncle
mt - mamillothalamic tract
mtg - mamillotegmental tract
OPT - olivary pretectal nuc
opt - optic tract
OT - nuc optic tract
pc - posterior commissure
PCg - post cingulate cortex
PMCo - posteromedial amygdala
Po - posterior thalamus
PP - peripeduncular nucleus
PR - prerubral field
RF - rhinal fissure

Abbreviations (Continued from previous page)

S - subiculum

scc - splenium corpus callosum

SCO - subcommissural organ

scp - sup cerebellar peduncle

SG - supragenicular thalamus

SNC - substantia nigra, compacta

SNR - substantia nigra, reticulada

str - superior thalamus radiation

SuM - supramamillary nucleus

sumx - supramamillary decussation

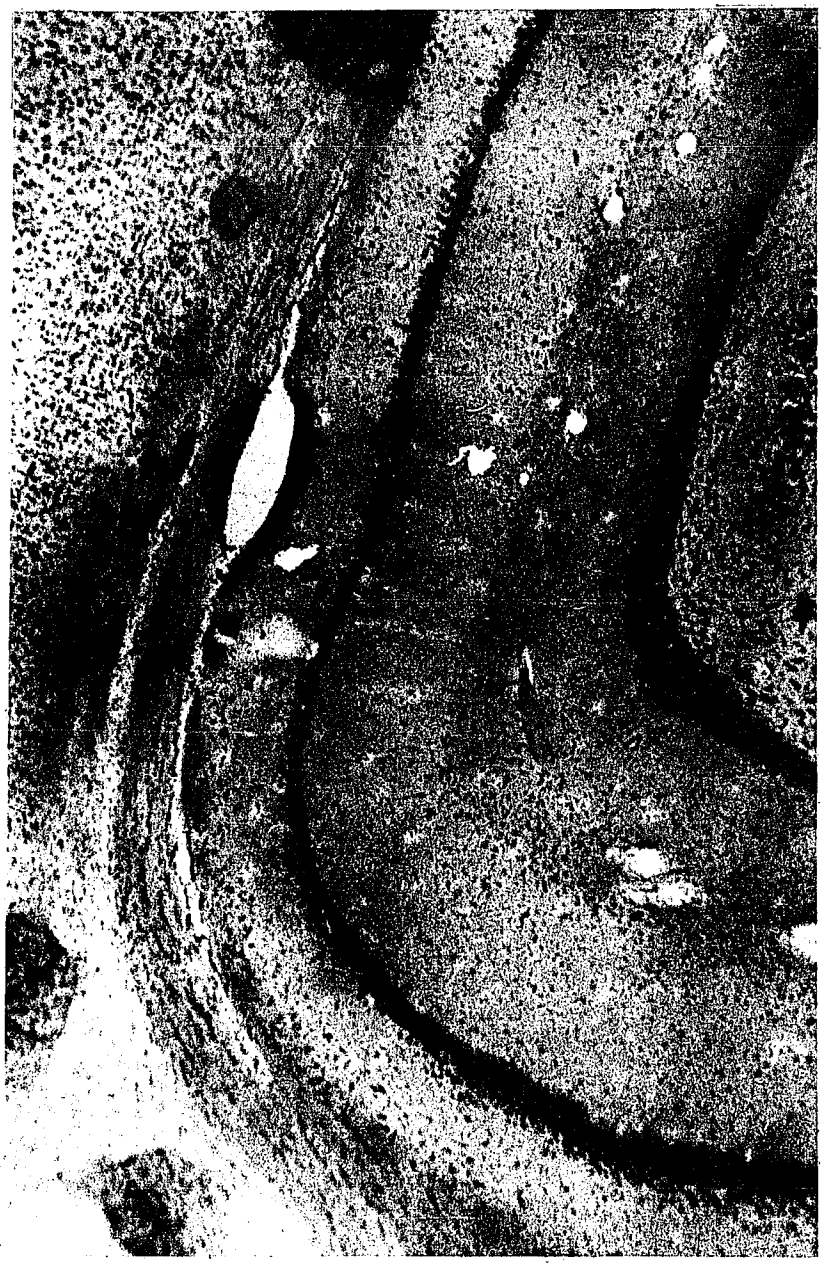
VLGMC - vent lat geniculate nuc, magnocellular

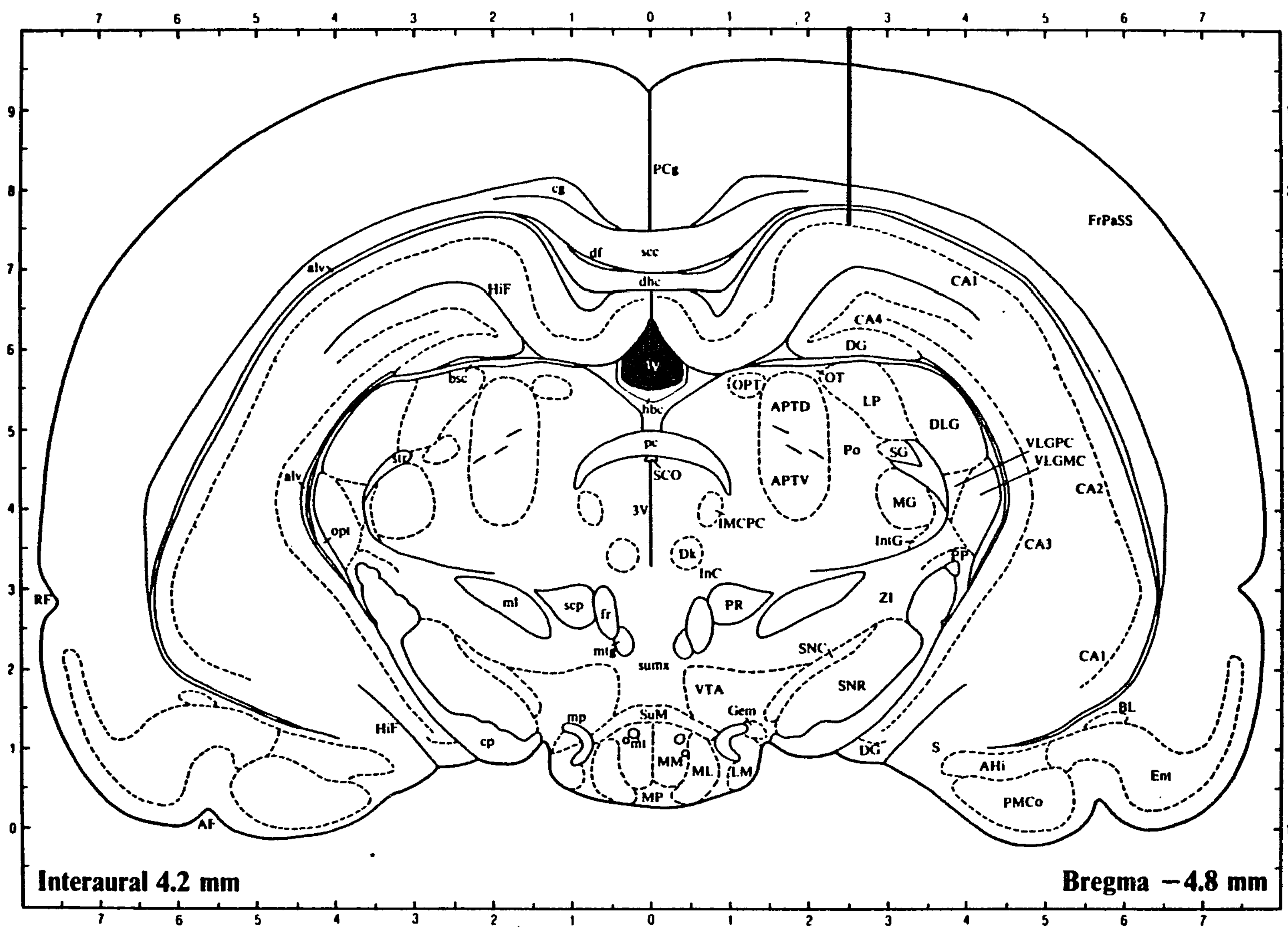
VLGPC - vent lat geniculate nuc, parvocellular

VTA - ventral tegmental area

ZI - zona incerta

Figure 29a





PCg
cg
FrPaSS
scc
df
dhc
alv
HiF
CA1
CA4
DG
1V
bsc
OPT
OT
APTD
LP
DLG
hbc
pc
Po
SG
VLGPC
VLGMC
str
SCO
APTV
MG
CA2
alv
opt
3V
IMCPC
IntG
CA3
Dk
InC
PP
RF
ml
scp
fr
PR
ZI
mtg
sumx
SNC
CA1
VTA
SNR
HiF
mp
SuM
Gem
BL
cp
mt
MM
ML
LM
DG
S
AHi
Ent
MP
PMCo
AF
Interaural 4.2 mm
Bregma −4.8 mm

Figure 29b

Figure 30 a) A photomicrograph showing the track left by the stimulating electrode in the ventral part of the fimbria as it joins the hippocampal commissure and crosses to the contralateral hippocampus. Magnification x 40. Scale: 260 uM. b) A schematic illustration of the approximate location of the stimulating electrode (Taken from Paxinos & Watson, 1982). The vertical line indicates the location of the electrode. Abbreviations:

3V - third ventricle
AA - anterior amygdala
ACg - anterior cingulate
ACo - ant cortical amygdala
AD - anterodorsal thalamus
AHy - anterior hypothalamus
AM - anteromedial thalamus
AV - anteroventral thalamus
B - basal nucleus of Meynert
BST - bed nuc stria terminalis
BSTPO - bed nuc str term preoptic
cc - corpus callosum
Ce - central amygdala
cg - cingulum
Cl - claustrum
Cpu - caudate putamen
CxA - ctx-amygdala transition
df - dorsal fornix
ec - external capsule
En - endopiriform nuc
f - fornix
fi - fimbria
FrPaM - motor frontoparietal ctx
FrPaSS - frontoparietal ss ctx
FStr - fundus striati
GP - globus pallidus
I - intercalated amygdala
ic - internal capsule
IG - induseum griseum
LH - lateral hypothalamus
lo - lateral olfactory tract
LOT - nuc lat olfactory tract
LOTD - nuc lat olf tract dorsalis
LV - lateral ventricle
mfb - med forebrain bundle
MPO - med preoptic area
ox- optic chiasm
Pa - paraventricular hypoth
PC - paracentral thalamus
Pe - periventricular hypoth

Abbreviations (Continued from previous page)

PO - primary olfactory cortex

PT - paratenial thalamus

Re - reuniens nucleus of thalamus

RF - rhinal fissure

Rh - rhomboid nucleus of thalamus

Rt - reticular nucleus of thalamus

SCh - suprachiasmatic nucleus

SFO - subfornical organ

SI - substantia innominata

sm - stria medullaris

SO - supraoptic nucleus

st - stria terminalis

TS - triangular septal nucleus

vhc - ventral hippocampal commissure

VP - ventral pallidum

Figure 30a

Figure 30b

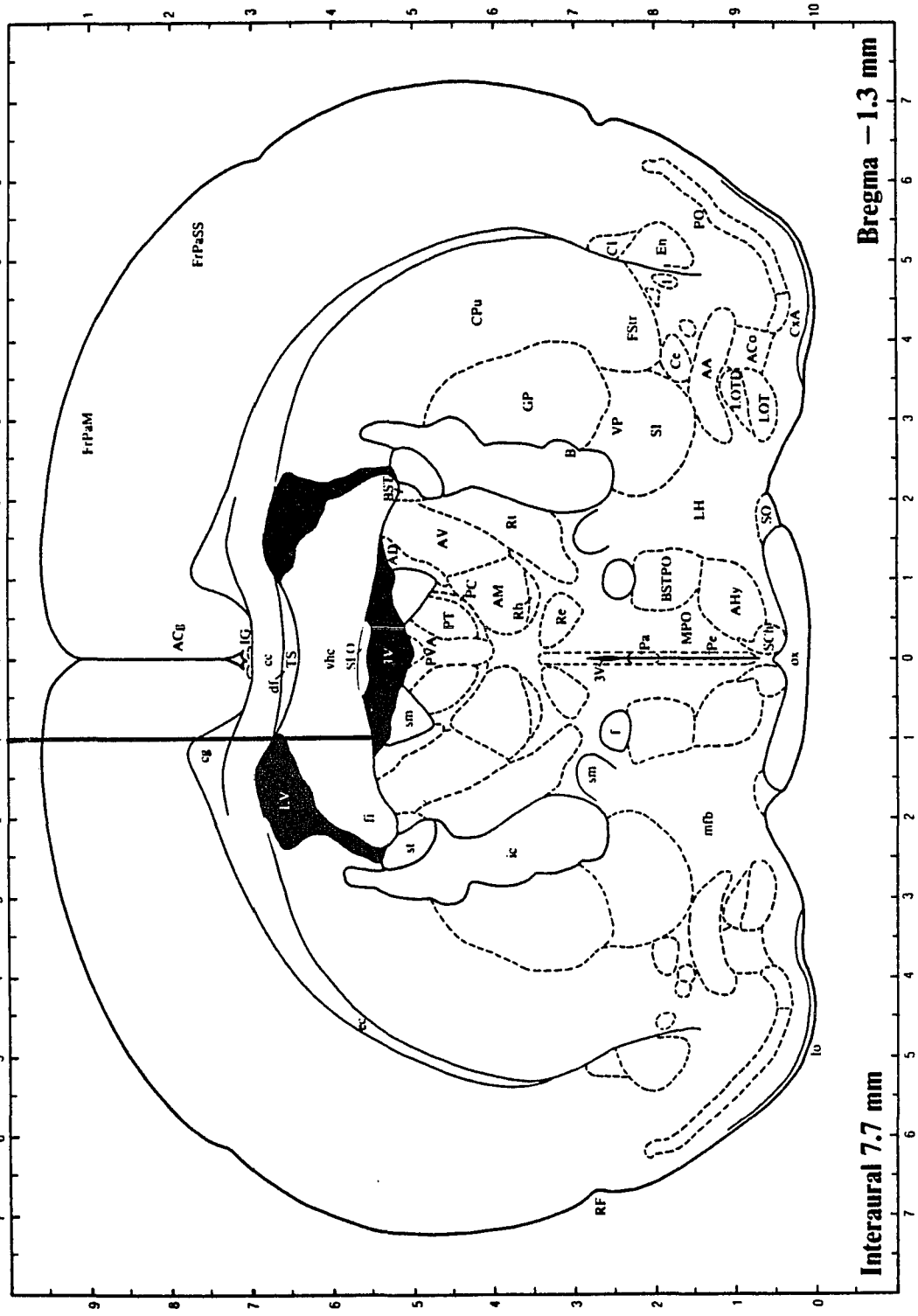

Bregma −1.3 mm
Interaural 7.7 mm
FrPaSS
FrPaM
ACg
IG
cg
cc
TS
df
vhc
SLO
LV
fi
sm
st
ic
RF
BST
AD
AV
Rt
PVA
PT
PC
AM
Rh
Re
3V
f
CPu
GP
B
VP
SI
FStr
Cc
AA
LOTd
LOT
ACo
Cl
En
PO
CxA
LH
BSTPO
MPO
Pa
Pe
AHy
SO
SCh
ox
mfb
Io

Figure 31 a) A photomicrograph showing the track left by the theta electrode, positioned in the midbrain in order to induce theta rhythm. Magnification: x 40. Scale: 260 uM. b) A schematic illustration showing the approximate location of the electrode (from Paxinos & watson, 1982). The vertical line indicates the location of the electrode.

Abbreviations:

4- trochlear nucleus
Aq - cerebral aqueduct
bas - basilar artery
bic - brachium inf colli
bp - brachium pontis
CG - central grey
CGD - central grey, dorsalis
CGM - central grey, medialis
CLi - caudal nucleus raphe
DpG - deep grey layer sup colli
DpWh - deep white sup colli
DR - dorsal raphe
Dsc - lamina dissecans EC
Ent - entorhinal cortex
InG - intermed gray sup colli
InWh - intermed white sup colli
lfp - longitudinal fasciculus
ll - lateral lemniscus
m5 - motor root trigeminalis
mcp - middle cerebral peduncle
Me5 - nuc mesenceph trigeminal
ml - medial lemniscus
mlf - med longitudinal fasciculus
MnR - median raphe
Op - optic nerve layer sup colli
Pas - parasubiculum
PBg - parabigheminal nucleus
Pn - pontine nuclei
PnO - pontine reticular nuc oral
Prs - presubiculum
RF - rhinal fissure
RR - retrorubral nuc
rs - rubrospinal tract
RSpl - retrosplenial cortex
S - subiculum
s5 - sensory root trigeminalis
Str17 - striate cortex 17
Str18(a) - striate cortex 18(a)
SuG - superficial gray sup colli
tfp- transverse fibers pons
xscp - decussation sup cere peduncle
Zo - zonal layer sup colliculus

Figure 31a

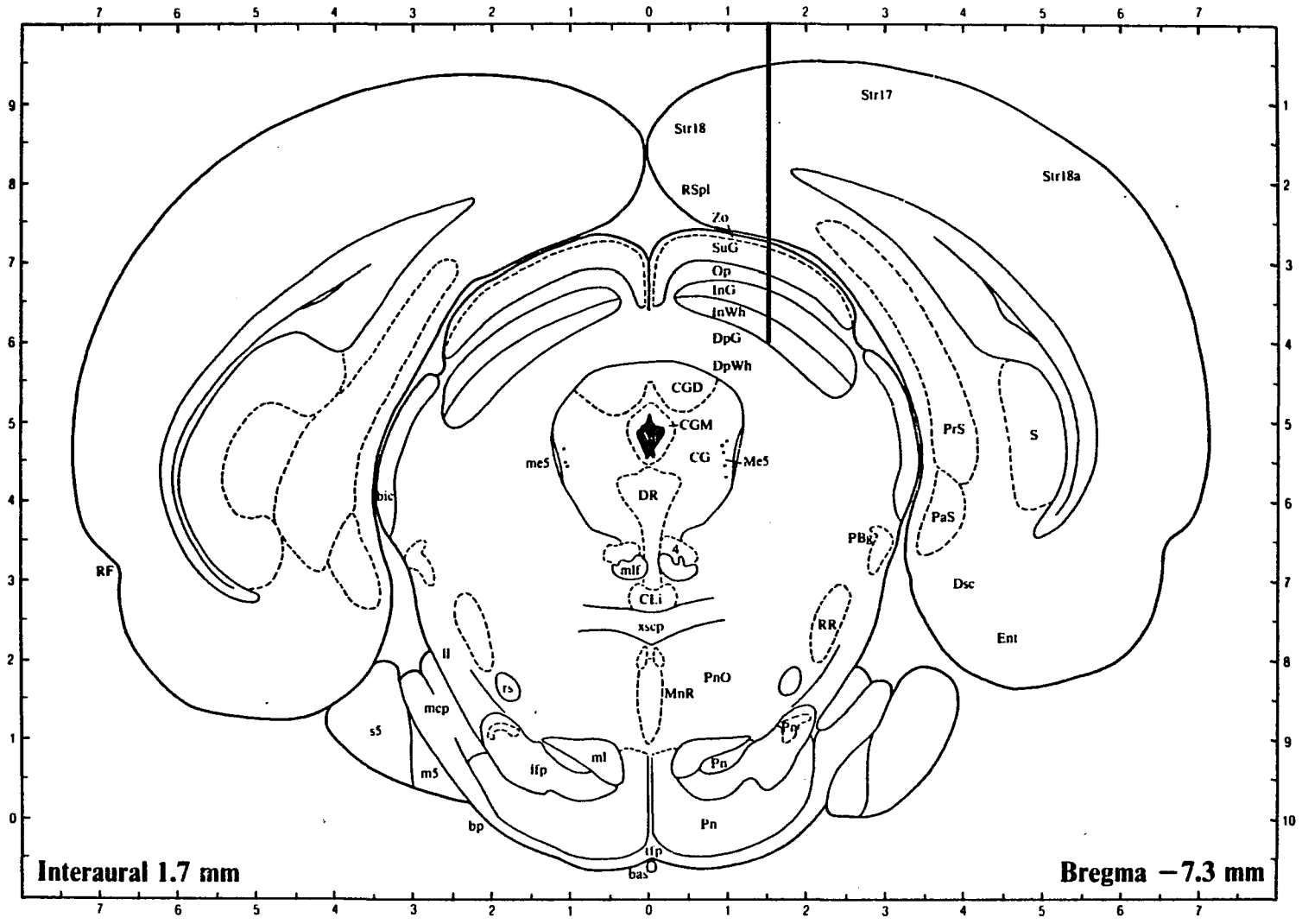

Str17
Str18
Str18a
RSpl
Zo
SuG
Op
InG
InWh
DpG
DpWh
CGD
CGM
CG
Me5
me5
DR
PrS
S
PaS
PBg
RF
mlf
4
CLi
xscp
RR
Dsc
Ent
ll
rs
PnO
MnR
mcp
s5
Pg
ml
m5
lfp
Pn
bp
tfp
bas
Interaural 1.7 mm
Bregma −7.3 mm

Figure 31b

Figure 32 A characteristic record of field potential data of an animal that was run in Condition 3 (tetanic stimulation applied on the positive peak of theta rhythm). There were no significant changes in the amplitude of the population spike in either Condition 1 (the application of tetanic stimulation to the commissural pathway without theta rhythm) or Condition 2 (the induction of theta rhythm in the hippocampus by midbrain stimulation) as compared to baseline. The application of tetanic stimuli at the positive peak of theta rhythm (Condition 3) produced reliable LTP of the spike of the field response.
Abbreviations: PRE - baseline C-1 - Condition 1

C-2 - Condition 2 C-3 - Condition 3

Filled arrowhead: LTP Stimulus

Empty arrowhead: Theta Stimulus

Figure 32

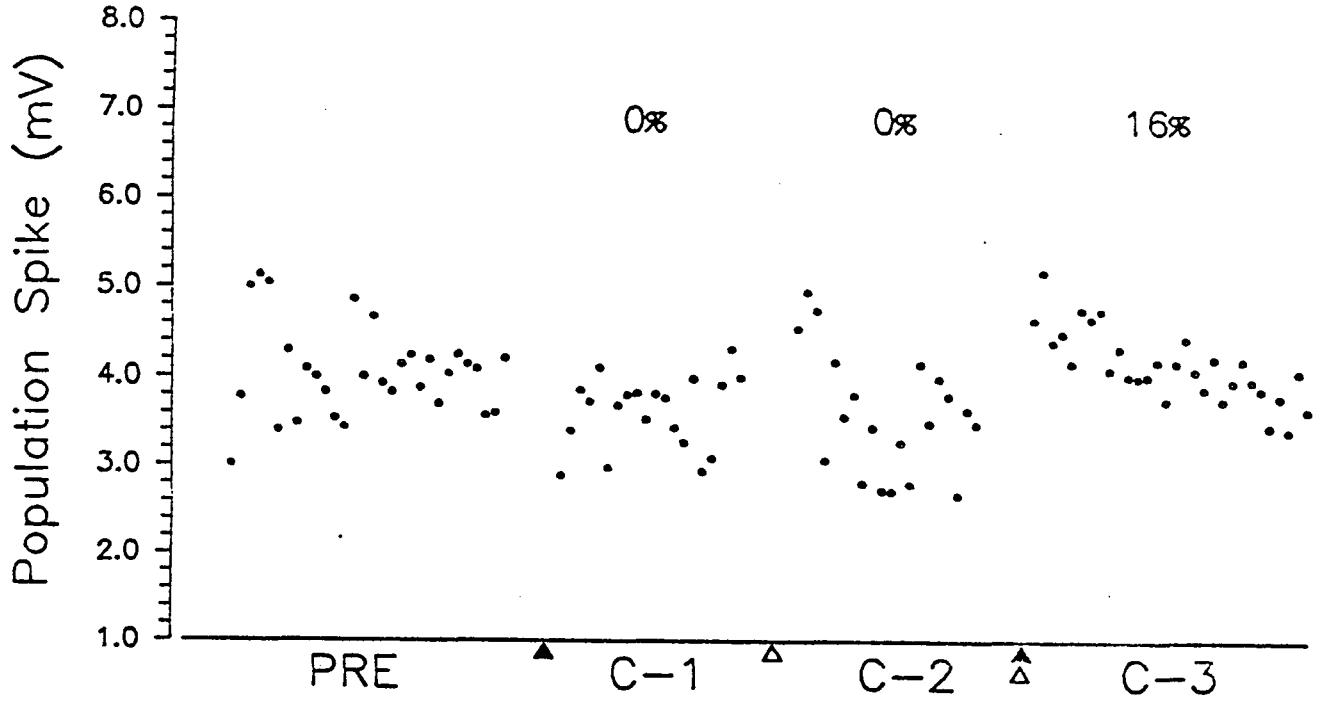


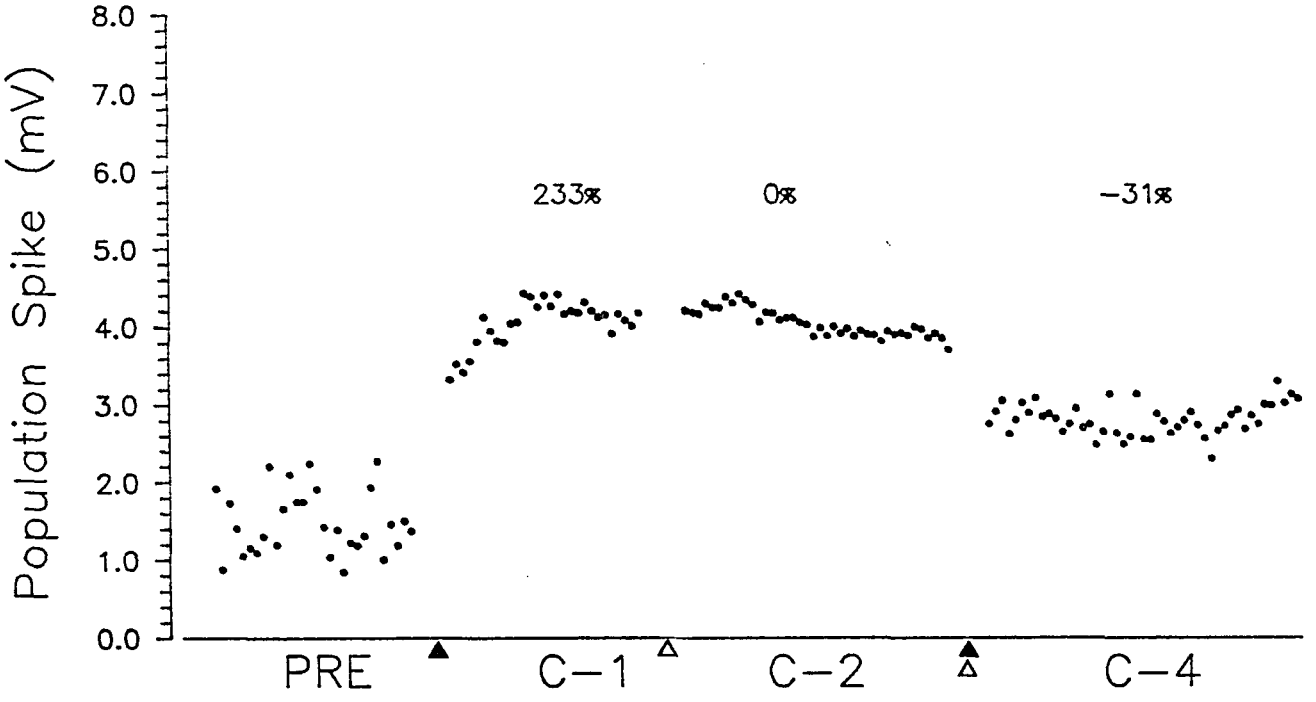
Figure 33 A characteristic record of field potential data of an animal that was run in Condition 4 (tetanic stimulation applied on the negative peak of theta rhythm). There was a potentiation of the amplitude of the population spike in Condition 1 (233%, $p<.001$). The application of tetanic stimuli at the negative peak of theta rhythm (Condition 4) produced a long-lasting depression (-31%, $p<.001$) of the spike of the field response.

Abbreviations: PRE - baseline C-1 - Condition 1
C-2 - Condition 2 C-3 - Condition 3

Filled arrowhead: LTP Stimulus

Empty arrowhead: Theta Stimulus

Figure 33


Population Spike (mV)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
233%
0%
-31%
PRE
C-1
C-2
C-4

Comparisons of the currents that were used in this experiment showed that LTP induction, concurrent with the positive phase of theta rhythm (Condition 3), required an average current that was 8.4% ± 13.1 higher than the current required to induce a minimal population spike. Inducing LTP without the presence of theta rhythm (Condition 1) necessitated a current that was, on the average 18.2 ± 14.2 higher than the minimum spike current. The difference between these two averages is not significant, due to the large scatter, (*T*-test=1.2, df=9, p>.05). Due to instability and great scatter, a comparison of the currents that induced LTP in Condition 4 to the LTP currents used in Conditions 1 and 3 was impossible.

Discussion

The surprising observation in this experiment, as compared to Experiment 2, is the extremely low threshold of LTP in the CA1 field of the urethanized rat. LTP was induced in Condition 1 in eleven animals, despite efforts to keep the current as low as possible. This sensitivity of the CA1 field potentials to tetanic stimulation is confounded with a characteristic instability of the population spike. The CA1 and dentate gyrus population spikes tend to fluctuate to a great extent, even in response to currents of constant intensity. After LTP, the spike tends to grow several order of magnitude, as compared to changes in the ESP slope (Teyler and Discenna, 1987). The natural instability of the spike, combined with its sensitivity to tetanic stimulation caused premature potentiation and great scatter that masked any possible contribution of theta phase in some of the animals.

The mechanisms by which LTP is modulated by theta rhythm in the CA1 field may be similar to those existing in the dentate gyrus. Thus, the post-synaptic depolarization that has recently been demonstrated to facilitate the induction of LTP (Wigstrom and Gustafsson, 1986) and the hyperpolarization that was found to block it (Malinow and Miller, 1986) may be related to the theta phase, as described in the Discussion of Experiment 2. The above mentioned studies and other studies reviewed in the Introduction suggest that LTP is produced if there is a conjuction of a presynaptic transmitter release and post synaptic depolarization. It has been argued that the NMDA channel will open only if these two events occur concurrently or within a narrow time window, and then contribute to LTP development. Similarly, theta phase can be associated with ionic currents and concentrations, thereby affecting LTP threshold. The precise effect of theta phase on the ionic balance inside and outside the cell should be clarified in future studies.

Similarly to the dentate gyrus of the freely moving rat, repetitive bursts of CA1 pyramidal cell action potentials do accompany theta rhythm in the two behaviors in which the rhythm appears, locomotor activity and REM sleep. In the curarized rabbit, Green, Maxwell, Schindler & Stumpf (1960) found that most CA1 pyramidal cells and dentate granule cells fire near the positive peak of theta. Bland, Andersen, Ganes and Sveen (1980), investigating the urethanized rabbit, and Buzsaki and Eidelberg (1983), studying the urethanized rat, reported that dentate granule cells and CA1 pyramidal cells are locked to negative phase of local theta. Since urethane has been shown to reverse the phase relationship found between theta and cell firing

(Fox, Wolfson and Ranck, 1983; Rudell and Fox, 1984), this finding may suggest that the positive phase of walking theta is associated with increased cell firing in the CA1 field. Recently, Brankack and Fox, (1987) performed current source density analysis of theta rhythm in freely moving rats, and found a phasic sink in s. radiatum whose peak was near the positive phase of the locally recorded theta. This suggests that the third synapse of the tri-synaptic circuit, which is activated by input coming from CA3 Schaffer collaterals and terminates on the basal dendrites of CA1 pyramidal cells in s. radiatum (Carpenter and Sutin, 1983), is preferentially activated at the positive peak of theta rhythm, suggesting a modulatory role of theta rhythm at that synapse.

The study of the relationship between theta phase and cell firing is complicated by the findings that pyramidal cells and interneurons are locked to different phases of theta. Two types of cells are found in the hippocampus: Complex-spike cells, which constitute 90% of total cells, and are probably pyramidal cells, and theta cells, which are presumed to be interneurons (Fox and Ranck, 1975). Complex spike cells fire in series of 2-11 action potentials, with decreasing amplitude, usually less than 2/sec. Theta cells fire single action potentials with shorter duration, and at a usual rate of 5/sec and higher. Theta cells increase their firing rate during theta and locomotion, while complex spike cells increase their firing rate when the rat is in a certain place in the field. Both types of cells are phase-locked to theta. Pyramidal cells reduce their firing rate in the presence of theta, unless the animal is in a certain location. In such a place field,

pyramidal cells firing increases and becomes rhythmic, phase locked to the local theta. Buzsaki, Leung and Vanderwolf, (1983), in freely moving rats, and Buzsaki and Eidelberg (1983) in the urethanized rat, found that CA1 complex spike (pyramidal) cells fire near the negative phase of the local theta, while CA1 interneurons fire near the positive phase. They suggested that the medial septal cells drive directly inhibitory interneurons in the CA1 field, and these modulate the pyramidal cells firing by rhythmically inhibiting the pyramidal cell soma. This explanation is offered to account for the reduced firing of pyramidal cells during theta and suggests that theta is produced in the CA1 field by interneurons. During locomotion in the place field, CA1 and dentate interneurons, and also granule cells increase their firing rate, but they are locked to the positive phase of theta (Buzsaki, 1986). On the other hand, Artemenko, (1972) who was studying the curarized rabbit, found that pyramidal cells firing is locked to the positive phase, while basket cells firing is locked to the negative phase. Similarly, Sinclair, Seto and Bland (1982), and Bland, Seto and Rowntree (1983) found in the freely walking rabbit, that CA1 theta cells (presumably interneurons) fire on the negative phase of the local theta. If CA1 inhibitory interneurons are relatively silent during the positive phase of theta, as found by Artemenko and Sinclair et al's, LTP threshold might be reduced, and its induction might be facilitated. On the other hand, tetanizing on the negative phase, when the interneurons are also active, might raise LTP threshold, making it more difficult to induce long lasting potentiation of the spike. One should keep in mind the differences among the types of theta, species and preparations that make it difficult to interpret the relationship

between theta phase and cell firing in this experiment. By stimulating the midbrain, we have tried to induce a stable, large amplitude and high frequency theta, that resembles the kind of theta seen in locomoting rats (Type 1 theta). However, the anesthetic that was used in this experiment (urethane) is known to induce theta that resembles the kind seen during immobility in rabbits and also in rats during stressful situations, when the rat is restrained (Type 2 theta). These two types of theta are different not only behaviorally and electrophysiologically, but pharmacologically as well. The possibility of theta rhythm being contaminated by urethane in this experiment can not be ruled out, and future pharmacological studies should clarify the nature of theta that was induced in this experiment.

The present results, taken in conjuction with observations in experiments 1 and 2 and studies in freely moving rats suggest that the naturally occurring theta rhythm is associated with the induction of LTP, in both areas of the hippocampus where theta generators exist, namely the dentate gyrus and the CA1 field, and where NMDA channels have been implicated with LTP induction.

8. General Discussion

Since Broca's description of the Limbic System in 1878 as the cortical area that is found near the margins ("limbus") of the cortical mantle and surrounds the brain stem, numerous theories have been offered to explain the function of this system. Broca himself suggested that the system is involved with olfaction, because its proximity to the pyriform cortex. Other theories have implicated the limbic system in emotional processes (Kluver and Bucy, 1939; Papez, 1937), visceral functioning (MacLean, 1949, 1954) and motivation (Jarrard, 1973).

In 1954, Scoville published his observations on 30 patients who underwent bilateral resection of medial temporal lobe. In 2 cases Scoville extended the resection backwards, and removed the hippocampus and parahippocampal gyrus bilaterally. Only these two patients showed global amnesia. Milner (1970) described the intellectual and memory functions of one of these patients (patient H.M.): The patient's language and overall intelligence are preserved, but he suffers from severe anterograde amnesia, and can remember material only as long as rehearsal is possible. Interference with rehearsal causes sharp drop in his recall, and forgetting develops rapidly. In addition, the patient suffers from retrograde amnesia that goes back 8 years before the surgery. Immediate memory is spared, and so is the acquisition and retention of simple motor tasks and certain procedures. Global amnesia was also reported in other reports and only in patients who have had bilateral removal of the hippocampus, suggesting that this structure is involved in mnemonic functions.

The search for the neurobiological substrate of memory in the

hippocampus has focused upon anatomical, physiological and chemical levels of inquiry. A leading candidate for a physiological model of memory is Long-Term Potentiation (LTP), a long-term enhancement of synaptic efficacy that results from intense tetanic stimulation of afferents to the hippocampal formation. This synaptic enhancement can last for hours and even days. However, successful induction of LTP requires long trains of intense, tetanic stimuli that are not within the physiological range. Theta rhythm is a physiological phenomenon unique to the hippocampus. It appears during species-specific behaviors, is highly regular, has the largest amplitude of all rhythms in the archicortex, and synchronizes the activity of large groups of neurons in the limbic system. These qualities of theta make it a putative mechanism that may be involved in LTP induction.

Theta appears in rabbits and cats during locomotion, exploration, orientation, and attention, during which the neocortex is desynchronized. When the orientation response is reduced, theta also disappears. In rats, theta appears during locomotion and exploration, but not during immobility. Thus, novel or meaningful stimuli or exploratory behavior activate the hippocampus and set it in theta mode. In this mode, cells in the entorhinal cortex, dentate gyrus and CA1 field begin to fire rhythmically, at theta rhythm periodicity, and are also phase-locked to the locally recorded theta. These observations suggest that theta rhythm periodicity and theta phase affect somehow the intracellular events in hippocampal cells that may contribute to plastic events in the hippocampus, such as LTP.

How is theta rhythm related to LTP ? Several mechanisms can be operative, as suggested by the present findings. Theta appears during

orientation and exploration, when the animal is confronted with a new stimulus. Under such conditions, theta can signal the hippocampus to start registering the information in its circuitry. Physiologically, this can be achieved by the medial septum, driven by brain stem nuclei, that comprise the ascending reticular activating system (Segal and Landis, 1974b; Swanson and Cowan, 1979). Cells in the medullary reticular formation are found to fire 1 sec before theta development (Klemm, 1970). The medial septum drives the dentate, CA1, and entorhinal theta generators, causing rhythmic depolarization of large groups of entorhinal and hippocampal cells at theta rhythm periodicity. Nature has build a mechanism in the hippocampal and entorhinal cells that is sensitive to theta rhythm periodicity. This mechanism is the NMDA subtype channel of the glutamate receptor. The NMDA channel is activated more slowly and its time course is longer than other documented glutamate channels. Calcium ion concentration within the NMDA channel accumulates to a certain optimal value after about 200-300 msec. At that time, tetanizing the channel will preferentially induce LTP, as shown in Experiment 1. Periodic depolarizations are necessary to activate the NMDA channel, in order for calcium concentration to remain at a certain optimal level. If during these rhythmic depolarizations, supra-modal information will arrive from association cortex through the perforant path, and will traverse the tri-synaptic circuit, there is a higher probability that LTP will be induced. Interestingly, a theta generator was not identified in the CA3 field of the hippocampus, and LTP there is not blocked by APV, the NMDA channel blocker (Cotman, Monaghan, Otterson and Storm-Mathisen, 1987). APV

blocks hippocampal LTP only in areas where a theta generator was documented, suggesting a close relationship between theta rhythm and the NMDA channel.

Another putative mechanism that relates theta rhythm and LTP is the theta phase. Cells in the entorhinal cortex and the hippocampus are locked to a certain theta phase, and show rhythmic oscillation between depolarization and hyperpolarization, each state being locked to a different theta phase. Systemic atropine, sufficient to eliminate theta, is found to eliminate rhythmic firing as well as phase-locking of hippocampal principal cells and interneurons (Stewart and Fox, 1987). Phase-locking of large groups of cells might act to synchronize their activities, so that information processing takes place in all these cells at certain times and not at others. An example of such a synchrony takes place in the olfactory system of the rodent. Rodents are macrosmatic animals, using their olfaction to guide their behavior in a large number of situations, beginning in food intake, movement and exploration, and ending in social and sexual behavior (see Schultz and Tapp, 1973, for review). The olfactory bulb projects to the cortical nuclei of the amygdala, pyriform cortex and both parts of the entorhinal cortex (Beckstead, 1978). From the entorhinal cortex, olfactory input arrives the hippocampus.

Behavioral arousal in the rat is accompanied by sniffing bouts in which there are cycles of protraction of the vibrissae and inhalation that are coordinated in a one-to-one fashion (Macrides, 1975). During sniffing, the vibrissae move rhythmically at theta periodicity, with a 1:1 correlation between a vibrissa twitch and a theta beat. Macrides (1975) reported that in hamsters, theta is always in the dorsal

hippocampus during sniffing, and it appears before sniffing and continues for several minutes later. There are 4-10 successive periods of entrainment of theta and sniffing. In every subject, there is a preferred temporal relationship between theta and the respiratory cycle of the sniffing. This phase-locking may create a rhythmic sensory input that arrives the hippocampus at a theta rhythm periodicity. In an odor discrimination task, theta appears during sniffing and sampling, with a 1:1 relation between the sniffing and theta (Macrides, Eichenbaum and Forbes, 1982). The rhythmic firing of the olfactory bulb neurons is explained by projections from the septum and diagonal band of Broca to the bulb, pyriform cortex, and amygdaloid cortex. In another study, (Eichenbaum, Kuperstein, Fagan and Nagode, 1987), sniffing was examined during locomotion, while theta was recorded from CA1 field. During approach to the odor, theta of 7-10 Hz is present. During sampling, its frequency decreases to 5-7 Hz, and its amplitude lowers. During the sampling and before approaching the reward, bouts of sniffing of 3-6 sniffings are recorded, also at 5-7 Hz. During approach, sniffing is concurrent with theta; during sampling, sniffing comes before theta. The correlations between sniffing and theta range between 0.73 and 0.90, and reach their highest value when sniffing comes before theta. These studies suggest that theta synchronizes the activity of cells in the olfactory bulb, entorhinal cortex and hippocampus, so that these systems will act together, allowing for the sensory input to arrive the hippocampus at a specified phase of theta. If the input will reach the hippocampal cells during their depolarization phase, and/or when the input depolarizes the cells at

theta rhythm periodicity, there is a higher probability for LTP to take place, because under these conditions its threshold is reduced, as shown in this dissertation. Theta rhythm can thus be conceived as a mechanism that vary the threshold of large groups of neurons to synaptic input (Komisaruk, 1970).

The vibrissae of the rat contribute to the processing of spatial information. Zucker and Welker (1969) analyzed unit activity in the trigeminal ganglion in response to mechanical stimulation of the vibrissae. Units were found that respond to a deflection of a single vibrissa. A closer analysis revealed that the vibrissae code qualities such as peripheral location, in addition to qualities of the deflection, such as: onset, termination, duration, velocity and amplitude. Vincent (1912; cited in Komisaruk, 1977) found that after removal of the vibrissae, rats have to drag their snout in order to localize themselves on an elevated maze. An additional lesion, done in the infra-orbital nerve, which relays tactile information from the snout and nose, was found to increase the number of errors performed in the maze. On a visual cliff task, Schiffman, Lore, Passafiume and Neeb (1970) found that rats with intact vibrissae show no preference to either side of the cliff. Removing the vibrissae causes the rats to rely on their vision, and under such circumstances they prefer to remain on the optically shallow side. As mentioned above, vibrissae movements accompany sniffing cycles and are performed at theta rhythm rhythmicity. These data suggest that vibrissae movements are paced also by the medial septum, similarly to olfactory bulb and hippocampal cells. Although a direct connection between the medial septum and the motor nucleus of the facial nerve has not been documented, medial

septum lesions have been found to reduce vibrissae movements or interfere with the rhythm of their movements (Brady & Nauta, 1953; Gray, Quintao & Aravjo-Silva, 1972). Blocking hippocampal theta rhythm by high-frequency stimulation of the medial septum blocks all vibrissae movements (Gray, 1971). These data raise the possibility that spatial, in addition to olfactory information, arrives the hippocampus in a rhythmical fashion, similarly to olfactory information. Such rhythmicity might allow spatial information to activate hippocampal synapses at certain phases, that are conductive to LTP formation.

Based on the studies mentioned above, several predictions can be made. Elimination of theta, by lesioning the medial septum, administration of anticholinergics, or transecting the fornix, will cause acquisition deficits, because input will arrive the hippocampus un-synchronized with the intracellular events in hippocampal cells that are most conductive to LTP formation. On a radial maze task, that is sensitive to hippocampal damage, animals with medial septal lesions had a slower rate of acquisition, but could eventually acquire the task. Hippocampal animals could not acquire the task at all (Mitchell, Rawlins, Steward, and Olton, 1982). Similarly, rats with medial septal lesions showed slower acquisition rate of a two-way avoidance task, but dorso-lateral lesions did not slow the acquisition rate (Sagvolden and Johnsrud, 1982). Similarly, lesioning the hippocampal connections with the lateral septum did not interfere with performance on a radial maze task (Jarrard, 1978). These findings highlight the possible contribution of theta rhythm to memory formation. The hippocampus projects to the lateral septum, which projects to the medial septum.

Cooling the ventral hippocampal commissure eliminates phasic firing of cells in the lateral septum, but rhythmicity is preserved in the medial septum, suggesting that the medial septum does not require hippocampal feedback (through the lateral septum) for its rhythmicity (Stewart and Fox, 1987). This explains why lesions in the medial septum (which eliminate hippocampal theta), and not in the lateral septum (which preserve hippocampal theta), cause the behavioral deficits mentioned above. Overall, lesions in the medial septum cause behavioral deficits that resemble the effect of hippocampal lesions. The lesioned animals are hyperactive, have difficulty in inhibiting their responses on a passive avoidance task, have difficulty in habituation and extinction and are more impaired on working and spatial memory tasks (Fried, 1972; Isaacson, 1982; Stanton, Thomas and Brito, 1984; Thomas, Brito, Stein and Berko, 1982; Winson, 1978).

Another evidence for the role of the medial septum in memory formation comes from studies on conditioning of the nictitating membrane in rabbits. In these experiment, an air stream is puffed to the eye of a rabbit. If 250 msec earlier, a tone will be presented, classical conditioning will develop, the tone eliciting now the extension of the nictitating membrane and closure of the eye lid. Hippocampal pyramidal cells develop a neuronal model of the behavioral response, firing at a rate similar to the extension rate of the membrane, and preceding it by 50 msec. Similar changes are seen in the lateral septum. The medial septum, however, shows a constant change of firing at the onset of the tone, but does not develop a neuronal model of the behavior. Medial septal cells also fire during un-paired presentations of the US and CS, whereas no changes are seen in the

activity of the hippocampus or lateral septum. This supports the idea that the medial septum acts as a arousal or orientation signal, signaling the hippocampus to start processing the incoming information (Berger and Thompson, 1977). As described in the Introduction, animals acquire this conditioning faster if their EEG is at a theta rhythm range (Berry and Thompson, 1978), suggesting that theta might facilitate acquisition of this task.

The phase locking of numerous neurons suggests that optimal information processing will take place only during certain times. What are the advantages of such a mechanism ? One possible advantage that was already mentioned, is to allow novel sensory input to arrive at times and periodicities that are conductive for LTP induction. Komisaruk (1977) suggests that such a rhythmic mechanism has advantages for animals with small brains, such as the rat. The rat is faced with a possible trade-off: to have as many neurons available as possible some of the time, or occupying many neurons busy in information processing all the time, thus leaving only a small number of neurons available for sudden, unexpected (life-threatening ?) stimuli. A rhythmic mechanism, such as theta rhythm, enables the performance of particular activities only at certain times, and have a maximal number of neurons available to process other kinds of information at other times. Theta rhythm assures that information processing will take place 5-7 times a second, making the neurons free during the remaining time.

Under certain optimal conditions, made available by the synchronizing and depolarizing effects of theta, LTP may be formed. How is LTP related to long-term storage in the hippocampus? As described

earlier, LTP is associated with structural changes in the hippocampus, such as enlargement of the dendritic spine, and thickening of the active zone. Such changes may be at the basis of long-term storage. Information reaches the hippocampus along highly topographically organized pathway: the perforant path. The lateral perforant path, carrying information from several supra-modal areas, occupies the distal third of the dendritic tree of the granule cells, while the medial perforant path carries supra-modal information (excluding olfactory information), and occupies the mid-third portion of the dendritic tree. When LTP is formed, structural changes take place at certain synapses, that are activated by a certain combination of inputs, perhaps depending on the supra-modal nature of the episode. When the animal encounters the same episode, the same set of synapses is activated again, but it responds now differently, because the synapses are potentiated. This potentiation implies a larger amount of transmitter release, formation of new receptors, decrease of spine resistance, growth of dendritic branches or all of these combined. These changes might provide a signal of familiarity of the episode. This signal is send back to association cortex, where presumably memories are stored, and activates the memory of the episode. The basic assumption is that the medial temporal lobe is a reservoir of random connections that are selectively strengthened by LTP (Halgren, 1984). This accounts, according to Halgren, for the effects of stimulation of the medial temporal lobe on memory experiences. Such stimulation leads to Deja Vu phenomena, and penetration of memory images to consciousness. It is suggested that association cortex generates these images as a response to the medial-temporal lobe stimulation.

A similar theory is suggested by Teyler and Discenna (1984). They suggest that the hippocampus stores a map of locations of cortical sites, or modules of the neocortex and other areas. Sensory information is registered in sensory and association areas in assemblies of cortical modules. The cortical module is a replicating structure with a changing anatomy from region to region. Information is registered and stored in time and space in arrays of modules, which connect with other modules. The hippocampus stores a map or index of these modules through LTP, which potentiates neocortical-limbic synapses. For every input, millions of modules are activated, and hippocampal sites, which contain the modules indexes, are also activated. The primary storage of an episode takes place through LTP. Re-activation of these modules in specific temporal-spatial pattern will imitate the original experience, and a match will be formed between the episode and the hippocampal index, leading to recognition. Such an index will be strengthened with repeated activation of neocortical-hippocampal pathways; otherwise, the index decays. If only a part of the index is activated, there is partial recognition. If the index threshold is passed, full recognition takes place. During recall, the hippocampus itself activates the index, producing a sense familiarity. This theory implies that the hippocampus monitors all the time the pattern of incoming cortical information to see if something will match an index. If the input will impinge on potentiated hippocampal synapses, recognition will take place. Repeated activation of the index causes an increment of a neocortical circuit, and the hippocampal index becomes redundant. This explains why old memories and certain well-rehearsed tasks are spared after hippocampal

lesions.

The effect of theta rhythm may not be only on sensory thresholds but also on motor output. As mentioned in the Introduction, theta is highly correlated with movement in the rat. Semba and Komisaruk (1978) found that rats press or release a lever at a certain theta phase. Lever release was made on the positive phase while lever press was made on more negative portions of the phase. Buno and Vellit (1977) also report that increase of theta amplitude precedes lever press in up to 1 sec., this happening only if the press was on a certain phase. Medial septal lesions eliminate theta and increase the rate of pressing, suggesting that the septum is exerting an inhibitory influence on the movement. This finding might explain why in septal and hippocampal animals, there is increase in activity in an open field, and inability to withold responses in tasks such as a passive avoidance task. Can theta modulate movement in a manner similar to its modulatory effect on LTP threshold? The phase-locking of movement to theta is one indication that such an effect is possible. Recently, an anatomical projection has been described that might subserve such an effect. CA1 cells project to the subiculum, from which projections are made to layers 4-6 of both divisions of the entorhinal cortex (Kohler, 1985). A retrograde analysis of entorhinal cortex connections has revealed that layer 4 in the lateral entorhinal cortex projects heavily to the motor cortex (Swanson and Kohler, 1986). The medial septum also projects to the entorhinal cortex, and theta was recorded in layers 3-6, and was found to be in phase with CA1 theta (Mitchell and Ranck, 1980). In addition, the lateral entorhinal cortex was found to project to the nucleus accumbens, caudate and putamen in the rat, suggesting a mechanism

through which the hippocampus and entorhinal cortex affect goal-oriented movement (Swanson and Kohler, 1986). As mentioned before, hippocampal and septal lesions cause disinhibition of responses, hyperactivity, and perseverations of responses. These might indicate inability to plan movements accordingly to environmental contingencies, and may result from the absence of the synchronizing effect of theta rhythm on hippocampal and entorhinal cells, on the one hand, and association cortex cells, on the other. Figure 34 represents the main structures that are suggested to be involved in memory functions in the rat brain, and the sites where theta might affect information processing.

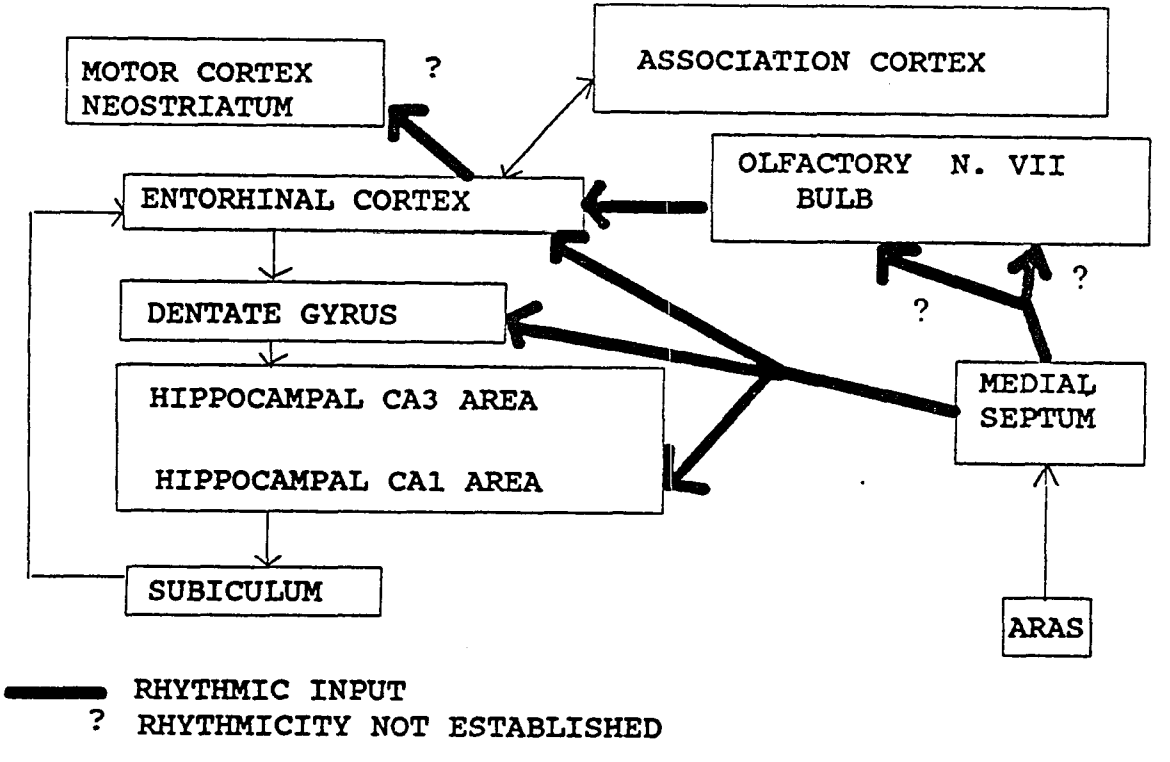
In order to fully understand the effect of theta on LTP threshold, it is essential to continue the present series of experiments in freely-moving animals. As mentioned before, theta rhythm changes according to the behavior of the animal, whether it is locomoting or immobile. In addition, theta is present during REM sleep. These theta rhythms are different pharmacologically and are also controlled by different regions of the medial septum. It is of great interest to see whether the effect on LTP threshold, seen in this dissertation, will be different under locomotion, immobility and REM theta. It is predicted that locomotion theta will have a more facilitatory effect on LTP than immobility theta in the rat, due to the exploratory nature of locomotion in the rat. Similar predictions can be made, concerning sniffing theta. These effects can change when studying the rabbit or the cat, where the behavioral characteristics of theta are different. Theta is also affected differentially by different drugs. A

pharmacological analysis of the kind of theta induced in this dissertation will enable us to better understand the behavioral significance of our findings.

It has been argued that theta represents an inactive hippocampus. Theta appears during orientation, and hippocampal stimulation was found to reduce the orientation response. This has suggested that the hippocampus functions to inhibit old memories from interfering with the present behavior, and theta appears whenever this inhibitory effect is absent. The findings of this dissertation argue strongly against this theory. We believe that theta represents an active hippocampus, which takes part in information processing and possible memory storage, through the facilitatory effects of theta rhythm on LTP induction.

Figure 34 An illustration of a basic memory circuit in the rat, as suggested by this dissertation, based on the known anatomy, physiology, and behavioral studies cited, with special emphasis on the contribution of theta rhythm to cell firing rhythmicity. The ascending reticular activating system (ARAS) activates the medial septum, and it, in turn, generates theta rhythm in the entorhinal cortex, dentate gyrus and hippocampus, driving cells in these regions to fire rhythmically at theta rhythm periodicity, being phase-locked to the local theta. Medial septum also induces rhythmicity in olfactory bulb cells and drives the seventh cranial nerve to activate rhythmically the vibrissae, both effects are relayed through pathways where rhythmicity was not yet established. Association cortex projects supra-modal information to the entorhinal cortex, from which it is projected topographically to the tri-synaptic circuit. At certain phases of theta, LTP can be formed within this circuit. Finally, output goes from the entorhinal cortex back to association cortex, and a putative rhythmic output drives motor cortex and neostriatum cells, to initiate movements. These movements are synchronized with environmental contingencies, as signaled by the olfactory bulb, the vibrissae, and stored information in the hippocampus and association cortex.

Figure 34


MOTOR CORTEX
NEOSTRIATUM
?
ASSOCIATION CORTEX
OLFACTORY N. VII
BULB
ENTORHINAL CORTEX
DENTATE GYRUS
HIPPOCAMPAL CA3 AREA
HIPPOCAMPAL CA1 AREA
SUBICULUM
?
?
MEDIAL
SEPTUM
ARAS
RHYTHMIC INPUT
? RHYTHMICITY NOT ESTABLISHED

9. References

Abraham, W.C., Bliss, T.V.P. & Goddard, G.V. (1985). Heterosynaptic changes accompany long-term but not short-term Potentiation of the perforant path in the anaesthetized rat. J. Physiol., 363, 335-349.

Adey, R.W. (1967). Hippocampal states and functional relations with cortical and subcortical systems in attention and learning. In R.W. Adey & T. Tokizane (Eds.) Structure and Function of the Limbic System, (pp. 222-254), North Holland: Elsevier.

Adey, R.W., Dunlop, L.W., and Hendrix, C.E. (1960). Hippocampal slow waves: Distribution and phase relations in the course of approach learning. Arch. Neurol., 3, 74- 90.

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

Amaral, D.G. (1987). Memory: The anatomical organization of candidate brain regions. In F. Plum (Ed.), Handbook of Physiology: Higher Functions of the Nervous System (pp. 211-294 Bethesda, MD: American Physiological Society.

Amaral, D.G. & Campbell, M.J. (1986). Transmitter systems in the primate dentate gyrus. Unpublished manuscript.

Amaral, D.G. & Kurz, J. (1985). An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. J. Comp. Neurol., 240, 37-59.

Andersen, P. (1978). Long-lasting facilitation of synaptic transmission. In Functions of the Septo-Hippocampal System. Ciba Symposium 58, North Holland: Elsevier.

Andersen, P. (1980). Participating neurons and mechanisms underlying theta activity in unanesthetized rabbits. Prog. Brain Res., 54, 371-380.

Andersen, P. (1982). Physiology. In L.W. Swanson, T.J. Teyler & R.F. Thompson (Eds.) Hippocampal Long-term Potentiation, (pp. 637-641). NRP Bulletin, Vol. 20.

Andersen, P., Bland, B.H., Myhrer, T., & Schwartzkroin, P.A. (1979). Septo-hippocampal pathway necessary for dentate theta production. Brain Res., 165, 13-22.

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

Andersen, P., Holmqvist, B., & Voorhoeve, P.E. (1966). Entorhinal activation of dentate granule cells. Acta Physiol. Scand., 66, 448-460.

Andersen, P. & Hvalby, O. (1986). Long-term potentiation. In R.L. Isaacson & K.H. Pribram (Eds.) The Hippocampus, Vol.4, (pp. 169-186), N.Y.: Plenum Press.

Apostol, G., & Creutzfeldt, O.D. (1974). Crosscorrelation between the activity of septal units and hippocampal EEG during arousal. Brain Res., 67, 65-75.

Artemenko, D.P. (1972). Role of hippocampal neurons in theta-wave generation. Neurophysiol., 4, 531-539.

Assaf, S.Y. & Miller, J.J. (1978). The role of raphe serotonine system in the control of septal unit activity and Hippocampal desynchronization. Neuroscience, 3, 539- 550.

Barnes, C.A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol., 93, 74-104.

Barnes, C.A. & McNaughton, B.L. (1985). An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. Behav. Neuroscience, 99, 1040-1048.

Barr, M.L. (1979). The Human Nervous System. N.Y.: Harper & Row.

Barrionuevo, G., Kelso, S.R., Johnston, D., & Brown, T.H. (1986). Conductance mechanism responsible for long-term potentiation in monosynaptic and isolated excitatory synaptic inputs to hippocampus. J. Neurophysiol., 55, 540-550.

Barrionuevo, G., Schottler, F., & Lynch, G. (1980). The effects of repetitive low frequency-stimulation on control and "potentiated" synaptic responses in the hippocampus. Life Science, 27, 2385-2391.

Baudry, M. & Lynch, G. (1982). Possible mechanisms of long-term potentiation: Role of glutamate receptors. In L.W. Swanson, T.J. Teyler & R.F. Thompson (Eds.) Hippocampal Long-term Potentiation NRP Bulletin, n.20.(pp. 663-671).

Baudry, M., & Lynch, G. (1984). Glutamate receptor regulation and the substrates of memory. In G. Lynch, J.L. McGaugh, & N.M. Weinberger (Eds.) Neurobiology of Learning and Memory, (pp. 431-447), N.Y.: Guilford Press.

Baudry, M., Oliver, M., Gregor, A., Wieraszto, A., & Lynch, G. (1980). Increase in glutamate receptors following repetitive electrical stimulation in hippocampal slices. Life Science, 27, 325-330.

Baxter, D.A., Bittner, G.D. & Brown, T.H. (1985). Quantal mechanism of long-term potentiation. Proc. Natl. Acad. Science, 82, 5978- 5982.

Beckstead, R.M. (1978). Afferent connections of the entorhinal area in the rat as demonstrated by retrograde cell-labeling with horseradish peroxidase. Brain Res., 152, 249-264.

Bennet, T.L. (1971). Hippocampal theta activity and behavior- A review. Comm. Behav. Biol., 6, 37-48.

Bennet, T.L. (1975). The electrical activity of the hippocampus and the process of attention. In R.L. Isaacson & K.H. Pribram (Eds.) The Hippocampus, Vol. 2, (pp. 71-99), N.Y.: Plenum.

Berger, T.W. (1984). Long-term potentiation of hippocampal synaptic transmission affects rate of behavioral learning. Science, 224, 627-629.

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

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

Bland, B.H. (1986). The physiology and pharmacology of hippocampal formation theta rhythm. Prog. Neurobiol., 26, 1-54.

Bland, B.H., Andersen, P., Ganes, T., & Sveen, O. (1980). Automated analysis of rhythmicity of physiologically identified hippocampal formation neurons. Exp. Brain Res., 38, 205-219.

Bland, B.H., Seto, M.G., & Rowntree, C.I. (1983). The relation of multiple hippocampal theta cell discharge rates to slow wave theta frequency. Physiol. Behav., 31, 111-117.

Bland, B.H., Seto, M.G., Sinclair, B.R., & Fraser, S.M. (1984). The pharmacology of hippocampal theta cells: Evidence that the sensory processing correlate is cholinergic. Brain Res., 299, 121-131.

Bland, B.H. & Whishaw, I.Q. (1976). Generators and topography of hippocampal theta (RSA) in the anaesthetized and freely moving rat. Brain Res., 118, 259-280.

Bliss, T.V.P. (1979). Synaptic plasticity in the hippocampus. Trends in Neuroscience, 1, 42-45.

Bliss, T.V.P. & Gardner-Medwin, A.R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the urethanized rabbit following stimulation of the perforant path. J. Physiol., 232, 357-374.

Bliss, T.V.P., Goddard, G.V. & Riives, M. (1983). Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. J. Physiol., 334, 475-481.

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

Brace, H.M., Jeffreys, J.G., & Mellanby, J. (1985). Long term changes in hippocampal physiology and learning ability of rats after intrahippocampal tetanus toxin. J. Physiol., 368, 343-357.

Brady, J.V. & Nauta, W.J.H. (1953). Subcortical mechanisms in emotional behavior: Affective changes following septal forebrain lesions in the albino rat. J. Comp. Physiol. Psychol., 46, 339-346.

Brankack, J. & Fox, S.E. (1987). Current sources for the alternating and sustained potentials of the hippocampal theta rhythms of the rat. Soc. Neurosci. Abstr., 13, 1331.

Broca, P. (1878). Anatomie comparee des circonvolutions cerebrale. Le grand lobe limbique et la scissure limbique dans la serie des mammilers. Rev. d'Anthrop., 1, 285-498.

Brodal, A. (1981) Neurological Anatomy. (3rd. ed.) N.Y.: Oxford University Press

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

Buno, W. & Vellit, J.C. (1977). Relationship of hippocampal theta cycles with bar pressing during self stimulation. Physiol. Behav., 19, 615-621.

Buzsaki, G. (1986). Generation of hippocampal EEG patterns. In R.L. Isaacson & K.H. Pribram (Eds.) The Hippocampus, Vol.4, (pp. 137-167), N.Y.:Plenum.

Buzsaki, G., & Eidelberg, E. (1983). Phase relations of hippocampal projection cells and interneurons to theta activity in the anesthetized rat. Brain Res., 266, 334- 339.

Buzsaki, G., Grastyan E., , Haubenreisen, J., Czopf, J., & Kellenyi, L. (1982). Hippocampal slow wave activity: Sources of controversy. In C. Ajmone-Marsan & H. Matthies (Eds.) Neuronal Plasticity and Memory Formation, (pp. 511-529), N.Y.:Raven Press.

Buzsaki, G., Leung, L-W, S., & Vanderwolf, C.H. (1983). Cellular bases of hippocampal EEG in the behaving rat. Brain. Res. Rev., 6, 139-171.

Carpenter, M.B. & Sutin, J. (1983). Human Neuroanatomy. (8th ed.), Baltimore: Williams & Wilkins.

Chronister R.B., & White, Jr, L.E. (1971). Fiberarchitecture of the Hippocampal, formation: Anatomy, projections, and structural significance. In R.L. Isaacson & K.H. Pribram (Eds.) The Hippocampus, Vol.1, (pp. 9-39), N.Y.: Plenum.

Collingridge, G.L. (1985). Long term potentiation in the hippocampus: Mechanisms of initiation and modulation by neurotransmitters. Trends in Pharmacological Science, 6, 407-411.

Collingridge, G.L. & Bliss, T.V.P. (1987). NMDA receptors- their role in long-term potentiation. Trends in Neurosciences, 10, 288-293.

Collingridge, G.L., Kehl, S.J., & McLennan (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol., 334, 33-46.

Colom, L.V., Ford, R.D. & Bland, B.H. (1987) Hippocampal formation neurons code the level of activation of the cholinergic septohippocampal pathway. Brain Res., 410, 12-20.

Cotman, C.W. & Iversen, L.L. (1987). Excitatory amino acids in the brain- focus on NMDA receptors. Trends in Neurosciences, 10, 263-265.

Cotman, C.W., Monaghan, D.T., Otterson, O.P. & Storm-Mathisen, J. (1987). Anatomical organization of excitatory amino acid receptors and their pathways. Trends in Neurosciences,10, 273-280.

Crowne, D.P. & Radcliffe, D.D. (1975). Some characteristics and functional relations of the electrical activity of the primate hippocampus and a hypothesis of hippocampal function. In R.L. Isaacson & K.H. Pribram (Eds.) The Hippocampus, Vol. 2, (pp. 185-206), N.Y.: Plenum Press.

Desmond, N.L. & Levy, W.B. (1986a). Changes in the numerical density of synaptic contacts with long-term potentiation in the hippocampal dentate gyrus. J. Comp. Neurol., 253, 466-475.

Desmond, N.L. & Levy, W.B. (1986b). Changes in the postsynaptic density with LTP in the dentate gyrus. J. Comp. Neurol., 253, 476- 482.

Diamond, D.M. & Rose, G.M. (1987). Induction of hippocampal long term potentiation in the awake rat using physiologically patterned stimulation. Soc. Neuroscience Abstr., 13, 975.

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

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

Dunwiddie, T., & Lynch, G. (1978). Long-term potentiation and depression of synaptic responses in the rat hippocampus: Localization and frequency dependency. J. Physiol., 276, 353-367.

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

Eccles, J.C. (1983). Calcium in long-term potentiation as a model for memory. Neuroscience, 10, 1071-1081.

Eichenbaum, H., Kuperstein, M., Fagan, A., & Nagode, J., Cue-sampling and Goal-approach correlates of hippocampal unit activity in rats performing an odor-discrimination task. J. Neurosci., 7, 716-732.

Feenstra, B.W.A. & Holsheimer, J. (1979). Dipole-like neuronal sources of theta rhythm in dorsal hippocampus, dentate gyrus and cingulate cortex of the urethane-anesthetized rat. EEG Clin. Neurophysiol., 47, 532-538.

Fifkova, E., & Van Harreveld, A. (1977). Long lasting morphological changes in dendritic spines of dentate granule cells following stimulation of the entorhinal area. J. Neurocytol., 6, 211-230.

Finch, D.M., Wong, E.E., Derian, E.L., Chen, X-H., Nowlin-Finch, N.L. & Brothers, L.A. (1986). Neurophysiology of the limbic system pathways in the rat: Projections from the amygdala to the entorhinal cortex. Brain Res., 397, 205-213.

Fox, S.E. & Ranck, Jr, J.B. (1975). Localization and anatomical identification of theta and complex spike cells in dorsal hippocampal formation of rats. Exp. Neurol., 49, 299-313.

Fox, S.E., Wolfson, S., & Ranck, Jr. J.B. (1983). Investigating the mechanisms of hippocampal theta rhythm: Approaches and progress. In W. seifert (Ed.) Neurobiology of the hippocampus, pp. 303-319, N.Y.: Plenum.

Fox, S.E., Wolfson, S. & Ranck, Jr. J.B. (1986). Hippocampal theta rhythm and the firing of neurons in walking and urethane anesthetized rats. Exp. Brain Res., 62, 495-508.

Fried, P.A. (1972). Septum and behavior: A review. Psychol. Bull., 78, 292-310.

Fujita, Y. & Sato, T. (1964). Intracellular records from hippocampal pyramidal cells in rabbit during theta rhythm activity. J. Neurophysiol., 27, 1011-1025.

Grastyan, E., Karmos, G., Ureczky, L., & Kellenyi, C. (1966). The hippocampal electrical correlates of the homeostatic regulation of motivation. EEG Clin. Neurophysiol., 21, 34-53.

Gray, J.A. (1971). medial septal lesions, hippocampal theta rhythm and the control of vibrissal movement in the freely moving rat. EEG Clin. Neurophysiol., 30, 187-197.

Gray, J.A., Quintao, L., & Aravjo-Silva, M.T. (1972). The partial reinforcement extinction effect in rats with medial septal lesions. Physiol. Behav., 8, 491-496.

Green, J.D. & Arduini, A. (1954). Hippocampal electrical activity in arousal. J. Neurophysiol., 17, 533-557.

Green, J.D., Maxwell, D.S., Schindler, W.J. & Stumpf, C. (1960> Rabbit EEG "theta" rhythm: Its anatomical source and relation to activity in single neurons. J. Neurophysiol., 23, 403-423.

Greenstein, Y.J., Pavlides, C. & Winson, J. (1988). Long-term potentiation in the dentate gyrus is preferentially induced at theta rhythm periodicity. Brain Res., 438, 331-334.

Gustafsson, B. & Wigstrom, H. (1986). Hippocampal long-lasting potentiation produced by pairing single volleys and brief conditioning tetani evoked in separate afferents. J. Neurosci., 6, 1575-1582.

Gustafsson, B., Wigstrom, H., Abraham, W.C. & Huang, Y.-Y. (1987). Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. J. Neurosci., 7, 774-780.

Halgren, E. (1984). Human hippocampal and amygdala recording and stimulation: Evidence for a neural model of recent memory. In: L.R. Squire & N. Butters (Eds.) Neuropsychology of memory, pp. 165-182, N.Y.: Guilford Press.

Halgren, E., Babb, T.L. & Crandall, P.H. (1978). Activity of human hippocampal formation and amygdala neurons during memory testing. EEG Clin. Neurophysiol., 45, 585-601.

Harris, E.W., Ganong, A.H. & Cotman, C.W. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Res., 323, 132-137.

Herron, C.E., Lester, R.A., Coan E.J., & Collingridge, G.L. (1985). Intracellular demonstration of an NMDA receptor mediated component of synaptic transmission in the rat hippocampus. Neurosci. Lett., 60, 19-23.

Herron, C.E., Lester, R.A., Coan, E.J. & Collingridge, G.L. (1986). Frequency-dependent involvement of NMDA receptors in the hippocampus: a novel synaptic mechanism. Nature, 322, 265-268.

Hesse, G.W. & Teyler, T.J. (1976). Reversible loss of hippocampal long-term potentiation following electroconvulsive seizures. Nature, 264, 562-564.

Higashima, M. & Yamamoto, C. (1985). Two components of long-term potentiation in Mossy fibers-induced excitation in hippocampus. Exp. Neurol., 90, 529-539.

Hjorth-Simonsen, A. (1974). Projection of the lateral part of the entorhinal area to the hippocampus and fascia dentata. J. Comp. Neurol., 146, 219-232.

Hjorth-Simonsen, A. & Jeune, B. (1972). origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. J. Comp. Neurol., 144, 215-232.

Holsheimer, J. (1982). Neuronal sources of the hippocampal EEG. Manuscript.

Hopkins, W.F. & Johnston, D. (1984). Frequency-dependent noradrenergic modulation of long-term potentiation in the hippocampus. Science, 226, 350-352.

Huang, Y.-Y., Wigstrom, H., & Gustafsson, B. (1987). Facilitated induction of hippocampal long-term potentiation in slices perfused with low concentration of magnesium. Neurosci., 22, 9-16.

Hubbard, J.I., Llinas, R., & Quastel, D.M.J. (1969). Electrophysiological analysis of synaptic transmission. Baltimore: Williams & Wilkins.

Irle, E., & Markowitsch, H.J. (1982). Connections of the hippocampal formation, mamillary bodies, anterior thalamus and cingulate cortex. Exp. Brain Res., 47, 79-94.

Isaacson, R.L. (1982). The limbic system. 2nd Ed. N.Y.: Plenum.

Jahr, C.E., & Stevens, C.F. (1987). Glutamate activates multiple single channel conductances in hippocampal neurons. Nature, 325, 522-525.

Jarrard, L.E. (1973). The hippocampus and motivation. Psychol. Bull., 79, 1-12.

Jarrard, L.E. (1978). Selective hippocampal lesions: Differential effects on performance by rats of a spatial task with preoperative versus postoperative training. J. Comp. Physiol. Psychol., 92, 1119-1127.

Jones, E.G., & Powell, T.P.S. (1970). An anatomical study of converging sensory pathways within the cerebral cortex of the monkey. Brain, 93, 793-820.

Klemm, W.R. (1970). Correlation of hippocampal theta rhythm, muscle activity, and brain stem reticular formation activity. Comm. Behav. Biol., 5, 147-151.

Kluver, H., & Barrera, E. (1953). A method for the combined staining of cells and fibers in the nervous system. J. Neuropathol. Exp. Neurol., 12, 400-403.

Kluver, H., & Bucy, P.C. (1939). Preliminary analysis of functions of the temporal lobe in monkeys. Arch. Neurol. Psychit., 42, 979-1000.

Kohler, C. (1985). Intrinsic projections of the retrohippocampal region in the rat brain. I. The subicular complex. J. Comp. Neurol., 236, 504-522.

Komisaruk, B.R. (1970). Synchrony between limbic system theta activity and rhythmical behavior in rats. J. Comp. Physiol. Psychol., 70, 482-492.

Komisaruk, B.R. (1977). The role of rhythmical brain activity in sensorimotor integration. Prog. Physiol. Psychol., 7, 55-90.

Lancaster, B. & Wheal, H.V. (1982). A comparative histological and electrophysiological study of some neurotoxins in the rat hippocampus. J. Comp. Neurol., 211, 105-114.

Larson, J. & Lynch, G. (1986). Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. Science, 232, 985-988.

Larson, J. & Lynch, G. (1987). The NMDA antagonist AP5 blocks a component of the postsynaptic response to theta burst stimulation and prevents LTP induction. Soc. Neurosci. Abstr., 13, 1662.

Larson, J., Wong, D., & Lynch, G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res., 368, 347-350.

Lee, K.S. (1983). Sustained modification of neuronal activity in the hippocampus and neocortex. In W. Seifert (Ed.) Neurobiology of the hippocampus, pp. 265-272, N.Y.:Academic Press.

Lee, K.S., Schottler, F., Oliver, M., & Lynch, G. (1980). Brief bursts of high frequency stimulation produce two types of structural changes in rat hippocampus. J. Neurophysiol., 44, 247-258.

Lee, W.L., Anwyl, R., Rowan, M. (1986). 4-Aminopyridine-mediated increase in long-term potentiation in CA1 of the rat hippocampus. Neurosci. Lett., 70, 106-109.

Lemon, R. (1984). Methods for neuronal recording in conscious animals. N.Y.: Wiley.

Leung, L.S., Lopes Da Silva, & Wadman, W.J. (1982). Spectral characteristics of hippocampal EEG in the freely moving rat. EEG Clin. Neurophysiol., 54, 203-229.

Leung, L.S. & Yim, C.Y. (1986). Intracellular records of theta rhythm in hippocampal CA1 cells of the rat. Brain Res., 367, 323-327.

Lomo, T. (1971). Potentiation of monosynaptic EPSPs in the perforant path-dentate granule cell synapse. Exp. Brain Res., 12, 46-63.

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

Lorente De No, R. (1934). Studies on the structure of the cerebral cortex. Continuation of the study of the ammonic system. J. Psychol. Neurol., 46, 113-177.

Lorente De No, R. (1947). A study of nerve physiology. Part 2. Vol. 132. The Rockefeller Institute.

Lynch, G. & Baudry, M. (1984). The biochemistry of memory: A new and specific hypothesis. Science, 224, 1057-1063.

Lynch. G.S., Gribkoff, V.K. & Deadwyler, S.A. (1976). Long-term potentiation is accompanied by a reduction in dendritic responsiveness to glutamic acid. Nature, 263, 151-153.

Lynch, G., Kessler, M., & Baudry, M. (1984). Correlated electrophysiological and biochemical studies of hippocampal slices. In R. Dingledine (Ed.) Brain slices, pp. 201-225, N.Y.: Plenum Press.

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

Lynch, M.A., Errington, M.L. & Bliss, T.V.P. (1985). Long-term potentiation of synaptic transmission in the dentate gyrus: Increased release of [14C] glutamate without increase in receptor binding. Neurosci. Lett, 62, 123-129.

Macador, A.W., Chalupa, L.M. & Lindsley, D.B. (1974). Differentiation of brain stem loci which affect hippocampal and neocortical electrical activity. Exp. Neurol., 43, 494-524.

Macador, O., Roig, J.A., Monti, J.M. & Budelli, R. (1970). The functional relationship between septal and hippocampal unit activity and hippocampal theta rhythm. Physiol. Behav., 5, 1443-1449.

MacDermott, A.B. & Dale, N. (1987). Receptors, ion channels and synaptic potentials underlying the integrative actions of excitatory amino acids. Trends Neurosci., 10, 280-284.

MacLean, P.D. Psychosomatic disease and the visceral brain. Recent developments bearing on the Papez theory of emotion. Psychosomat. Med., 11, 338-353.

MacLean, P.D. (1954). The limbic system and its hippocampal formation. Studies in animals and their possible relation to man. J. Neurosurg., 11, 29-45.

Macrides, F. (1975). Temporal relationship between hippocampal slow waves and exploratory sniffing in hamsters. Behav. Biol., 14, 295-308.

Macrides, F., Eichenbaum, H.B. & Forbes, W.B. (1982). Temporal relationship between sniffing and the limbic theta rhythm during odor discrimination reversal learning. J. Neurosci., 2, 1705-1717.

Malinow, R. & Miller, J.P. (1986). Postsynaptic hyperpolarization during conditioning reversibly blocks induction of long-term potentiation. Nature, 320, 529-530.

McNaughton, B.L. (1983). Activity dependent modulation of hippocampal synaptic efficacy: Some implications for memory processes. In W. Seifert (Ed.) Neurobiology of the hippocampus, pp. 233-252, N.Y.: Academic Press.

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

Meibach, R.C. & Siegel, A. (1977). Efferent connections of the septal area in the rat. An analysis utilizing retrograde and anterograde transport methods. Brain Res., 119, 1-20.

Mesulam, M.M., Mufson, E.J., Wainer, B.H., & Levey, A.I. (1983). Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience, 10, 1185-1201.

Milner, B. (1970). Memory and the medial temporal regions of the brain. In: K.H. Pribram & D.E. Broadbent (Eds.) Biology of memory, pp. 29-50, N.Y.: Academic Press.

Mitchell, S.J. & Ranck, Jr, J.B. (1980). Generation of theta rhythm in medial entorhinal cortex of freely moving rats. Brain Res., 189, 49-66.

Mitchell, S.J., Rawlins, J.N.P., Steward, O., & Olton, D.S. (1982). Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial maze behavior in rats. J. Neurosci., 2, 292-302.

Monmaur, P. (1982). Hippocampal theta rhythms from CA1 and dentate gyrus generators during paradoxical sleep of the rat: Differential alterations after septal lesions. Physiol. Behav., 28, 467-471.

Monmaur, P., Houcine, O., & Delacour, J. (1979). Experimental dissociation between wakefulness and paradoxical sleep hippocampal theta rhythm. Physiol. Behav., 23, 471-479.

Montoya, C.P. & Sainsbury, R.S. The effects of entorhinal cortex lesions on type 1 and type 2 theta. unpublished manuscript.

Morris, R.G.M., Anderson, E., Lynch, G.S. & Baudry, M. (1986). Selective impairment of learning and blockade of long-term Potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature, 319, 774-776.

Mosko, S., Lynch, G. & Cotman, C.W. (1973). The distribution of septal projections to the hippocampus of the rat. J. Comp. Neurol., 152, 163-174.

Nauta, W.J.H., & Freitag, M. (1986). Fundamental Neuroanatomy. N.Y.: W.H. Freeman.

Nicholson, C. (1979). Generation and analysis of extracellular field potentials. In: Electrophysiological techniques, pp. 93-147, Washington: Society for Neuroscience.

Pandya, D.N. & Kuypers, H.G.J.M. (1969). Corticortical connections in the rhesus monkey. Brain Res., 13, 13-36.

Paxinos, G., & Watson, C. (1982). The rat brain in stereotaxic coordinates. N.Y.: Academic Press

Petsche, H., Gogolak, G. & van Zwieten, P.A. (1965). Rhythmicity of septal discharges at various levels of reticular excitation. EEG Clin. Neurophysiol., 19, 25-33.

Papez, J.W. (1937). A proposed mechanism of emotion. Arch. Neurol. Psychit., 38, 725-743.

Pavlides, C., Greenstein, Y.J., Grudman, M. & Winson, J. (1988). Long-term potentiation in the dentate gyrus is induced preferentially on the positive phase of theta rhythm. Brain Res., 439, 383-387.

Petsche, H., Stumpf, C. & Gogolak, G. (1962). The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. EEG Clin. Neurophysiol., 14, 202-211.

Phillips, M.I. (1973). Unit activity recording in freely moving animals: Some principles and theory. In M.I. Phillips (Ed.) Brain unit activity during behavior. pp: 5-40, Springfield: C.C. Thomas.

Pockett, S., & Lippold, O.C. (1986). Long-term potentiation in hippocampal slices. Exp. Neurol., 91, 481-487.

Pole, P. & Mannier, M. (1970). An activation mechanism in the ponto-bulbar raphe system of the rabbit. Brain Res., 22, 47-61.

Powell, E.H. & Hines, G. (1975). Septohippocampal interface. In Isaacson, R.L. & K.H. Pribram (Eds.) The Hippocampus, Vol. 1, pp. 41-59, N.Y.: Plenum Press.

Quirk, G.J. & Ranck, Jr., J.B. (1986). Firing of single cells in entorhinal cortex is location specific and phase locked to hippocampal theta rhythm. Soc. Neurosci. Abstr., 12, 1524.

Racine, R.J. & Kairiss, E.W. (1987). Long-term potentiation phenomena: The search for the mechanisms underlying memory storage processes. In; N.W. Milgram, C.M. Macleod & T.L Petit (Eds.) Neuroplasticity, learning and memory, pp. 173-197, N.Y.: A. R. Liss.

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

Ramon Y Cajal (1893). The Structure of Ammon's Horn. Springfield: C.C. Thomas. [Estructura del asta de Ammon. Anal. Soc. Esp. Hist. Nat. Madrid, 22, 53-114].

Robinson, T.E. (1980). Hippocampal rhythmical slow activity (RSA:theta): A critical analysis of selected studies and discussion of possible species differences. Brain Res. Rev., 2, 69-101.

Rose, G. (1983). Physiological and behavioral characteristics of dentate granule cells. In W. Seifert (Ed.) Neurobiology of the hippocampus, pp. 449-472, N.Y.: Plenum.

Rose, G., Diamond, D. & Lynch, G.S. (1983). dentate granule cells in the rat hippocampal formation have the behavioral characteristics of theta neurons. Brain Res., 266, 29-37.

Rose, G.M. & Dunwiddie, T.V. (1986). Induction of hippocampal long-term Potentiation using physiologically patterned stimulation. Neurosci. Lett., 69, 244-248.

Rosene, D.L. & Van Heusen, G.W. (1977). Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey. Science, 198, 315-317.

Rudell, A.P. & Fox, S.E. (1984). Hippocampal excitability related to the phase of the theta rhythm in urethanized rats. Brain Res., 294, 350-353.

Rudell, A.P., Fox, S.E., & Ranck, Jr. J.B. (1980). Hippocampal excitability phase-locked to the theta rhythm in walking rats. Exp. Neurol., 68, 87-96.

Sagvolden, T. & Johnsrud, G (1982). Two-way active avoidance learning following medial, dorsolateral, or total septal lesions in rats: Effect of intensity of discontinuous shock. Behav. Neural Biol., 35, 17-32.

Sainsbury, R.S., Heynen, A., & Montoya, C.P. (1987). Behavioral correlates of hippocampal type 2 theta in the rat. Physiol. Behav., 39, 513-519.

Schiffman, H.R., Lore, R., Passafiume, J. & Neeb, R. (1970). Role of vibrissae for depth perception in the rat (Rattus Norvegicus). Animal Behav., 18, 290-292.

Schultz, E.F. & Tapp, J.T. (1973). Olfactory control of behavior in rats. Psychol. Bull., 79, 21-44.

Schuster, T., Krug, M., Plaschke, M., Schulz, E., Wagner, M., & Wenzel, J. (1986). Ultrastructural changes of hippocampal neurons and synapses following long-term potentiation- a morphometric study. In H. Matthies (Ed.) Learning and memory, pp. 115-118, Pergamon Press.

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

Segal, M. & Landis, S. (1974b). Afferents to the septal area of the rat studied with the methods of retrograde axonal transport of horseradish peroxidase. Brain Res., 82, 263-268.

Semba, K. & Komisaruk, B.R. (1978). Phase of the theta wave in relation to different limb movement in awake rats. EEG Clin. Neurophysiol., 44, 61-71.

Shepherd, G. (1979). The Synaptic Organization of the Brain. N.Y.: Oxford U. Press.

Sinclair, B.R., Seto, M.G., & Bland, B.H. (1982). Theta cells in CA1 and dentate layers of hippocampal formation: Relation to slow-wave activity and motor behavior in the freely moving rabbit. J. Neurophysiol., 48, 1214-1225.

Skrede, K.K. & malthe-Sorenssen, D. (1981). Increased resting and evoked release of transmitter following repetitive electrical tetanization in the hippocampus: A biochemical correlate to long-lasting synaptic potentiation. Brain Res., 208, 436-441.

Smith, S.J. (1987). Progress on LTP at hippocampal synapses. A post-synaptic Ca^{2+} trigger for memory storage? Trends Neurosci., 10, 142-144.

Stanton, M.E., Thomas, G.J., & Brito, G.N.O. (1984). Posterodorsal septal lesions impair performance on both shift and stay working memory tasks. Behav. Neurosci., 98, 405-415.

Stanton, P.K. & Sarvey, J.M. (1984). Blockade of long-term potentiation in rat hippocampal CA1 region by inhibition of protein synthesis. J. Neurosci., 4, 3080-3088.

Staubli, U., & Lynch, G. (1987). Stable hippocampal long-term potentiation elicited by 'theta' pattern stimulation. Brain Res., 435, 227-234.

Steward, O. (1976). Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. J. Comp. Neurol., 167, 285-314.

Steward, O. & Scoville, S. (1976). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. J. Comp. Neurol., 169, 347-370.

Stewart, M. & Fox, S.E. (1987). Phases of firing of medial and lateral septal neurons and the importance of hippocampal feedback. Soc. Neurosci. Abstr., 13, 1331.

Swanson, L.W. (1978). The anatomical organization of septo-hippocampal projections. In. Functions of the septo-hippocampal system. pp. 25-48. North Holland: CIBA Foundation Symposium.

Swanson, L.W. (1982). Anatomy. In L.W. Swanson, T.J. Teyler & R.F. Thompson (Eds.) Hippocampal long-term potentiation, NRP Bull. Vol.20.

Swanson, L.W. (1983). The hippocampus and the concept of the limbic system. In W. Seifert (Ed.) Neurobiology of the Hippocampus, pp. 3-19, N.Y.: Academic Press.

Swanson, L.W. & Cowan, W.M.(1977). An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J. Comp. Neurol., 172, 49-84.

Swanson, L.W. & Cowan, W.M. (1979). The connections of the septal region in the rat. J. Comp. Neurol., 186, 621-656.

Swanson, L.W. & Kohler, C. (1986). Anatomical evidence for direct projections from entorhinal area to the entire cortical mantle in the rat. J. Neurosci., 6, 3010-3023.

Swanson, L.W., Sawchenko, P.E., & Cowan, W.M. (1981). Evidence for collateral projections by neurons in Ammon's horn, the dentate gyrus and the subiculum: A multiple retrograde labeling study in the rat. J. Neuroscience, 1, 548-559.

Teyler, T.J. & Discenna, P. (1984). Long-term Potentiation as a candidate mnemonic device. Brain Res. Rev., 7, 15-28.

Teyler, T.J. & Discenna, P. (1987). Long-term potentiation. Ann. Rev. Neurosci., 10, 131-161.

Thomas, G.J., Brito, G.N.O., Stein, D.P., Berko, J.K. (1982). memory and septo-hippocampal connections in rats. J. Comp. Physiol. Psychol., 96, 339-347.

Tombol, T. & Petsche, H. (1969). The histological organization of the pacemaker for the hippocampal theta rhythm in the rabbit. Brain Res., 12, 414-426.

Turner, R.W., Baimbridge, K.G. & Miller, H. (1982). Calcium-induced long-term Potentiation in the hippocampus. Neuroscience, 7, 1411-1416.

Van Heusen, G.W. (1982). The parahippocampal gyrus. Trend. Neurosci., 5, 345-350.

Van Hoesen, G.W., Pandya, D.N. & Butters, N. (1972). Cortical afferents to the entorhinal cortex of the rhesus monkey. Science, 175, 1471-1473.

Vanderwolf, C.H., Kramis, R., Gillespie, L.A., & Bland, B.H. (1975). Hippocampal rhythmic slow activity and neocortical low-voltage fast activity: Relation to behavior. In R.L. Isaacson and K.H. Pribram (Eds.) The Hippocampus, Vol. 1, pp. 101-128, N.Y.: Plenum.

Vanderwolf, C.H., Leung, L.W.S. & Cooley, R.K. (1985). Pathways through cingulate, neo- and entorhinal cortices mediate atropine-resistant hippocampal rhythmical slow activity. Brain Res., 347, 58-73.

Vertes, R.P. (1977). Selective firing of rat pontine gigantocellular neurons during movements and REM sleep. Brain Res., 128, 146-152.

Vertes, R.P. (1980). Brain stem activation of the hippocampus: A role for the magnocellular reticular formation and the MLF. EEG Clin. Neurophysiol., 50, 48-58.

Vertes, R.P. (1981). An analysis of ascending brain stem systems involved in hippocampal synchronization and desynchronization. J. Neurophysiol., 46, 1140-1159.

Vertes, R.P. (1982). Brain stem generation of the hippocampal EEG. Prog. Neurobiol., 19, 159-186.

Vertes, R.P. (1986). Brainstem modulation if the hippocampus. In R.L. Isaacson & K.H. Pribram (Eds.) The hippocampus, Vol. 4, pp. 41-75.

Wenzel, J., & Matthies, H. (1985). Morphological changes in the Hippocampal formation accompanying memory formation and long-term potentiation. In N.M. Weinberger, J.L. McGaugh, & G. Lynch (Eds.) Memory systems of the brain, pp. 150-170, N.Y.: Guilford Press.

Wigstrom, H. & Gustafsson, B. (1984). A possible correlate of the postsynaptic condition for long-lasting potentiation in the guinea pig hippocampus in vitro. Neurosci. Lett., 44, 327-332.

Wigstrom, H. & Gustafsson, B. (1986). Postsynaptic control of hippocampal long-term potentiation. J. Physiol. (Paris), 81, 228-236.

Wigstrom, H., Gustafsson, B. & Huang, Y.-Y. (1985). A synaptic potential following single volleys in the hippocampal CA1 region possibly involved in the induction of long-lasting potentiation. Acta Physiol. Scand., 124, 475-478.

Wigstrom, H., Gustafsson, B., Huang, Y.-Y., & Abraham, W.C. (1986). Hippocampal long-term potentiation is induced by pairing single afferent volleys with intracellularly injected depolarizing current pulses. Acta Physiol. Scand., 126, 317-319.

Winson, J. (1972). interspecies differences in the occurrence of theta. Behavioral Biol., 7, 479-487.

Winson, J. (1974). Patterns of hippocampal theta rhythm in the freely moving rat. EEG Clin. Neurophysiol., 36, 291-301.

Winson, J. (1976). Hippocampal theta rhythm. I. Depth profiles in the curarized rat. Brain Res., 103, 57-70.

Winson, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science, 201, 160-163.

Wyss, J.M. (1981). An autoradiographic study of the afferent connections of the entorhinal cortex in the rat. J. Comp. Neurol., 199, 495-512.

Zucker, E. & Welker, W.I. (1969). Coding of somatic sensory input by vibrissae neurons in the rat's trigeminal ganglion. Brain Res., 12, 138-156.