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The Role of the Hypothalamus in Hippocampal Synchrony

by

Scott D. Oddie

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- Nuclear 0610
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Statistics 0463

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- Africaine 0331
- Canadienne 0334
- États-Unis 0337
- Européenne 0335
- Moyen-orientale 0333
- Latino-américaine 0336
- Asie, Australie et Océanie 0332

Histoire des sciences 0585
Loisirs 0814
Planification urbaine et régionale 0999
Science politique
- Généralités 0615
- Administration publique 0617
- Droit et relations internationales 0616

Sociologie
- Généralités 0626
- Aide et bien-être social 0630
- Criminologie et établissements pénitentiaires 0627
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- Études de l' individu et de la famille 0628
- Études des relations interethniques et des relations raciales 0631
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- Anatomie 0287
- Biologie (Statistiques) 0308
- Biologie moléculaire 0307
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- Écologie 0329
- Entomologie 0353
- Génétique 0369
- Limnologie 0793
- Microbiologie 0410
- Neurologie 0317
- Océanographie 0416
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- Medicale 0760

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Paléobotanique 0345
Paléoécologie 0426
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Palynologie 0427

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Sciences de l'environnement 0768
Sciences de la santé
- Généralités 0566
- Administration des hôpitaux 0769
- Alimentation et nutrition 0570
- Audiologie 0300
- Chimiothérapie 0992
- Dentisterie 0567
- Développement humain 0758
- Enseignement 0350
- Immunologie 0982
- Loisirs 0575
- Médecine du travail et thérapie 0354
- Médecine et chirurgie 0564
- Obstétrique et gynécologie 0380
- Ophtalmologie 0381
- Orthophonie 0460
- Pathologie 0571
- Pharmacie 0572
- Pharmacologie 0419
- Physiothérapie 0382
- Radiologie 0574
- Santé mentale 0347
- Santé publique 0573
- Soins infirmiers 0569
- Toxicologie 0383

SCIENCES PHYSIQUES
Sciences Pures

Chimie
- Généralités 0485
- Biochimie 487
- Chimie agricole 0749
- Chimie analytique 0486
- Chimie minérale 0488
- Chimie nucléaire 0738
- Chimie organique 0490
- Chimie pharmaceutique 0491
- Physique 0494
- PolymÇres 0495
- Radiation 0754

Mathématiques 0405
Physique
- Généralités 0605
- Acoustique 0986
- Astronomie et astrophysique 0606
- Électronique et électricité 0607
- Fluides et plasma 0759
- Météorologie 0608
- Optique 0752
- Particules (Physique nucléaire) 0798
- Physique atomique 0748
- Physique de l'état solide 0611
- Physique moléculaire 0609
- Physique nucléaire 0610
- Radiation 0756

Statistiques 0463

Sciences Appliqués Et Technologie

Informatique 0984
Ingénierie
- Généralités 0537
- Agricole 0539
- Automobile 0540
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- Génie aérospatial 0538
- Génie chimique 0542
- Génie civil 0543
- Génie électronique et électrique 0544
- Génie industriel 0546
- Génie mécanique 0548
- Génie nucléaire 0552
- Ingénierie des systèmes 0790
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Psychologie du comportement 0384
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Psychologie sociale 0451
Psychométrie 0632

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "The Role of the Hypothalamus in Hippocampal Synchrony" submitted by Scott D. Oddie in partial fulfillment of the requirements for the degree of Master of Science.

Dr. Brian H. Bland (Supervisor)
Department of Psychology

Dr. Jos J. Eggermont
Department of Psychology

Dr. Don Kline
Department of Psychology

Dr. Stuart Coupland
Department of Paediatrics

Date September 15, 1992

Abstract

This thesis examined the role of the posterior hypothalamus in the ascending hippocampal synchronizing system in rats. In the first experiment, an intrahippocampal infusion of carbachol resulted in hippocampal theta field activity (θ) and electrical stimulation in the posterior hypothalamic (PH-SUM) region modulated this θ. Subsequent infusion of procaine into the PH-SUM (hypothalamic inactivation) had no affect on intrahippocampal carbachol-induced θ yet rendered the PH-SUM stimulation ineffective. As the PH-SUM recovered from inactivation, stimulation first elicited θ frequencies lower than the pre-procaine values and then these elicited frequencies slowly recovered to pre-procaine values. In the second experiment, hypothalamic inactivation abolished spontaneous θ, θ elicited by tail pinch and θ produced by PH-SUM stimulation. Spectral analysis revealed that after inactivation, the amplitude of hippocampal θ recovered rapidly to pre-procaine values whereas θ frequency recovered slowly. In the third experiment, intraseptal application of carbachol resulted in hippocampal θ and PH-SUM stimulation modulated this θ. Subsequent hypothalamic inactivation significantly reduced the amplitude of θ. In the fourth experiment, electrical stimulation in both the PH-SUM region and pontis oralis resulted in hippocampal θ. Intrahypothalamic application of carbachol resulted in hippocampal θ which could be modulated by PH-SUM and pontis oralis stimulation. Subsequent inactivation of the PH-SUM failed to abolish θ yet significantly reduced its amplitude and blocked the influence of pons stimulation. The results support the role of limbic cortex in producing and maintaining hippocampal synchrony (theta) which allows sensory-motor integration for appropriate behaviour.

Acknowledgments

There are so many people who have affected my life in significant ways that it is difficult to know where to begin. I would first like to thank Brian and Cheryl Bland. Brian's enthusiasm for research and dedication to science are comparable to none. You are the best Chief and so is your lab! Cheryl has been an inspiration since my wife and I first met her. You radiate a warmth which permeates us all and provides a comfort second only to my own mother. Thank you ever so much.

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I want to thank my Grandmother, Pheobe Oddie, for purchasing my first computer for me. The computer catapulted me into a world with which this thesis heavily relied upon. Saving the best until last, my final acknowledgment goes to my parents, Colin and Elsie Oddie. The influence that your guidance, support and love has had on my life couldn't possibly be measured. I can honestly say that your contributions will affect me until the day I die. My only hope is that I am able to provide the same for my children.

Dedication

I dedicate this thesis to significant people in my past, present and future. In the past, I dedicate this thesis to the memory of my Grandfather, Gordon Thomas Oddie. My Grandfather suffered a stroke when I was only nine years old and I remember his courage and stamina. My Grandfather taught me many important things and stimulated the inquisitive nature that I possess of the world and everything in it. In the present, I dedicate this thesis to my best friend and lifemate Jacqueline Oddie. Jackie stuck with me through many hard times when we barely seemed to be muddling through. I couldn't have managed through it with anyone but her. Jackie is also my main source of strength when I am running low and has backed this adventure from its inception. Thanks Jackie, I love you. In the future, I dedicate this thesis to my, as yet, unborn child. I am sure you will have an exciting and significant impact on my life (I can't wait until you get here).

Table of Contents

List of Figures

Introduction

I. *Overview*

A wide variety of functions have been attributed to limbic cortex: perception of time, expression of emotion, sensory-motor integration, learning and memory. As a result, a vast quantity of research has accumulated in an attempt to provide support for the various theories concerning the function of this region. The integrity of limbic cortex is thought to be essential for sensory-motor integration (Bland, 1986). Learning and memory deficits associated with limbic structure damage are well documented (Milner, 1970; Squire, 1986). Degeneration of this region occurs in Alzheimer's disease, an insidious disease which obstructs learning and memory (Katzman and Terry, 1983). Further, stimulation of the limbic cortex has the ability to produce long term potentiation, a phenomenon thought to underlie learning and memory (Kuba and Kumamoto, 1990).

An important structure of limbic cortex is the hippocampal formation and the surrounding parahippocampal cortex (Mishkin, Malamut, and Bachevalier, 1984; Milner, 1970). The hippocampal formation, produces a unique field potential known as rhythmical slow wave activity (RSA) or theta, a sinusoidal like signal. This activity is the largest amplitude rhythmicity produced in the mammalian brain. Thus, a promising endeavour is to examine the electrophysiological properties of the hippocampal formation and the mechanisms which generate theta in an attempt to understand the functional significance of limbic structures.

At this point I would like to summarize some of the properties of hippocampal theta field activity and speculate on their relevance in the hope that it might clarify the topics to follow. Briefly, the hippocampus produces three main forms of field activity; theta, LIA and beta activity. At a behavioural level, theta has been divided into two types; type I theta is related to mobility and can be recorded in the hippocampal formation while the animal engages in voluntary forms of movement such as running, walking, rearing, postural shifts, head movements, etc. Type II theta is related to immobility and seen while an animal is immobile yet processing relevant sensory stimulation (e.g., tones, whistles, light flashes, stroking the animal's

fur, etc.). The term "relevant sensory stimulation" is used operationally, to denote sensory stimuli which an animal processes which may result in the initiation of voluntary motor behaviour. LIA is recorded in the hippocampal formation when an animal engages in automatic forms of motor behaviour such as grooming, licking, feeding, etc. At a pharmacological level the two types of theta have distinct neurotransmitters mediating activity within each system. Type I theta is thought to be produced by a serotonergic system and is blocked by anaesthetics. Type II theta is resistant to many anaesthetics, is produced by a cholinergic system and susceptible to muscarinic blockade. Beta field activity occurs at frequencies within the 30 - 70 Hz range. Although it too has limited behavioural correlates, in this thesis examination of hippocampal field activity was limited to theta and LIA.

One theory which has implicated the hippocampal formation as a site for sensory motor integration has been purported by Bland (1986). The theory was not meant to represent the sole function of the hippocampal formation, but rather one of several possible activities in which the hippocampal formation engages. In this thesis, the medial septum, hypothalamus and pons are regions which are hypothesized to convey levels of ascending excitation to the hippocampal formation which result in changes in the amplitude and frequency of hippocampal theta field activity. These changes in hippocampal theta field activity are thought to play a role in determining appropriate sensory-motor behaviour of the animal.

II. *Anatomy of the Hippocampal Formation*

i) *Limbic Cortex*

Unlike other cortical areas of the brain whose functions are well delineated (e.g., visual and motor cortex), the function of limbic cortex has been somewhat of an enigma. Even this cortical region's nomenclature is indicative of this in the sense that the term "limbic" refers to an anatomic feature of the cortical region, rather than a functional feature. The term "limbic" was derived from Broca's anatomic description of the structures nearest the border (limbus) of the neocortex near the base of the cerebral hemisphere. Broca found the development of this cortical region to be relatively constant in the brains of all mammals. Broca's "le grand lobe limbique"

included the subcallosal, cingulate, and parahippocampal gyri and the underlying hippocampus and dentate gyrus.

The limbic structures were originally thought to subserve the sense of smell, but were later linked to a role in emotive mechanisms (Papez, 1937). Papez related emotional disturbances in his patients to damage of the hippocampus and the cingulate gyrus. He felt a closed circuit existed which processed information from the hippocampus, the site responsible for organizing programs of emotional expression, to the mammillary bodies of the hypothalamus, the site responsible for the expression of emotions. The information then passed to the anterior nuclei of the thalamus, then to cingulate gyrus, the site responsible for subjective emotional states, and finally again to the hippocampus. This came to be known as Papez's circuit. Later, MacLean (1952) hypothesized that limbic structures integrated signals from the external sensory and internal emotive worlds. From these descriptions, it can be seen that the limbic system consists not only of cortical components, but also of subcortical structures. The latter include, besides the hypothalamus and anterior nuclei of the thalamus, the septal nuclei, the preoptic area, the amygdala, and the nucleus accumbens, a part of the basal ganglia. This thesis focuses primarily on limbic structures which convey ascending information from a region of the brainstem, the pontis oralis (pons), to a region of the hypothalamus (the posterior hypothalamic - supramammillary complex (PH-SUM)), the medial septum, and finally to the hippocampal formation.

ii) ***Hippocampal Formation: Intrinsic Circuitry***

Based on phylogenetic and cytoarchitectonic data, the cortex of the mammalian telencephalon can be subdivided into two major categories: the allocortex and neocortex. The allocortex has one or two cell layers, whereas the neocortex has five cell layers (II-VI) and a superficial molecular layer (I). The allocortex can further be subdivided into the archicortex or hippocampal formation and paleocortex or piriform cortex.

A vast quantity of detailed research has focused on the anatomy of the hippocampal formation. For the purpose of this thesis, I refer mainly to the more recent reviews and papers which address major aspects of hippocampal formation

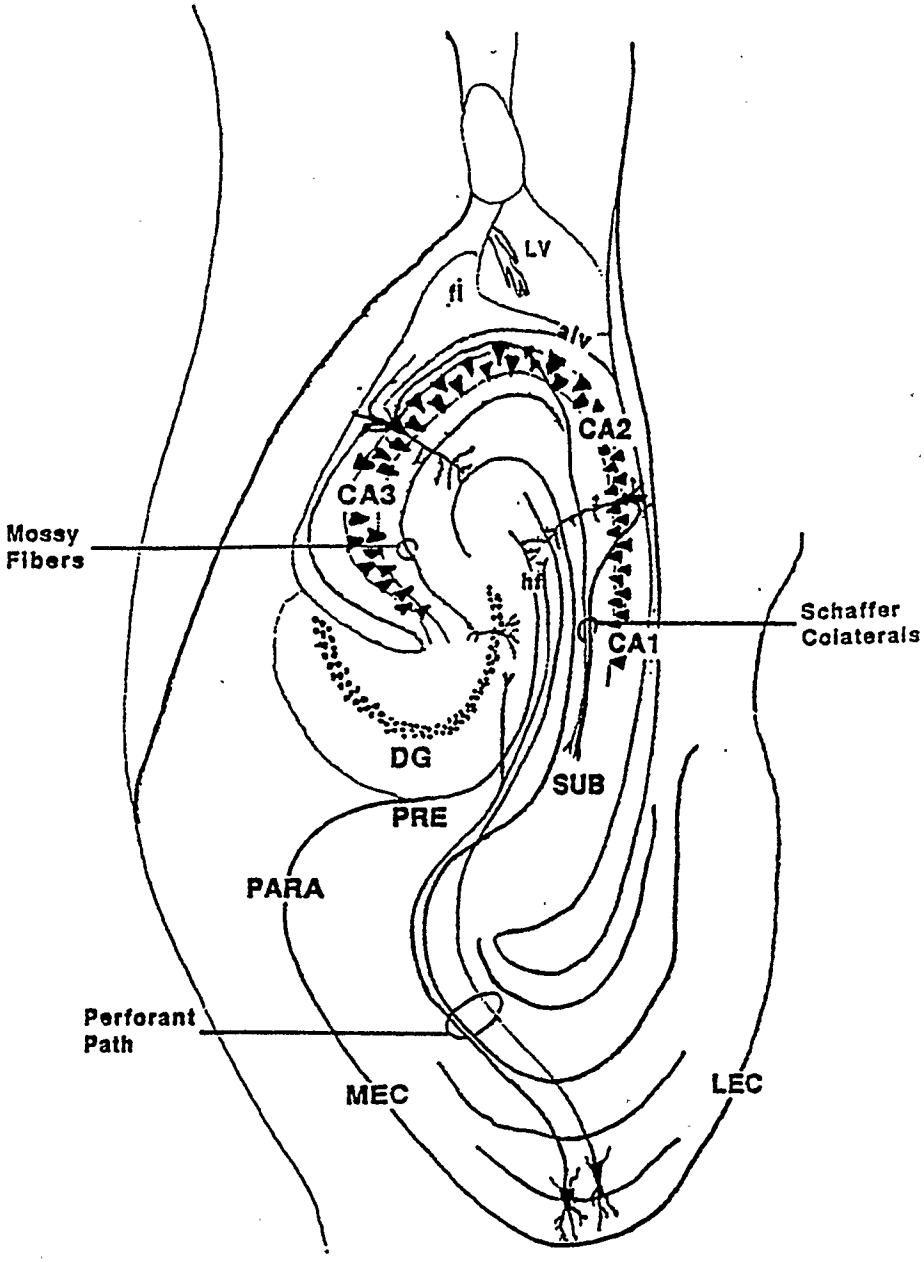
morphology (Swanson, Köhler, and Björklund, 1987; Amaral and Witter, 1989; Lopes Da Silva, Witter, Boeijinga, and Lohman, 1991). The term hippocampus in Greek means "seahorse" and refers to the gross appearance of the structure to early anatomists. When viewed in an appropriate horizontal section, the hippocampal formation resembles a seahorse lying along the inferior horn of the lateral ventricle (see Figure 1).

The hippocampal formation consists of two C-shaped interlocking cell layers: the granular cell layer of the dentate gyrus and the pyramidal cell layer of the *cornu ammonis* (hippocampus proper). While opinions differ, the hippocampal formation has been commonly thought to be comprised of four major divisions: the entorhinal cortex (which consists of the lateral and medial regions), the subicular complex (which consists of the parasubiculum, presubiculum, and subiculum) the fields of the *cornu ammonis* (CA1-CA4), and the dentate gyrus (see Figure 1). Throughout this thesis I refer to the hippocampal formation simply as the hippocampus.

The major divisions of the hippocampus are linked by a trisynaptic pathway conferring an intrinsic circuitry on the hippocampal formation. The basic connections were determined in the now classic Golgi studies of Ramón y Cajal (cf. Lorente de No, 1934). The dentate gyrus receives its major input from the entorhinal cortex via the perforant pathway. The granule cells of the dentate gyrus project via their mossy fibers to the CA3 field of the hippocampus proper. Pyramidal cells of the CA3 field give rise to collateralized axons that terminate within CA3 as associational connections and also provide the major input to the CA1 field of the hippocampus, the so-called Schaffer collaterals. This intrinsic circuitry has come to be known as the trisynaptic pathway and is shown in Figure 1. More recently, Yeckel and Berger (1990) have redefined the anatomy of the trisynaptic pathway and have shown that pathways from entorhinal cortex terminate not only in the dentate, but in the CA3 and CA1 subfields as well. They feel that these monosynaptic pathways are important because the magnitude of cellular response in the three subfields and the time delay to record those responses after entorhinal cortex stimulation were identical. Current ideology of the trisynaptic pathway suggests that afferents from entorhinal

Figure 1. A drawing to show the major features of the hippocampal formation in the rat. The drawing is of a horizontal section and denotes the trisynaptic pathway (Perforant Path, Mossy Fibers, Schaffer Colaterals).

Abbreviations: CA1, *cornu ammonis* field 1; CA2, *cornu ammonis* field 2; CA3, *cornu ammonis* field 3; DG, dentate gyrus; LEC, lateral entorhinal cortex; LV, lateral ventricle; MEC, medial entorhinal cortex; PARA, parasubiculum; PRE, presubiculum; SUB, subiculum; alv, alveus; fi, fimbria; hf, hippocampal fissure.

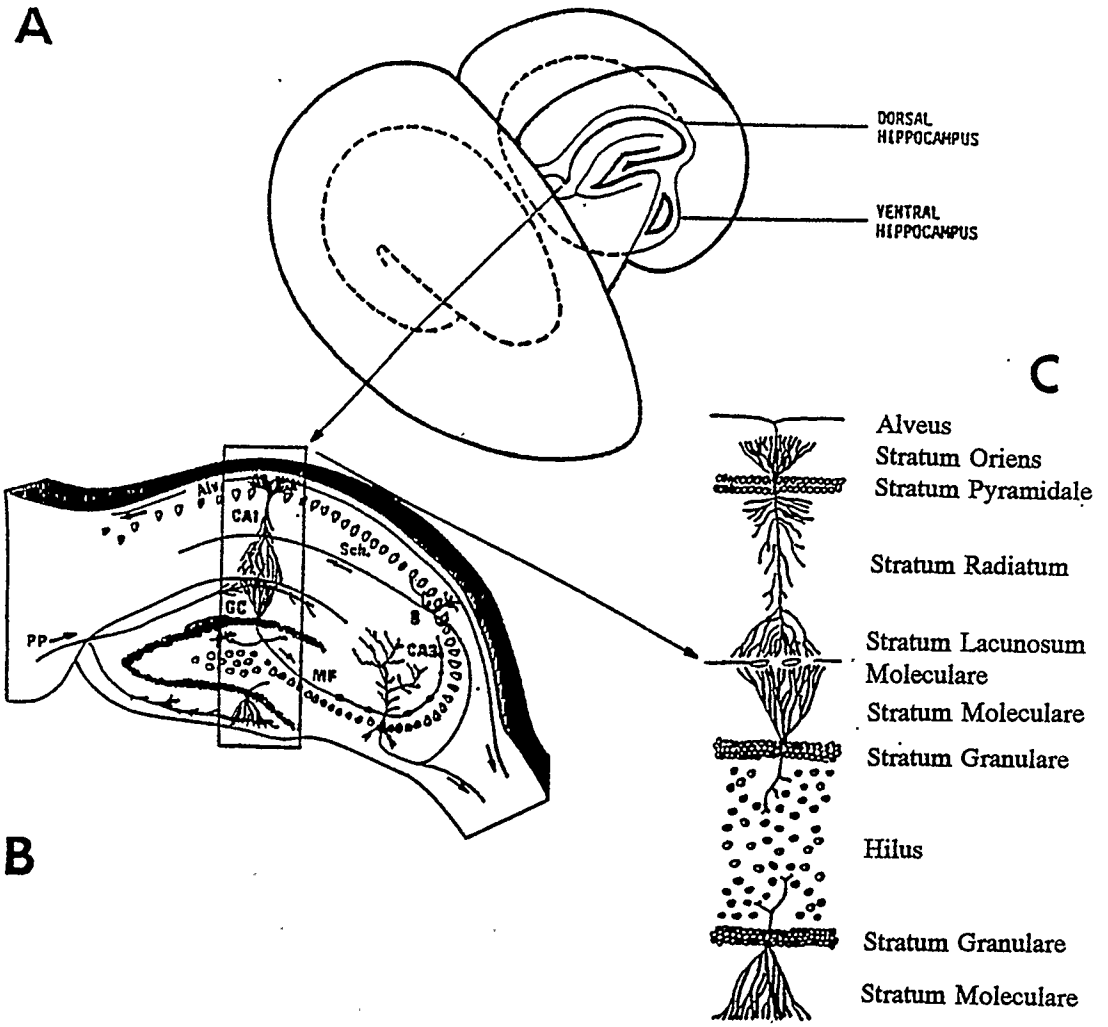

LV
fi
alv
CA2
CA3
Mossy
Fibers
hf
Schaffer
Colaterals
CA1
DG
SUB
PRE
PARA
Perforant
Path
MEC
LEC

cortex project mainly to the dentate, which in turn then conveys information to the *cornu ammonis* via the mossy fibers.

A three dimensional perspective of the hippocampal formation's orientation in the rat brain, its intrinsic circuitry and unique cytoarchitecture are provided in Figure 2, panels A, B, and C, respectively. A slice taken perpendicular to the longitudinal axis of the hippocampal formation (the so-called septo-temporal axis) is shown in panel B. Not only is each slice or lamella interconnected by the trisynaptic pathway noted above, but each also exhibits a longitudinal associational organization (Andersen, Bliss, and Skrede, 1971). Within all fields of the hippocampus a large number of interneurons are present. These interneurons give rise to widespread axonal arborizations, not only along the transverse axis but also along the longitudinal axis. Therefore, interaction among cells is along both axes of the hippocampus.

The interlocking C-shape morphology of the hippocampus provides a layered cytoarchitecture as shown in Figure 2 panel C. The dorsal layer is the alveus and consists of the axons of pyramidal cells and incoming fibers. Beneath this is the stratum oriens, a layer that contains the tufts of the basal dendritic arborizations of the pyramidal cells. This layer also contains a number of different cell body types and the collaterals of the axons of CA3 pyramidal cells which run parallel to the Schaffer collaterals of the stratum radiatum. Next, the stratum pyramidale is formed by the densely packed cell bodies of the pyramidal cells. Still moving ventrally, the stratum radiatum is characterized by sparse cell bodies and several fiber systems; the most important of which is formed by the Schaffer collaterals and form synapses with the dendrites of the CA1 pyramidal cells. In the next layer lies the stratum lacunosum moleculare, which consists mainly of bundles of parallel fibers, some of which are collaterals of pyramidal cells, while others are extrinsic to the hippocampus. Continuing ventrally, the stratum moleculare lies directly adjacent to the hippocampal fissure and contains mainly fibers and dendritic terminals. These fibers and dendritic terminals arise out of the dorsally projecting basal dendritic branches of stratum granulare cells. Next, as just mentioned, lies the stratum granulare and is so named

Figure 2. Anatomy of the hippocampal formation. Part A depicts the gross appearance of the intact hippocampus in the rat. Part B depicts a slice taken perpendicular to the septotemporal axis (lamella). Part C show the various layers of the hippocampus and dentate gyrus after transection.


A
DORSAL HIPPOCAMPUS
VENTRAL HIPPOCAMPUS
B
Alv
CA1
Sch.
GC
PP
MF
CA3
C
Alveus
Stratum Oriens
Stratum Pyramidale
Stratum Radiatum
Stratum Lacunosum Moleculare
Stratum Moleculare
Stratum Granulare
Hilus
Stratum Granulare
Stratum Moleculare

for the closely packed granule cells it contains. Their axons project into the hilus and form a thick bundle, which innervates both mossy cells residing in the hilus and CA3 pyramids (the so-called mossy fibers). The stratum granulare continues with the curvature of the dentate gyrus to form an upper and lower blade. The hilus resides in between these layers. In this region many cell types can be identified and have been aptly termed polymorphic cells. There also is a large number of interneurons in this region.

There are three main inputs to hippocampal formation: (1) the perforant path as noted above, (2) the septal inputs arising mainly from the medial septum, and (3) the commissural fibers originating in the contralateral hippocampus. These inputs were initially documented by Blackstad (1956) in his degeneration studies. More recently it has been shown that a relatively small injection of tritiated amino acid, involving $< 15\%$ of the hilar region along its septotemporal axis, led to labelling of 70% of the contralateral dentate gyrus along the same axis (Van Groen and Wyss, 1988). Thus the latter of the three inputs is also thought to be extensive.

Taken collectively, the hippocampus and dentate gyrus consist of monolayered structures with synaptic inputs arranged in a precise laminated manner. Not only is the intrinsic circuitry extensively organized within a lamella along the transverse axis, but longitudinal projections among lamella and commissural connections are also extensive. Much of this intrinsic circuitry is excitatory in nature, as will be addressed in the pharmacology section to follow. Such extensive circuity permits small areas of activation within a restricted site of the hippocampus to produce enhanced activation of the entire structure. This is a property of the hippocampal formation and its circuitry which will be exploited in this thesis.

iii) ***Extrinsic Afferent/Efferent Pathways***

a) ***Entorhinal Cortex***

The importance of the entorhinal cortex (EC) projection to the hippocampal formation, via the perforant path, has already been mentioned. Of all projections, the EC gives rise to the most prominent input to the hippocampal formation. The Golgi studies of Ramón y Cajal (cf. Lorente de No (1934)) and Lorente de No (1934), and

the degeneration studies of Blackstad (1956) revealed that the fibers of the perforant pathway terminate in the molecular layer of the dentate gyrus, the *cornu ammonis*, and subiculum, although the densest was to the dentate gyrus (Witter, 1989).

The entorhinal projection arises mainly from layers II and III of the EC and reach the hippocampal formation by way of the perforant path and angular bundle (see Figure 1). The medial entorhinal cortex (MEC) projection terminates in the middle third of the molecular layer, whereas the afferents from the lateral entorhinal cortex (LEC) terminate in the outer two thirds of this layer. The MEC fibers also project close to the border of CA3, whereas the LEA fibers terminate closer to the subicular border. It should be noted that the EC afferents project to the same fields of the contralateral hippocampus.

The EC also receives efferent projections from the hippocampus which mainly arise from the subiculum. The majority of subicular fibers terminate in the MEC, however, this projection is strictly ipsilateral in rats (Swanson and Cowan, 1977). To summarize, a main input to the hippocampus originates in the entorhinal cortex and terminates largely in the dentate gyrus. However, recent evidence suggests monosynaptic connections to the other fields of the hippocampus as well. From the dentate gyrus, neural activity is processed along the trisynaptic pathway of the hippocampus and ultimately is sent back to the entorhinal cortex, a major efferent of the hippocampal formation.

b) ***Septum and Hippocampus***

The septal region is found between the anterior horns of the lateral ventricles and courses to form a part of the anterior border of the preoptic region of the hypothalamus. The septal region can be partitioned into four main divisions based upon its cytoarchitecture and afferent-efferent connections (Swanson and Cowan, 1979): (1) the lateral septal nucleus which can be divided into dorsal, intermediate, and ventral parts, (2) the medial division consisting of the medial septal nucleus and the nucleus of the diagonal band of Broca, (3) the posterior division consisting of the septofimbrial and triangular nuclei, and (4) the ventral division consisting of the bed nuclei of the stria terminalis and the small bed nucleus of the anterior commissure.

Generally, the lateral, medial and posterior divisions project more densely to the hippocampal formation whereas the ventral region sends more projections to the amygdala. This thesis is concerned with the medial septal nucleus and the vertical limb of the diagonal band. Hereafter they are considered a single complex (medial septum - diagonal band of Broca complex). I will refer to this region more simply as the medial septum and at times as the septum.

The septum and hippocampal formation share many reciprocal connections which have been studied extensively (for a review see Swanson *et al.*, 1987). The medial septum and vertical diagonal band afferents are thought to be a critical relay for ascending activation of the hippocampal formation (Smythe, Christie, Colom, Lawson, and Bland, 1991; Smythe, Colom and Bland, 1992). The projections from the medial septal nucleus reach all subdivisions of the hippocampus, whereas those from the nucleus of the diagonal band mainly terminate in the subiculum (Nyakas, Luiten, Spencer, and Traber, 1987). Afferents to the dentate gyrus from the medial septum terminate mostly in the hilar region, although some fibers are found within the cell layer (Nyakas *et al.*, 1987). In the *cornu ammonis*, the septal fibers terminate in the stratum radiatum and the stratum oriens of CA3 and CA1.

Nyakas *et al.* (1987) have shown that two main fiber systems project from the medial septum to the hippocampus. These inputs leave the septum via the fimbrial and fornix pathways. The first fiber system consists of thick, coarse fibers with large terminal boutons. This system innervates CA3, the hilus and the stratum lacunosum-moleculare of CA1. The second system has delicate, thin fibres with numerous *en passant* varicosities. This system exhibited a restricted laminar pattern of efferents which were more dense in the CA1 subpyramidal and dentate supragranular molecular zones. As will be pointed out in the pharmacology section to follow, the two systems are thought to mediate different neurochemicals; one cholinergic, the other GABAergic.

The septal afferents to the hippocampus are critical for the production of theta field activity. The septum has been traditionally thought of as the pacemaker of

hippocampal theta activity (Petsche, Stumpf, and Gogolak, 1962). This notion is explored in the experiments contained in this thesis and further elaboration of the septal role to hippocampal function will follow.

Efferent projections from the hippocampal formation to the septum are also extensive. They arise from neurons along the entire longitudinal axis of the *cornu ammonis* and subiculum (Lopes da Silva *et al.*, 1990). These fibers terminate mainly in the lateral septal nucleus in the rat. CA3 sends symmetrical, bilateral projections, whereas the CA1 field only sends ipsilateral efferents to the septal region. The projections from subiculum to the septum are also ipsilateral. Projections from nonpyramidal neurons in the hilar region and *cornu ammonis* to the medial septum and contralateral hippocampus have also been demonstrated (Schwerdtfeger and Buhl, 1986). Such cells were reported to function not only as local circuitry neurons (such as interneurons), but had collaterals terminating in rather distant targets as well. Lopes da Silva *et al.*, (1990) also report that non pyramidal cells of field CA3 give rise to a small projection which terminates in the medial septal nucleus. Medial septal afferents to the EC are prominent. Alonso and Köhler (1984) demonstrated reciprocal connections between the septum and EC using anterograde and retrograde axonal transport methods in rats. Septal afferents to the EC were to layers II and IV of the MEC and LEC and give off collaterals to other parts of the hippocampal region. EC projections to the septum were traced through the fimbria to terminal fields in the lateral septum and vertical limb of the diagonal band of Broca.

c) ***PH-SUM Complex***

There are a number of reports which suggest that certain cell groups in the diencephalon send their afferent projections to the hippocampal formation (Wyss, Swanson, and Cowan, 1979; Riley and Moore, 1981; Dent, Galvin, Stanfield, and Cowan, 1983). The hypothalamic region contains many of these cell groups and has been considered a crucial part of the limbic circuitry for this reason. Wyss *et al.*, (1979) were the first to demonstrate that the hypothalamic region had distinctive inputs to the hippocampal formation. These afferents were from the dorsomedial

hypothalamic nucleus (DMH), the lateral and posterior hypothalamic (PH) areas, the ventral premammillary nucleus, the supramammilliary region (SUM), as well as from parts of the tuberomammillary and lateral and medial mammillary nuclei. This thesis focuses on a diencephalic region comprised of the DMH, PH, and SUM referred to throughout the thesis as the PH-SUM complex. This region of cells has only recently been brought to the attention of researchers as a functional complex by Bland and Colom (1992) and Vertes, Colom, Bland and Oddie (1992). As will be seen, the dorsomedial posterior hypothalamus (DMPH) which Bland has investigated for some time and the SUM which Vertes has intently examined share some common properties in their association to hippocampal theta field activity. For these reasons the two groups of nuclei have been merged in terms of their functional significance in the generation of hippocampal field activity.

Using electrophysiological techniques, Bland (1971) identified a region of cells situated between the dorsomedial and posterior hypothalamic nuclei directly in the region of the PH-SUM. He found that these cells, when stimulated electrically, had a distinct affect on the hippocampal field activity and behaviour of rats. Anatomically, cells in the PH-SUM region span from the medial wall of the hypothalamus to just dorsal the ventromedial nucleus. The anterior hypothalamic area and paraventricular nucleus form its rostral border and parts of the posterior hypothalamus and supramammillary nuclei form its caudal border.

Afferents from the PH-SUM complex to the hippocampus terminate mainly on the somata and proximal dendrites of dentate granule cells (Dent *et al.*, 1983). Ter Horst and Luiten (1986;1987) injected anterograde tracers into PH-SUM neurons and showed that these cells also terminate in all fields of the *cornu ammonis*; the densest projection to CA3. The same investigators demonstrated that the afferents were both ipsilateral and contralateral to the injection site. These authors also noted that PH-SUM neurons projected extensively to the lateral septum.

Direct anatomical evidence demonstrating hypothalamic afferents to the medial septal area is scarce. Tracing studies have focused mainly on hypothalamic projections which send direct afferents to the hippocampal formation and not on

afferents to the septal nuclei. In the latter case, the hypothalamus could influence hippocampal activity ultimately via afferents it sends to the septal region. Including the afferents from the PH-SUM complex to the lateral septum, Swanson has shown that the PH-SUM complex may also send afferents to the medial septum (cf. Gray, 1982). The influence which the PH-SUM complex has on the septum via these afferents, and ultimately the influence the PH-SUM complex may have on hippocampal function via the septal afferents to the hippocampal formation, constitute a key focus of this thesis.

Hypothalamic projections ascend via the fimbria-dorsal fornix system (Swanson *et al.*, 1987). Recall that the septal afferents also ascended through this system. Interestingly, these pathways seem to follow the trajectory of the medial forebrain bundle (MFB) which originates in the brainstem.

d) *Brainstem*

Much of the anatomical data which detail the projections from the brainstem to hypothalamus, septum and hippocampal formation has been reviewed comprehensively by Vertes (1981, 1982, 1988). Vertes concludes that a system which synchronizes hippocampal field activity originates in the pontine reticular formation, specifically the nucleus pontis oralis (pons). He also noted a desynchronizing input which originated in the medial raphe nucleus. Both systems ascend via the MFB to the septum.

Although direct brainstem afferents to the hippocampal formation have been demonstrated (Wyss *et al.*, 1979; Riley *et al.*, 1981), brainstem influences on the hippocampus are thought to be conveyed mainly via the septum and hypothalamus. This suggests that brainstem afferents to the septal nuclei, especially the lateral and medial nuclei as well as the vertical limb of the diagonal band must be considerable. Studying injections of a retrograde tracer into the medial septum, Vertes (1988) has also shown labelling in the PH-SUM complex and pontis oralis. He demonstrated that labelling in the pons was sparse which suggested that an intervening nucleus between the pons and septum must be involved. The PH-SUM complex was hypothesized by him to be a candidate for the intervening cell group. This thesis examines the connections among the limbic structures, using electrophysiological techniques,

starting in the pons through to the hypothalamus, se ^^^Z 316^I^\ finally hippocampus in an attempt to attach some functional significance to these structures.

e) ***Ascending Synchronizing System***

This thesis considers the extrinsic brainstem inputs to the hippocampal formation as a system whose components influence hippocampal theta field activity. The pathway originates in the pons, ascends to the PH-SUM through to the septum, and finally to the hippocampal formation. The detailed topic of extrinsic influences on hippocampal field activity will be given in the section on electrophysiology, having thus far alluded to these limbic regions as having the ability to "influence" hippocampal theta field activity. Electrical stimulation at different levels of the ascending system has the ability to synchronize hippocampal field activity. This synchronized activity has been referred to as rhythmical slow activity (RSA) or theta. The remaining details of hippocampal electrophysiology will follow and the reader should focus on the ability of this system to influence hippocampal field activity. The terminology used to refer to this concept will be the ascending hippocampal synchronizing system. This nomenclature has been used by Bland (1992) in an attempt to attach functional significance to limbic structures, rather than anatomical characteristics.

III. ***Electrophysiology of Hippocampal Field Activity***

i) ***Theta field activity***

The cytoarchitecture of the hippocampus makes it capable of generating distinct electrophysiological field activities. Jung and Kornmüller (1938) were the first to make reference to the rhythmical field potential recorded in the hippocampus of rabbits during stimulation of their peripheral nerves. It wasn't until some years later when Green and Arduini (1954) made a systematic description of the field potential which they found in the hippocampi of rabbits, cats and monkeys. They recorded a rhythmic, sinusoidal pattern with a frequency range of 4 - 7 Hz in urethane-anaesthetized animals. This field activity was termed theta since its frequency was similar to the theta range (4-7 Hz) defined for neocortical regions. The range of frequencies in freely moving rodents is 3 - 12 Hz. Thus the term theta

is a misnomer but is so well entrenched that it continues to be used. The rhythmical field activity has also been called rhythmical slow activity (RSA) (Vanderwolf, 1969).

The first extensive study which examined the relationship between hippocampal field activity and behaviour in rats was done by Vanderwolf (1969). Vanderwolf noted that specific types of behaviour in rats correlated with distinct forms of hippocampal field activity. He described three forms of electrical activity recorded in the hippocampus: (1) rhythmical slow activity (RSA - theta), (2) large amplitude irregular activity (LIA), and (3) small amplitude irregular activity. He also categorized the animal's behaviour into two main types which he denoted as type 1 and type 2 behaviour. Type 1 behaviours were forms of voluntary movement such as walking, running, rearing, jumping, head movements and manipulating objects; all of these behaviours were accompanied by hippocampal theta field activity of 7 - 12 Hz. Type 2 behaviours were involuntary or more automatic forms of movement such as grooming, licking, chewing, scratching, and sniffing; all of these behaviours were accompanied by hippocampal LIA.

Given these distinctions, the main focus in the literature has been on these two types of hippocampal field activity: theta and LIA. As an animal shifts from voluntary to more automatic forms of behaviour (almost its entire behavioural repertoire) these field activities predominate in the hippocampus. In addition to theta and LIA, Vanderwolf, Leung, Baker and Stewart (1985) have recently described a third form of hippocampal field activity known as beta which has a frequency of 20-70 Hz. Beta also seems to be correlated with certain behaviours, but currently not much is known about it. This thesis focuses on hippocampal theta and LIA.

The hippocampal theta field activity which occurs during voluntary movement has been referred to as type I theta and is related to voluntary motor movements made by an animal. There is a second type of theta which occurs during immobility. This form of theta has been referred to as type II theta (Kramis, Vanderwolf and Bland, 1975) and has been shown to occur when an animal is processing sensory stimulation which is relevant to the animal, in that it results in the animal initiating or changing

its ongoing motor behaviour (Bland, 1986). Type II theta has been shown to occur during visual, auditory, and tactile stimulation.

At appropriate levels of urethane anaesthesia, rats will cycle between LIA and type II theta field activity. This type of spontaneous field activity has operationally been defined as spontaneous LIA and spontaneous theta. Hippocampal type II theta field activity can also be produced by pinching the tail of a urethane anaesthetized rat. I refer to this type of hippocampal theta field activity as sensory-induced or tail pinch theta. The ability to elicit hippocampal theta activity with this form of stimulation reinforces the notion that type II theta is related to sensory processing. A distinction between the two types of hippocampal theta field activity is that type II theta has been shown to have a lower frequency range than type I theta field activity (3 - 8 Hz vs. 7 - 12 Hz, respectively) although there is some overlap. This was one of the first indications that the two thetas are subserved by different systems.

The pharmacology of type I and II theta field activity has also been determined to be distinct, and is discussed in the section to follow on the pharmacology of hippocampal field activity. However, it should be mentioned here that type II theta is mainly cholinergic in nature and sensitive to cholinergic blockade while type I theta field activity is thought to be serotonergic (Vanderwolf and Baker, 1986). Type I theta is sensitive to anaesthetics and cannot be recorded in urethane anaesthetized animals. The pharmacological distinctions of the two types of theta is briefly presented here since it reinforces the idea that there are two theta systems; one related to sensory processing and one related to motor processing. Based on these findings, the hippocampal formation has been postulated to be a site for the integration of sensory and motor information (Bland, 1986).

ii) ***Theta Profiles and Mechanisms of Theta Generation***

a) ***Profiles and Dipoles***

The cytoarchitecture of the hippocampus has evolved to allow the structure to produce a field potential known as theta. The amplitude and phase of theta field activity recorded differs as one passes the recording electrode through the laminar structure of the hippocampal formation. This technique of recording the field activity

while moving ventrally through a structure is referred to as a depth profile. In curarized rats, Winson (1976) showed that the depth profile of hippocampal theta changed as the recording electrode was moved ventrally through the different layers of the hippocampus. He found theta in the dorsal region of the stratum radiatum, but as the electrode continued ventrally there was a null zone where no theta was recorded, and eventually a second theta peak was found. The second peak was ventral the hippocampal fissure and had the following characteristics: 1) it was 180° out of phase with the theta found above the stratum radiatum; and 2) it was approximately twice the amplitude. Other researchers have confirmed the findings of Winson in urethane anaesthetized rats and cats (Green and Rawlins, 1979; Bland, Sainsbury and Creery, 1979). The results of these depth profiles taken together suggest that there are two zones of theta generation, one located in the stratum oriens of CA1, and a second located in the stratum moleculare of the dentate.

To complicate matters further, it was found that the depth profile in freely moving rats was different (Winson, 1974; Bland and Whishaw, 1976). Winson found amplitude maxima in both the CA1 region and the area ventral to the hippocampal fissure (dentate). The two signals were out of phase and CA1 theta had a lower amplitude, however, the second profile differed from the one discussed above in that the phase shift was gradual (over 400 μm) rather than rapid, and there was no null zone below the pyramidal cell layer. This was later verified by Bland *et al.* (1976) as well as Feenstra and Holsheimer (1979). A similar profile has been confirmed in rabbits and cats (Bland, Andersen, and Ganes, 1975; Bland, Sainsbury, and Creery, 1979, respectively). All reports seem to conform to the notion that there are two regions of theta generation, one located in the stratum oriens of CA1, and the other located in the stratum moleculare of the dentate gyrus. The only difference was in the phase shift and null zone.

An explanation of the phase shift was first purported by Feenstra *et al.*, (1979). They developed a double dipole model which explained the two sources of theta. In the model each source had a dipole centered at the site where a phase reversal was encountered. The amplitude of the field potential recorded depended

upon the strength of the source and the location of the recording electrode. However, the model did not sufficiently explain the gradual phase shift seen in the profile of freely moving rats. Buzsaki, Leung, and Vanderwolf (1983) proposed a model of theta profiles which suggested that two inputs onto a CA1 pyramidal cell were involved; distal and proximal. In this model, it was proposed that the distal inputs governed movement related type I theta and the proximal inputs generated the type II sensory related theta. Although the model accounted for the absence of the null zone in freely moving preparations, they assumed that the inputs arrived synchronously, which did not provide an adequate description of the gradual phase shift. In an attempt to explain the gradual phase shift, Leung (1984a) demonstrated that it was the relative contributions of the type I motor system and type II sensory system which determined the type of shift. Activation of type II theta by itself produced a rapid 180° phase shift. When activation of the type I system was added during the occurrence of type II theta, a gradual phase shift was seen. Leung (1984b) also provided a mathematical model which explained the differences seen in the phase shift across different preparations.

Leung's model proposes two spatially distinct yet overlapping dipole fields. The field potential which shows a quick phase reversal and the presence of a null zone was thought to be generated by somatic (proximal) inhibition and was referred to as dipole I. Dipole I has an amplitude maxima at the basal dendritic and the distal apical dendritic layers, with a distinct null zone and phase reversal at the apical side of the CA1 pyramidal cell layer. The second dipole (dipole II) was produced by rhythmic distal dendritic excitation and was phase shifted 30° - 90° from dipole I (somatic inhibition). This time delay was thought to account for the gradual phase shift seen in the freely moving rat. Further, it was suggested that dipole I resulted from the type II theta input which originated from the cholinergic septohippocampal pathway. Dipole II accounted for the type I theta input, probably originating in both the septum and entorhinal cortex. Later, support for Leung's model was found by Buzsaki, Czopf, Kondakor, and Kellenyi (1986). They looked at depth profiles through the hippocampal formation and using current-source density (CSD) analysis

determined differential affects of urethane and atropine on the two theta systems. Theta in the CA1-dentate axis had power maxima at about the hippocampal fissure, hilus, outer molecular layer of the dentate and in stratum oriens of CA1. A gradual shift of phase occurred in stratum radiatum of CA1. CSD analysis revealed multiple sinks and sources which showed cyclic changes with theta in the CA1-dentate axis. When the cholinergic pathway from the septum was blocked by atropine a null zone in the middle of stratum radiatum of CA1 occurred and a steepened phase shift was prominent. Under urethane anaesthesia a null zone was present in the inner stratum radiatum associated with a sudden phase reversal. Thus, the gradual phase shift appears to result from the additive effect of multiple dipoles produced by the synaptic inputs from two separate theta systems. Either system can be influenced in a manner which correspondingly determines the amplitude maxima seen in the profile as well as the type of phase shift.

Lesion studies examining the hippocampal generators reinforce the view discussed above. Whishaw and Sutherland (1982) eliminated CA3 and CA4 using kainic acid injections in rats. They found theta amplitude, frequency, loci of maxima and null zones to be normal despite virtually complete removal of CA3-CA4 fields. As well, both the atropine-resistant (type I theta) and atropine-sensitive (type II theta) forms of theta were present. Thus, the generators located in CA1 and dentate did not depend on circuitry involving the CA3 or CA4 pyramidal cells. The synchronous input to the generators was thought to be from the septum and entorhinal cortex. The findings reaffirm the notion that theta generation occurs in two regions of the hippocampal formation; CA1 and dentate.

b) *Synaptic Potentials Underlying Theta Generation*

Research which has focused on determining the type of synaptic potentials which underlie theta generation is somewhat limited and controversial. There are three main possibilities: 1) theta is produced by rhythmic inhibition of the cells within the *cornu ammonis* and dentate gyrus (inhibitory post synaptic potentials (IPSPs)); 2) theta is produced by rhythmic excitation of hippocampal cells (excitatory post synaptic potentials (EPSPs)); and 3) theta is produced by a combination of excitatory and

inhibitory potentials. Most studies examining this topic utilize intracellular techniques because of their ability to manipulate the membrane potential and reveal the types of synaptic potentials involved in theta generation.

Fujita and Sato (1964) recorded intracellularly from pyramidal cells in the *cornu ammonis* in rabbits and found rhythmic changes in the membrane potential which were synchronized with extracellular theta. They called the oscillation "intracellular theta." Intracellular theta has recently been demonstrated to occur in both phasic theta-on and theta-off cells in the urethane anaesthetized rat (Konopacki, Bland, Colom and Oddie, 1992). Theta-related cell activity is discussed in the following section. Fujita and Sato (1964) also demonstrated that when the cell was hyperpolarized past the reversal potential of K^+, the phase relation of intracellular theta with respect to extracellular theta did not change. The authors stated that if IPSPs underlie intracellular theta, then hyperpolarizing current should have shifted the phase of the intracellular theta. This was not the case. Hyperpolarization of the cell resulted in a greater peak amplitude of intracellular theta rhythm and depolarization attenuated the amplitude. From this they concluded that EPSPs were responsible for the generation of intracellular theta. In support of this, Núñez, García-Austt and Buño (1987), and Muñoz, Núñez and García-Austt (1990) have indicated that rhythmic EPSPs play a prominent role in theta genesis. Their evidence showed increases and decreases of intracellular theta amplitudes with hyperpolarizing and depolarizing currents, respectively, without revealing and changes in the phase of intracellular theta. Further, Núñez, García-Austt and Buño (1990) have shown in CA1 and CA3 pyramidal neurons that hyperpolarizing current injections and Cl^- diffusion both decreased IPSP amplitude. Taken together these studies suggest that rhythmic EPSPs underlie the generation of theta. These findings have been disputed by others.

Another group which demonstrated intracellular theta was Fox, Wolfson and Ranck (1980) who recorded membrane oscillations in CA3 pyramids. They posited that IPSPs were generating the oscillations because when they increased intracellular chloride concentrations, the phase was shifted 90°. They attributed this shift to a

reversal of IPSPs to depolarizing potentials. One would have predicted the phase shift to be 180° however, not 90°, and as a result, they suggested that mechanisms other than just IPSPs were involved. Leung and Yim (1986) stimulated the alveus to produce IPSPs in CA1. They showed that hyperpolarizing current, beyond the reversal potential, caused a phase change in the intracellular theta indicating that theta results from rhythmic modulation of IPSPs. They were not able to rule out the contribution of EPSPs. These studies show reduced intracellular theta amplitude and decreased peak amplitude of synaptically evoked IPSPs at more negative membrane potentials. They further revealed that IPSP amplitude was reduced and eventually reversed during intracellular chloride ion diffusion. Taken together these findings suggest that rhythmic IPSPs are responsible for the genesis of theta in the hippocampal formation. Thus, discrepancies as to whether theta originates from rhythmic EPSPs or IPSPs or both are abundant suggesting further research is needed to clarify the issue.

iii) ***Cellular Correlates of Hippocampal Theta Generation***

Although the present thesis does not report recordings of single cell activity, knowledge of their behaviour is important to an understanding of hippocampal theta field activity. The summation of the synaptic potentials impinging on single cells contributes to the production of hippocampal field activity. Further, cells discharging within the hippocampal formation as well as within some of the nuclei which send afferents to the hippocampus show interesting relationships to the hippocampal field activity.

a) ***Hippocampal Formation***

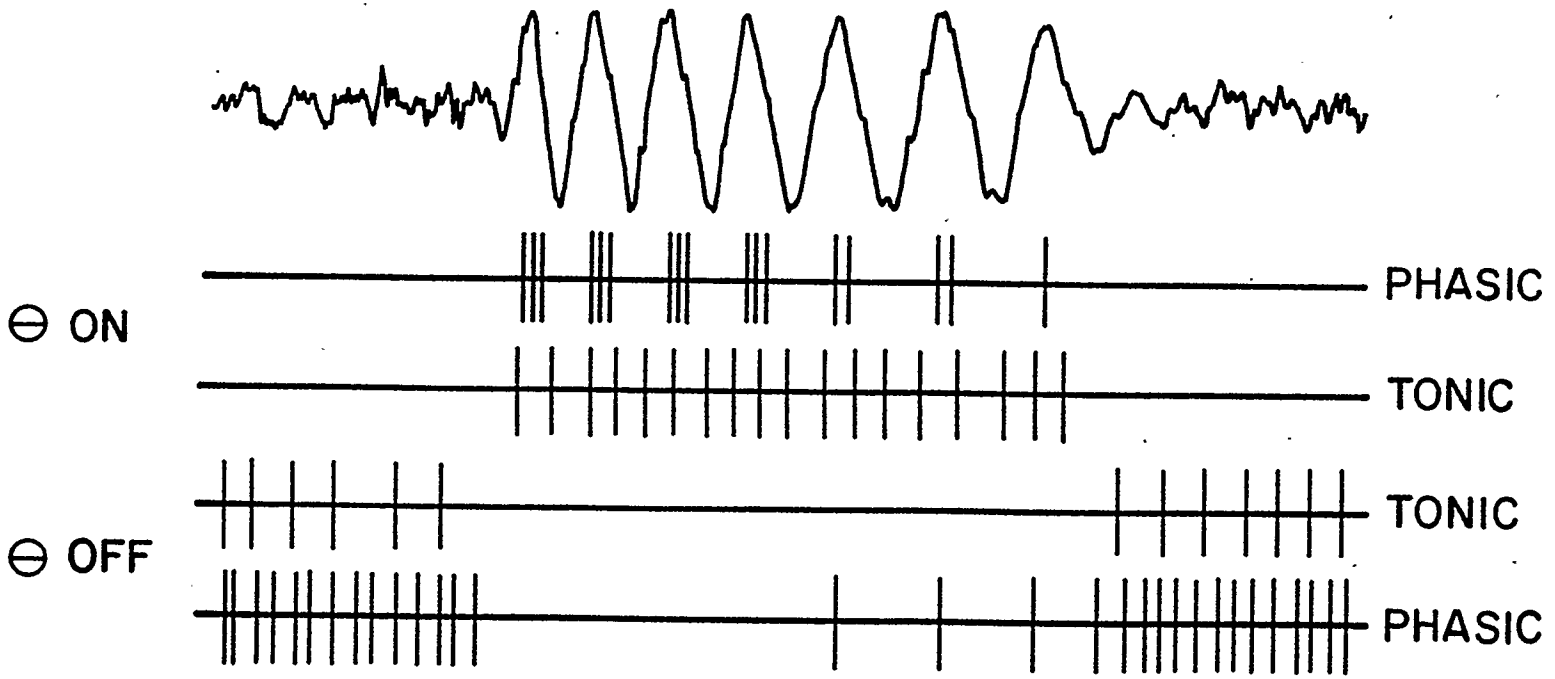
As stated above, a major portion of theta field activity represents the summation of synaptic potentials impinging on a large number of cells. The activity of single cells or units also show unique relationships to hippocampal formation field activity. Theta-related cells have been found in all fields of the *cornu ammonis* and dentate and although researchers have termed them differently (Garcia-Sánchez, Buño, Guentes and Garcia-Austt, 1978; Fox and Ranck, 1981; Buzáki *et al.*, 1983), the data support the notion of two classes of theta-related cells.

Colom and Bland (1987) have described the discharge properties of cells in the hippocampal formation in relation to the spontaneously occurring field activity. Based on these observations they have developed a classification for theta-related cells. Figure 3 demonstrates the cell classifications they have provided for the hippocampus. The top trace of figure 3 provides an analog of hippocampal field activity showing LIA (the desynchronized activity at the beginning and end of the trace) and theta activity (the synchronous activity in the middle of the trace), recorded in the hippocampus. Cells were classified as either theta-on or theta-off. Theta-on cells, as their name suggests, discharged during the occurrence of theta in the hippocampal formation. These cells could either be phasic, that is fire rhythmically and in a constant phase with the theta wave, or tonic, which simply increase their discharge rate in a non-rhythmic manner during the presence of theta. Notice also the decreasing discharge rates which accompany decreasing frequencies of theta. Theta-off cells, as their name suggests, stopped firing when theta occurred and discharged during LIA. They also had phasic and tonic divisions (Bland and Colom, 1989). The cell classifications have been documented in both urethane anaesthetized rats and partially in freely moving rabbits (Colom, Roquet and Bland, 1989). Interestingly, the classifications also apply to other limbic structures.

b) *Medial Septum*

Petsche *et al.*, (1962) originally studied the relationship of medial septal cells to hippocampal theta field activity. They described two types of units in the region and classified them as A-units and B-units. A-units discharged randomly. B-units were rhythmic and discharged in bursts phase locked to hippocampal theta while they fired irregularly during LIA. Out of this paper evolved the notion of the medial septum as the "pacemaker" for hippocampal theta field activity. More on this theory will follow but it should be mentioned here that the pacemaker hypothesis has come under considerable scrutiny and no longer appears to be a tenable theory (Stewart and Fox, 1990; Colom, Nassif-Caudarella, Dickson, Smythe and Bland, 1991; Bland and Colom, 1992).

Figure 3. A diagram depicting the classifications of theta-related cells. The top line is the slow wave line illustrating hippocampal LIA and theta field activity. The top two cell lines represents the discharge patterns of phasic and tonic theta-on cells respectively which occurred simultaneously during the two types of slow wave activity. The bottom two cells lines represents the discharge patterns of phasic and tonic theta-off cells respectively which occurred simultaneously during the two types of slow wave activity.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Gaztelu and Buño (1982) were also able to find distinct bursting characteristics of septal cells. They reported three types of cell discharges related to hippocampal theta field activity. Type 1 cells discharged rhythmically in phase with hippocampal theta. The second type of cells (Type 2) were non-rhythmic and discharged during the presence of hippocampal theta whereas the type 3 cells were non-rhythmic and unrelated to hippocampal theta field activity. Ford, Colom and Bland (1989) recorded cells in the medial septum in the region of the vertical limb of the diagonal band of Broca (MS/VDBB). They were able to demonstrate that the cells in this region could be classified using their criteria for theta-related cells in the hippocampal formation. Cells were classified as theta-on or theta-off with phasic and tonic subdivisions for both. In summary, the discharge behaviour of cells in the septum is related to hippocampal theta field activity. Theta-related cells can be classified in a manner similar to hippocampal cells. This suggests that the cell types of Colom and Bland's classifications may be a general characteristic of the organization of the limbic cortex.

c) *PH-SUM Complex*

The importance of the PH-SUM complex in terms of its afferent input to the septal and hippocampal regions was discussed previously. Recall that electrical stimulation in this region produces hippocampal theta (Bland, 1971). Considering the role of this hypothalamic region in the ascending synchronizing system, it is not surprising that Kirk and McNaughton (1991) have recently demonstrated the presence of phasic theta-on cells in the SUM. Further, they infused procaine (a local anaesthetic) into the septum and abolished hippocampal theta field activity, yet demonstrated that the rhythmicity of the SUM cells was unaffected. It would be of interest to determine if tonic theta-on cells as well as tonic and phasic theta-off cells are also present in this region of the hypothalamus.

d) *Brainstem*

I have previously noted that the ascending hippocampal synchronizing system originates in the pons and have discussed its importance in terms of anatomy as well as its ability to evoke hippocampal theta field activity when stimulated electrically. Recently, Nuñez, de Andrés and García-Austt (1991) examined the discharge

characteristics of reticularis pontis oralis (RPO) neurons during hippocampal theta rhythm evoked by sensory stimulation. They found that of the cells recorded, 63.9% increased their discharge rate while 20.8% decreased their firing rate. All cells discharged in a non-rhythmic manner. Furthermore, they demonstrated that microinjections of carbachol (a cholinergic agonist) into the pons region produced hippocampal synchrony (theta) and activated these cells; the effect was blocked by atropine. In terms of Bland's classification scheme, the findings suggest the presence of tonic theta-on, tonic theta-off and nonrelated cells in the pons region.

e) ***Entorhinal cortex***

Dickson and Bland (1991) have recently provided evidence that theta-on and theta-off cells are present in the entorhinal cortex. Dickson and Bland (1992) have also shown that entorhinal theta-on cells increased their discharge rates in response to electrical stimulation of the posterior hypothalamus where theta-off cells decreased their discharge rates. Thus, the PH-SUM region has influence on the discharge characteristics of entorhinal theta-related cells.

iv) ***Extrinsic Influences on Field and Cell Activity***

Under urethane anaesthesia, rats produce both spontaneous theta and theta in response to a tail-pinch. Type II theta can also be produced by electrically stimulating the nuclei which send afferents to the hippocampus. This demonstrated that activation of the ascending system can confer synchrony on the hippocampal field activity. Interestingly, as noted earlier, electrical stimulation of other nuclei desynchronized hippocampal field activity (LIA). Thus the limbic cortex appears to have afferents which are capable of producing synchrony or desynchrony. Furthermore, electrical stimulation can influence theta-related cell activity.

Green and Arduini's (1954) now classic paper was one of the first to demonstrate that electrical stimulation in a number of subcortical structures resulted in theta field activity in the hippocampus. They demonstrated that when electrical stimulation was delivered to the brainstem reticular formation, medial hypothalamus and medial thalamus, theta resulted in the hippocampal formation.

I mentioned previously that Bland (1971) electrically stimulated a group of midline nuclei in freely moving rats which he referred to as the dorsomedial-posterior hypothalamus. Stimulation of this region produced head movements, postural shifts, rearing, walking, running or jumping; all movements were associated with hippocampal theta field activity. Further, he demonstrated that increasing intensities of electrical stimulation produced increasing intensities of motor behaviour. For example, when a rat was placed in a running wheel, increasing intensities of stimulation resulted in increased running speeds (Bland and Vanderwolf, 1972). Similar effects were seen on a jump avoidance task.

As was previously discussed, stimulation at each level of the ascending system results in synchronized hippocampal field activity. At this point I will provide details on each level in the ascending system individually and review the research which examines the extrinsic influence that area has on hippocampal field activity. I will start with the septum and work "downstream", so to speak, to the hypothalamus and then to the pons.

a) ***Septum***

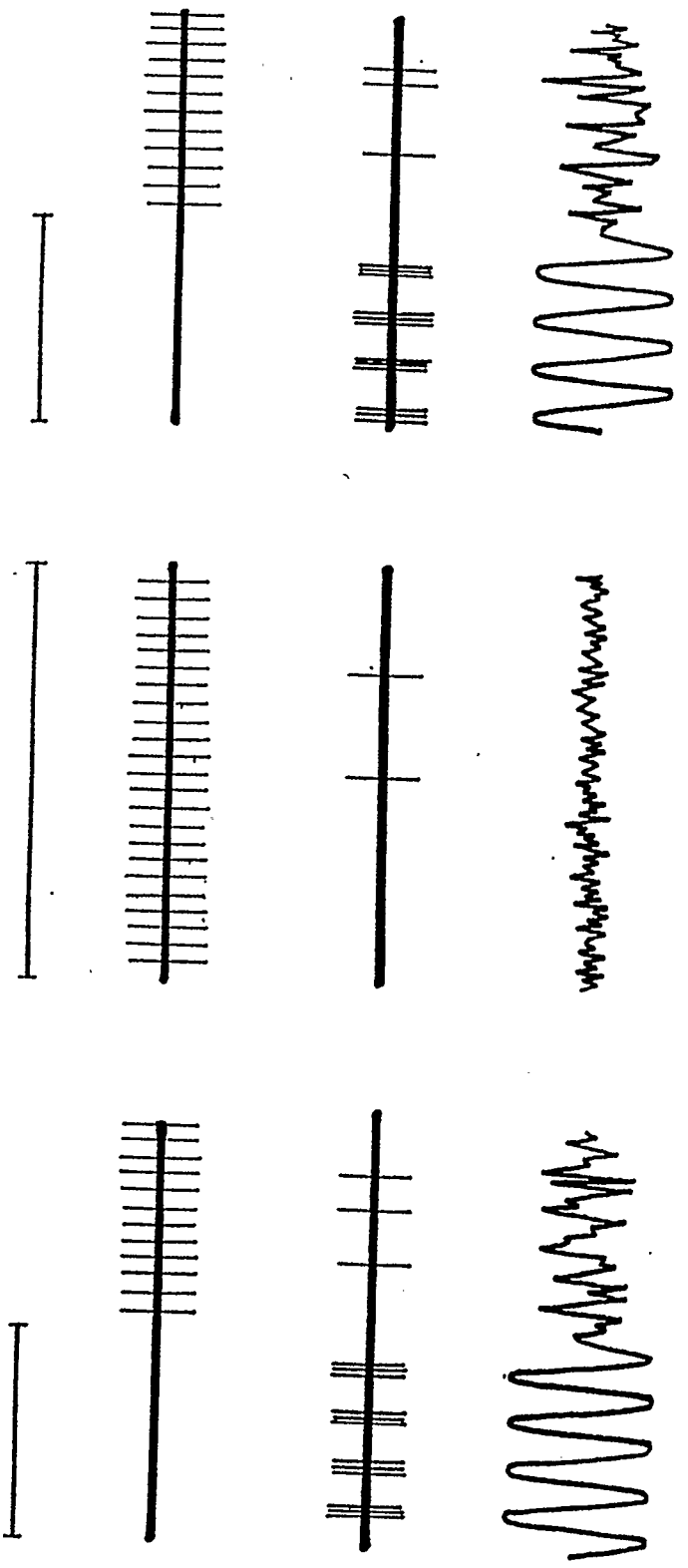
Considering the critical role of the septum in the generation of hippocampal theta field activity, it should be no surprise that electrical stimulation of the septum results in synchronized hippocampal field activity (Ball and Gray, 1971; Kramis and Routtenberg, 1977; Kramis and Vanderwolf, 1980). It has been firmly established that electrical stimulation of the septum results in frequency-specific hippocampal theta field activity. There is an important difference between theta elicited in this way from the septal area and theta elicited by stimulation of the other brain regions mentioned above. In the latter case, theta is elicited by high-frequency stimulation (100 Hz) and as the voltage of the applied current increases, so does the frequency of theta. Only with septal stimulation does each pulse of stimulation produce a corresponding wave in the hippocampal field activity, so that the elicited theta frequency is identical to the frequency of the applied current: 3 Hz stimulation produces 3 Hz theta, 6 Hz produces 6 Hz and so on. One caveat is that the stimulation frequencies must be within the range of normal physiological theta

frequencies (3-12 Hz). High stimulation frequencies produce irregular hippocampal field activity (Ball and Gray, 1971).

The medial septum has been called the pacemaker of hippocampal theta (Petsche *et al.*, 1962). These rhythmic cells were found to be in phase with hippocampal theta field activity and because their destruction apparently abolished hippocampal theta activity, this was a plausible hypothesis (Green *et al.*, 1954; Petsche *et al.*, 1962; Kolb and Whishaw, 1977; Sainsbury and Bland, 1981). Another method revealing the extrinsic influence of the septum on hippocampal field activity has been to temporarily anaesthetize the septum with procaine, a local anaesthetic (Colom, Nassif-Caudarella and Bland, 1990; Smythe *et al.*, 1991). I refer to this technique as reversible inactivation.

Smythe and his associates (1991) were able to record field and cellular activity in the hippocampal formation during the reversible inactivation of the septum. The results of this experiment on hippocampal field and cellular activity are summarized in Figure 4. The figure depicts hippocampal field activity (top trace), theta-on cell activity (middle trace) and theta-off cell activity (bottom trace) prior to inactivation (left column), during septal inactivation (center column) and following recovery of the septum (right column). It can be seen that septal inactivation abolished hippocampal theta, dramatically decreased the discharge of the theta-on cell, and released inhibition of a theta-off cell, even during high intensities of hypothalamic stimulation (stimulation is indicated at the bottom of the figure). Furthermore, as the septum recovered from the procaine injection, some interesting trends were recorded. The amplitude and frequency of hippocampal theta field activity had very different time courses of recovery. Theta frequency was quick to recover while theta amplitude recovered gradually. The frequencies of theta which were evoked by hypothalamic stimulation prior to septal inactivation were nearly identical to the frequencies evoked as the septum recovered, yet at largely attenuated amplitudes. That is, in the early stages of recovery from the local anaesthetic, there were enough cells to translate the frequency of theta in response to varying intensities of hypothalamic stimulation, but

Figure 4. Diagrammatic summary of the effects of reversible septal inactivation on hippocampal theta field activity and LIA. The top line is the slow wave line illustrating hippocampal LIA and theta field activity prior to inactivation (left column), during maximal inactivation of the septum (center column), and following recovery form septal inactivation (right column). The two cell lines illustrates the discharge patterns of theta-on and theta-off cells respectively. The bottom trace indicates periods of hypothalamic stimulation.



there were apparently too few cells to produce the normal amplitude of hippocampal theta. These findings are very relevant to the present thesis.

In keeping with the topic of this section, extrinsic influences mediated by the septum on the hippocampal formation, I should state that, to the best of my knowledge, no one has yet demonstrated the influence of septal stimulation on hippocampal theta-on or -off cells. I think this is an important pursuit for the following reason. Theta-related cells in the hippocampus have been shown to discharge during type I motor behaviour and during sensory processing in freely moving rabbits (Sinclair, Seto, and Bland, 1982; Mizumori, Barnes, and McNaughton, 1990). As already stated, electrical stimulation in the hypothalamus and brainstem modulates type I motor behaviour. However, this is not the case for septal stimulation. Kramis and Routtenberg (1977) and Kramis and Vanderwolf (1980) have shown that septal stimulation can evoke hippocampal theta during both movement and immobility. Behaviours normally correlated with the occurrence of hippocampal LIA (immobility, grooming, chewing, etc.) continued during elicitation of hippocampal theta. The behaviour was dissociated from the hippocampal field activity during septal stimulation. As mentioned, this is not the case for hypothalamic stimulation and given this reasoning, septal stimulation may not necessarily influence theta-related cells.

Taken together the above findings suggest that rhythmic cellular discharges of the septum are essential for the generation of hippocampal theta field activity. However, this is not the case. Theta-like activity has been recorded *in vitro*, in the septally deafferented brain slice preparation (Konopacki, MacIver, Roth, and Bland, 1987; Bland, Colom, Konopacki and Roth, 1988). Furthermore, Colom *et al.*, (1991) have demonstrated theta-like oscillations in the hippocampus of rats *in vivo* during septal inactivation. These findings suggest that the pacemaker hypothesis is no longer a tenable theory.

b) *PH-SUM*

The posterior hypothalamic region has been shown to be involved in theta generation. High frequency trains of stimulation produce hippocampal theta field

activity and increasing intensities of stimulation cause corresponding increases in theta frequency (Green and Arduini, 1954; Bland 1971; Bland and Vanderwolf 1972; Kramis and Vanderwolf, 1980). Hypothalamic stimulation also influences the behavior of freely moving rats (Bland, 1971). Hippocampal theta-on cells are activated by hypothalamic stimulation, whereas theta-off cells are inactivated by stimulation (Colom, Ford and Bland, 1987). Similarly, septal theta-on cells are activated by hypothalamic stimulation, whereas theta-off cells are inactivated by stimulation (Bland, Colom and Ford, 1990). Furthermore, Gottesmann (1992) has recently shown that midhypothalamic transections in rats abolished hippocampal theta activity and suggested that a trigger zone for hippocampal theta rhythm may be the posterior hypothalamus. The current thesis further explores this notion.

c) *Brainstem*

The ascending hippocampal synchronizing system originates in the pons region (Vertes, 1981, 1982, 1988). Further, a desynchronizing system originates in the median raphe of the brainstem. Electrical stimulation of these regions produces hippocampal synchrony or desynchrony, respectively. The influence of electrical stimulation in the pons region on theta-related cells in the hippocampus, septum or hypothalamus has yet to be examined. This thesis exploits the ability of pons stimulation to elicit hippocampal theta via its synaptic connections with the hypothalamus, septum and hippocampus.

III. *Pharmacology*

i) *Hippocampal Theta Field Activity*

Much of the pharmacology underlying hippocampal theta field activity has already been discussed in previous passages of this thesis. Based upon pharmacological evidence, there appears to be two distinct types of hippocampal field activity (Bland, 1986). Kramis *et al.*, (1975) demonstrated in rats and rabbits that type II theta was abolished by injections of atropine sulphate, whereas type I theta was unaffected by atropine. Thus, type II theta has been shown to be mediated by acetylcholine, specifically by muscarinic receptors. Muscarinic antagonists, such as atropine sulphate and scopolamine have been shown to abolish type II theta, whereas

agonists such as carbachol and physostigmine elicit type II theta (Rowntree and Bland, 1986). Type II theta can occur independently (e.g., when an animal is immobile and processing relevant sensory stimuli) or can be co-activated with type I theta during voluntary motor behaviour. However, it should not be assumed that type I theta (sensitive to anaesthetics) cannot be recorded under conditions of urethane anaesthesia. Stewart and Fox (1989a) have detected a small component of atropine-resistant theta in urethane-anaesthetized rats following treatment with atropine. The consensus remains that type I theta is sensitive to anaesthetics and is not predominant in the hippocampal field activity of acute preparations. More recent evidence has suggested that hippocampal type II theta is dependent on the coactivation of septal cholinergic and GABAergic projections (Smythe *et al.*, 1991, 1992).

Determining the neurotransmitter(s) mediating type I theta, which occurs during voluntary motor behaviour (Vanderwolf, 1969), have been problematic. Only recently has evidence accumulated which has suggested that type I theta is mediated by serotonin. Vanderwolf and Baker (1986) depleted up to 93% of the serotonin in rats. They found that the animals displayed excellent theta during type I motor behaviours. However, when atropine was administered to these animals, all theta activity was abolished in the hippocampus. They argued the theta which remained in the hippocampus following serotonin depletion was type II cholinergically mediated theta occurring during type I movements. The evidence suggested that type I theta was mediated by serotonin. Since then, further studies which have eliminated serotonin in the central nervous system have found similar results (Vanderwolf, Leung, Baker, and Stewart, 1989; Peck and Vanderwolf, 1991).

ii) *Hippocampal Cell Activity*

Studies which have examined the pharmacology of theta-related cell activity have drawn conclusions similar to those based on the pharmacology of hippocampal theta field activity. Systemic administration of cholinergic agonists, such as eserine or carbachol, elicit hippocampal theta activity and activate phasic and tonic theta-on cells in the hippocampus of urethane anaesthetized rats (Bland and Colom, 1988). The subsequent administration of atropine sulphate abolished both the theta field

activity and accompanying cellular activation. Nicotine was found to result in 20-30 second bouts of theta field activity, but phasic and tonic theta-on cell activity was suppressed during these bouts.

Bland and Colom (1989) examined phasic and tonic theta-off cells in the hippocampus of urethane anaesthetized rats prior to and after the administration of cholinergic agents. They found that eserine produced a reduction in the discharge rate of phasic theta-off cells and totally abolished tonic theta-off cell discharge. The administration of atropine abolished all theta field activity previously elicited by eserine. Furthermore, hypothalamic stimulation and sensory input in the form of a tail pinch following the atropine injection could not elicit theta activity, yet the discharge rates of phasic and tonic theta-off cells were still reduced. Thus, it appeared that theta-off cells are associated with ongoing cholinergically elicited field activity but were still inhibited by ascending inputs in the absence of cholinergically induced theta field activity. This suggests that while theta-off cells are involved in theta circuitry, they are not directly mediated by cholinergic synapses.

In freely moving rabbits, atropine abolished theta-on cell discharge which accompanied type II sensory processing (Bland, Seto, Sinclair and Fraser, 1984). The same theta-on cells continued to discharge rhythmically during type I theta, yet the number of discharges were reduced. Buzsáki *et al.*, (1983) have also reported the persistence of rhythmical cell discharge in the freely moving rat following administration of atropine. They too reported significant reduction in the cell discharge rate following cholinergic blockade suggesting that the cells received inputs from two distinct theta systems.

iii) ***Septum***

The cholinergic nature of the septohippocampal projection has received strong support. Lesions of the septum result in a loss of acetylcholinesterase (AChE) staining in hippocampus and decreases in hippocampal choline acetyltransferase (CAT) activity (Lewis and Shute, 1967). Stimulation of the septum causes release of acetylcholine in the hippocampus (Dudar, 1975). Alonso *et al.* (1984) also demonstrated that a number of septal projections to the EC contained acetylcholine

while others were devoid of the neurotransmitter which suggested that the septo-entorhinal projection consisted of a cholinergic as well as a noncholinergic component.

The septum not only provides much of the cholinergic innervation to the hippocampus but many septal cells are also cholinoceptive. Recently, Monmaur and Breton (1991) microinfused carbachol and atropine into the medial septum of freely moving rats. Intraseptal injections of carbachol elicited theta in the hippocampal formation which occurred during immobility and what are normally type 2 LIA-related behaviours. Subsequent intraseptal injection of atropine abolished theta accompanying immobility but not theta accompanying voluntary movement (type I atropine-resistant theta). Lawson and Bland (1991a,b) independently corroborated the findings of Monmaur and Breton, and further, evaluated the effect of septal inactivation in freely moving rats. Procaine inactivation of the septum abolished both types of hippocampal theta field activity. They also found that during the recovery period following septal inactivation, theta frequency recovered rapidly compared to the slower recovery of amplitude. This was exactly what Smythe, *et al.*, (1991) had reported following septal inactivation in the acute urethane anaesthetized rat.

The existence of a GABAergic input, as well as a cholinergic one from the septum, has recently been demonstrated. Freund and Antal (1988) have shown that GABA containing afferents originating in the septum innervate most of the GABA containing interneurons in the hippocampus. Activation of these neurons in the septum leads to disinhibition of principal neurons in the hippocampal formation and so this pathway is probably crucial in the induction of hippocampal theta field activity.

With respect to septal unit activity, Petsche *et al.*, (1962) revealed that septal units were activated by intravenous administration of eserine. More recently, Stewart and Fox, (1989b) demonstrated two populations of rhythmically bursting neurons in the medial septum of rats. Of the rhythmically bursting cells recorded, 66.7% continued to burst at theta field frequencies following the administration of intravenous atropine; the remaining 33.3% of the cells lost their rhythmic discharge pattern which accompanied the loss of hippocampal theta field activity. Colom and

Bland (1991a) have found similar results following systemic administration of atropine in urethane anaesthetized rats. Seventy-one percent of phasic theta cells recorded in the septum continued to discharge rhythmically, while the remaining 28.1% became irregular following atropine. These data suggested that rhythmic cells of the septum are composed of at least two distinct types, and that both may contribute to the production of theta in the hippocampus. It is possible that the atropine resistant rhythmic cells may mediate the type I atropine resistant hippocampal theta seen by Lawson and Bland (1991b) in freely moving rats during movement. Further research exploring this issue is necessary.

iv) ***Hypothalamus and Pons***

It has been postulated that the posterior hypothalamus receives dense cholinergic innervation from the pons region (Shute and Lewis, 1967), however, the cholinoceptive nature of the PH-SUM has yet to be demonstrated. Carbachol infusion into the pons region resulted in hippocampal synchrony verifying the cholinoceptive nature of the pons region (Nuñez *et al.*, 1991).

Besides the studies already mentioned, there is a paucity of research examining the pharmacology of the hypothalamus and its cells. Kirk *et al.*, (1991) made no mention of the pharmacology of the rhythmic theta-on cells they recorded in the SUM. Recently, Brudzynski, McLachlan, Bihari and Girvin (1991) reported that iontophoretic application of carbachol in the anterior hypothalamic/preoptic area revealed three populations of cells. Extracellular unit recordings revealed that of 61 neurons, 70% decreased their discharge rate, 15% increased and 15% remained unchanged by the carbachol ejection. The results suggest that cells in this region have predominantly M2 muscarinic receptors which are known to cause membrane hyperpolarization and decrease neuronal excitability. However, the relevance of these results to the present thesis should be interpreted with caution for a number of reasons: 1) no mention was made to hippocampal field recordings in the study, 2) the region of investigation is anterior to the PH-SUM complex investigated in this study, and 3) drawing analogies on muscarinic receptor types in the two regions is tenuous.

Whether or not these findings are applicable to the PH-SUM investigated here is a matter of further investigation.

IV. *Overview*

The experiments in this thesis attempted to evaluate a number of questions which have not been examined previously. There is strong evidence that the PH-SUM has a prominent extrinsic influence on hippocampal theta field activity, as do the septum and pons. However, the nature of the control which the pons, PH-SUM and septum exert in producing and maintaining the amplitude and frequency of hippocampal field activity is not known. Smythe *et al.*, (1991) revealed that septal integrity was important for hippocampal theta generation utilizing reversible septal inactivation. The technique provides the ability to monitor the recovery of a region over time revealing important aspects of its function. With respect to the medial septum, as the region recovered from procaine inactivation, the frequency of hippocampal theta recovered rapidly whereas the amplitude of hippocampal theta field activity recovered slowly across the same time course. This thesis examines the effect of **reversible hypothalamic inactivation** on hippocampal field activity in an attempt to reveal its influence on hippocampal theta field activity.

Since the PH-SUM is thought to be an important component of the ascending system (Bland, 1992; Vertes *et al.*, 1992) and an intervening region between the medial septum and pons, electrical stimulation of the PH-SUM in the urethane anaesthetized rat was used to test the effectiveness of hypothalamic inactivation. Pons stimulation was used to test the role of the PH-SUM in the ascending pathway. That is, since the pons is a "downstream" influence and the PH-SUM is thought to be the next upstream region in the ascending synchronizing system, then following hypothalamic inactivation, pons stimulation should no longer affect the hippocampal field activity. This was also examined in these experiments.

The thesis is comprised of a series of four experiments which progressed as a result of examination of the ascending hippocampal synchronizing system. The first experiment examined the effect of hypothalamic stimulation on intrahippocampal carbachol-induced theta field activity in the urethane anaesthetized rat. This was done

to determine if the extrinsic influence the hypothalamic stimulation provided on hippocampal circuitry could modulate hippocampal theta elicited by intrahippocampal carbachol infusion. Following that, the effect of hypothalamic inactivation on hippocampal theta field activity was explored. **Septal** inactivation has been shown to abolish intrahippocampal carbachol-induced theta field activity, demonstrating its importance in the ascending system. Is the integrity of the PH-SUM complex essential for hippocampal theta field activity? The second experiment evaluated the effect of hypothalamic inactivation on spontaneously occurring theta and theta related to sensory processing (tail pinch induced theta field activity). These studies would determine if the hypothalamus was a critical relay for ascending sensory information needed for hippocampal formation sensory-motor integration. The third experiment evaluated the effect of hypothalamic stimulation and inactivation on hippocampal theta field activity which was elicited by intraseptal infusions of carbachol. And finally, the fourth experiment evaluated the cholinoceptive nature of the PH-SUM by: 1) determining if carbachol infusions in the PH-SUM elicited hippocampal theta field activity as do pons and septum; 2) investigating the effect of hypothalamic inactivation on the hippocampal theta field activity induced by carbachol infusion in the PH-SUM; 3) determining if the reversible inactivation of the PH-SUM blocked the effects of electrical stimulation of the pons in producing hippocampal theta field activity.

In summary, the questions addressed in this thesis were an attempt to clarify the role of the PH-SUM complex in mediating the synchronizing influences of the ascending brain stem pathway on the hippocampal formation.

Methods - Experiment 1

i) *Objectives*

The first experiment evaluated the effect of reversible inactivation of the PH-SUM region by microinfusion of procaine on hippocampal theta elicited by intrahippocampal microinfusion of carbachol. Frequency and amplitude measurements of hippocampal field activity were provided by spectral analysis and were made in the pre-procaine condition, during the period of maximal suppression of

the PH-SUM, and during the recovery period. The efficacy of inactivation of the hypothalamus and its recovery was tested by electrical stimulation of the region.

ii) ***Subjects***

Five male Long Evans rats weighing between 300 and 550 g served as subjects. They were supplied by the Psychology Department vivarium at the University of Calgary. The rats were housed in groups of up to five in clear polycarbonate cages, and were given Purina rat chow and water *ad libitum*. The vivarium was maintained on a normal light cycle, on at 08:00 hours and off at 20:00 hours. Experiments were started between 07:00 and 10:00 hours and completed between 17:00 and 20:00 hours.

iii) ***Apparatus***

Extracellular field activity was recorded using tungsten microelectrodes with a resistance of 0.3 - 1.0 Mohms. The electrode was sharpened electrolytically and insulated with Kynar. A standard micromanipulator was used to position the electrode in the stratum moleculare of the dentate gyrus and served as the theta reference. Electrical activity recorded by the tungsten electrode was split and led into two Grass P511 AC preamplifiers. One preamplifier was used to separate unit activity from field activity by setting the 1/2 amplitude low filter at 300 Hz and the 1/2 amplitude high filter at 3 kHz. This filter configuration allowed only high frequency signals to pass. Since action potential durations are on the order of 0.5 to 2.0 msec (500-2000 Hz), this preamplifier was used to filter and amplify unit recordings. The other preamplifier received an identical signal (gain 200X), but was passed through 1/2 amplitude filters settings of 1 Hz (low) and 35 Hz (high). This allowed only low frequency signals to pass through the filter. Since theta occurs in the range of 2-9 Hz in the urethane-anaesthetized rat, this preamplifier was used to record ongoing field activity at the electrode site in the dentate gyrus.

To aid in electrode placement the signals passing out of the preamplifiers were fed through an audio monitor (Grass AM5), so that activity of cells in the neocortical layers overlying the hippocampus, the *cornu ammonis* cell layer in the hippocampus, and single cells within the hippocampus could be heard. This aided in determining

optimal positioning of the theta reference electrode in an attempt to maximize amplitude of the theta signal.

Field activity from the electrode was displayed on a model 7D Grass polygraph and also on a Tektronix storage oscilloscope so that the reference signal could be observed throughout an experiment. Field activity and oral commentaries were recorded on VHS cassette tapes using a TEAC XR-30, 7-channel FM tape recorder.

Electrical stimulation of the PH-SUM nuclei of the hypothalamus (hence forth I will refer to these hypothalamic nuclei as the hypothalamus) and drug infusions into the same site were delivered using a chemotrode. The chemotrode was bipolar and constructed by epoxying two lengths of 250 μm stainless steel wire along each side of a 30 g stainless steel tubing with 2-Ton epoxy (Devon). Each end of wire was soldered to a male subminiature connector, which facilitated attachment to a Grass SIU 4678 stimulation isolation unit (SIU) and a Grass CC UIA constant current unit. A Grass S44 stimulator provided a voltage output and was set to deliver 0.1 msec duration pulses at 100 Hz. The stimulator was connected to the SIU in order to reduce stimulation artifact in the recording. Its output then passed through to the constant current unit, to change its voltage output to a current intensity (which was varied by altering the voltage setting on the S44). The 30 gauge cannula of the chemotrode was attached to a gas tight microsyringe and contained procaine hydrochloride to be infused into the hypothalamus. Thus the chemotrode provided a means by which electrical stimulation of the hypothalamus could be applied and pharmacological infusions into the same site could be delivered. In this case, procaine hydrochloride, a local anaesthetic was infused into the hypothalamus to suppress its function temporarily (hypothalamic inactivation). Since the chemotrode delivered the infusion of procaine and stimulated the region electrically, it provided a means by which the extent and duration of hypothalamic inactivation could be tested, that is, by stimulating the region electrically at high intensities following the procaine infusion to determine if and when the stimulation had an influence on hippocampal field activity.

Infusions into the hypothalamus and hippocampus were performed using a Harvard Apparatus Infusion pump (model 22). One 10 μl Hamilton Microliter/Gas tight syringe (#701) was connected via PE 30 intramedic tubing to the chemotrode placed in the hypothalamus and a second to a cannula placed in the hippocampus. The hippocampal cannula was constructed from a 26 g needle (Chromatographic Specialties Inc.). The 10 μl syringe was placed onto the pump and the flow rate set to 1 μl/min for procaine infusion through the chemotrode into the hypothalamus and 0.5 μl/min for carbachol infusion into the hippocampus.

During an experiment the rat was maintained at 38° C using a Harvard Instruments Servosystem, which monitored the animal's core temperature via a rectal probe, and controlled a heating blanket positioned beneath the animal. Pin electrodes were positioned subcutaneously in the forelimbs of the rat in order to monitor heart rate, which was used to assess the animal's condition, as well as depth of anaesthesia throughout the duration of the experiment.

iv) ***Surgery***

On the day of an experiment, an animal was selected from the vivarium, weighed, and initially anaesthetized in an airtight chamber with a 4% oxygen (Medigas) and halothane (MTO Pharmaceuticals) mixture. Under halothane anaesthesia, an upper thoracic incision was made. Using blunt dissection the left jugular vein was located and separated from its adhering tissue. A small angular incision was snipped in the jugular vein through which a catheter (polysylastic tubing) was inserted and secured in place with suturing silk. The animal was then switched to urethane anaesthesia (0.8 g/ml in NaCl) which was administered via the jugular catheter. The incision was sutured and the animal was placed into the stereotaxic apparatus.

Once the animal was placed in the stereotaxic apparatus, a mid-sagittal incision was made in the animal's scalp to reveal bregma and lambda. The skin was tied back to the ear bars with suturing silk and the periosteum was retracted. Dorsal-ventral (D-V) coordinates for bregma and lambda were obtained and the skull was levelled to horizontal. Anterior-posterior (A-P) and lateral (L) coordinates for bregma were

obtained and reference electrode and chemotrode placement sites were determined and marked on the skull. Holes were drilled in the skull at these sites. Next to the placement sites, additional holes were drilled and jeweller's screws were fixed to the skull in these holes. These screws provided an anchor to which the electrode placements could be secured with dental acrylic.

An uninsulated tungsten electrode was placed in the anterior cortex and secured to the skull using a jeweller's screw and dental acrylic. This served as the indifferent electrode. Next a tungsten microelectrode was placed at - 4.2 mm A-P, $\pm$ 2.2 L and $\approx$ 2.5 D-V to bregma. Upon establishing a maximum amplitude of the theta signal, typically in the upper blade of the stratum moleculare, the microelectrode was cemented to a jeweller's screw with dental acrylic. This electrode served as the theta reference electrode.

The hypothalamic chemotrode was filled with procaine hydrochloride and then carefully positioned, typically within the same hemisphere as the reference electrode, at -3.3 mm A-P (from bregma), $\pm$ 0.2-0.4 mm L (from bregma), and $\approx$ 7.4 - 7.6 mm ventral to the dural surface in the PH-SUM complex of the hypothalamus. The final site selected produced optimal driving of theta which was operationally defined as the site where threshold levels of electrical stimulation (0.3 mA) produced low frequency theta (2-3 Hz). Optimal driving was demonstrated when increasing intensities of electrical stimulation manifested a linear increase in evoked theta frequency. Also, behavioural signs such as vibrissae movement, increased rate of respiration, and pupil dilation were evident only during optimal driving and served as indicators of good chemotrode placement. The placement of the chemotrode sometimes took as much as two hours because of the sensitivity of the hippocampal theta field activity to perturbation by the placement of the chemotrode. Sometimes this caused an attenuation in the amplitude of the theta and LIA signals which did or did not recover with time. Where attenuation resulted in a peak to trough hippocampal theta amplitude less than 1.0 mV, a second reference electrode was placed in the contralateral hemisphere at the same coordinates. This routinely resulted in a larger reference theta signal (1.0-2.0 mV peak to trough). Experiments

not meeting these amplitude criteria were terminated (Robinson, 1980). Upon successful placement of the chemotrode in an optimal driving site, it was cemented in place with dental acrylic. Finally, a hole was drilled contralateral to the reference electrode at -3.3 mm A-P, ± 2.2 mm L and a cannula was placed 2.5 D-V in the upper blade of the dentate gyrus.

The surgical preparation referred to above, usually took on the order of 4-5 hours. Following this, the rat was maintained at a deeper level of anaesthesia throughout the remainder of the experiment. Since rats spontaneously cycle between theta and LIA at lighter levels of anaesthesia and since sub-threshold levels of stimulation were being examined, spontaneously occurring theta could have been mistaken for theta evoked by sub-threshold electrical stimulation. A deep level of anaesthesia controlled for this.

v) ***Drugs Administered***

Vacuum filtered distilled water was used to prepare solutions. Carbachol was mixed at a concentration of 10 $\mu g/\mu l$ in distilled water. Procaine hydrochloride was mixed in distilled water to make a 10% solution by weight. Urethane was mixed with a 7% NaCl solution at 0.8 g/ml. All of the drugs were purchased from the Sigma Chemical Company, St. Louis, MO and were stored at 0-5° C.

vi) ***Experimental Procedure***

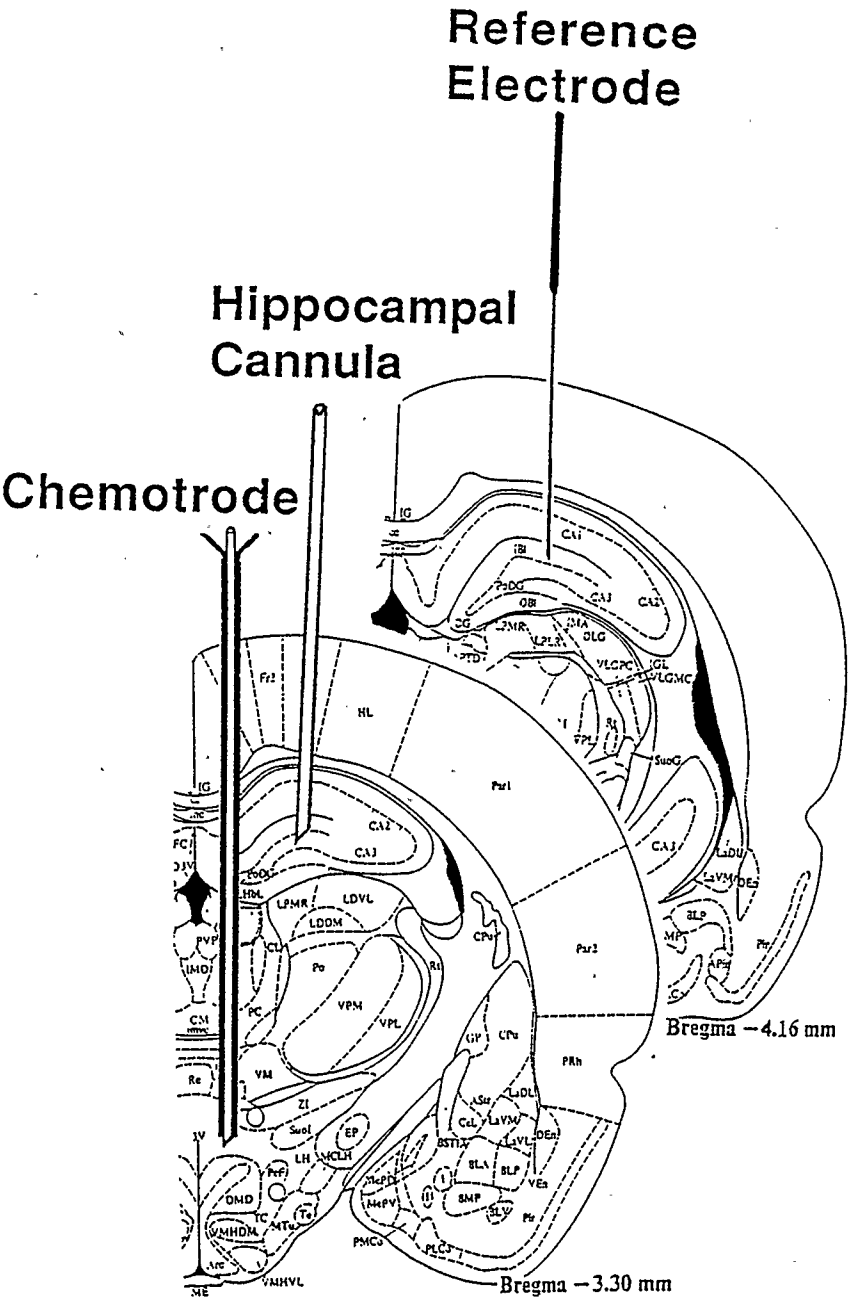
The experiment employed a 2-way repeated measures design which assessed the effects of electrical stimulation and inactivation of the hypothalamus on intrahippocampal carbachol-induced theta. Field activity in the hippocampus was compared across the following conditions: 1) baseline, 2) following a carbachol infusion into the hippocampus (referred to as the carbachol condition), 3) 10 minutes post-procaine, 4) 30 minutes post-procaine, 4) 45 minutes post-procaine, and 6) 60 minutes after procaine inactivation of the hypothalamus. This provided six conditions for the repeated measure factor which was referred to as the inactivation effect.

Measures of spontaneous theta and theta elicited by carbachol served as two of the dependent measures in each condition. Spontaneous theta was operationally defined as theta which occurred while the rat cycled between LIA and theta at an

appropriate level of urethane anaesthesia. With regard to carbachol elicited theta, as mentioned earlier, the intrinsic circuitry of the hippocampus is such that a small infusion of intrahippocampal carbachol results in bilateral hippocampal theta field activity for prolonged periods (up to five hours). I will use the term, carbachol theta, to refer to theta which is evoked by carbachol infusion into the hippocampus, septum or PH-SUM. Furthermore, field activity recorded while a stimulation block was applied to the hypothalamus provided the remaining dependent measures. Six blocks of stimulation intensities were used for each experiment; one block for each of the above noted conditions. Each block contained two sets of stimulus intensities to be delivered to the hypothalamus via the chemotrode. Stimulation intensities used were 0.1, 0.3, 0.5, 0.7, and 0.9 mA. As well, the stimulation intensities were randomly generated within a set using a PC microcomputer and a GWBASIC program which provided a printout of the six blocks used in an experiment. A stimulus trial at any given intensity had a duration of 10 seconds and was followed by an inter-stimulus interval of 10-20 seconds. Given this, each stimulation block contained 10 trials of stimuli and took up to five minutes total duration (including inter-stimulus intervals). Figure 5 provides a diagrammatic representation of the experimental recording and stimulation apparatus used for this design.

Once optimal driving of theta had been established with hypothalamic stimulation and the theta amplitude deemed acceptable, the experiment began. At least two samples of spontaneous theta with a duration of 10 seconds were recorded. Following these recordings, the animal was deeply anaesthetized in order to record baseline samples of field activity elicited in the hippocampus during electrical stimulation of the hypothalamus. Deep anaesthesia was operationally defined in a number of ways. 1) Consistent shallow and slow breathing. 2) No vibrissae movement was evident and a firm tail-pinch failed to produce any behavioural arousal. 3) A firm tail-pinch failed to evoke any hippocampal theta. 4) No spontaneous theta was evident immediately following a five-second bout of 0.9 mA stimulation in hypothalamus. Upon establishment of deep anaesthesia, the first block of electrical stimuli were delivered via the chemotrode to the hypothalamic region.

Figure 5. A diagram which illustrates the placement of the recording electrode used to record hippocampal theta field activity (reference electrode), the cannula used to infuse carbachol into the hippocampus and elicit theta field activity, and the chemotrode used to electrically stimulate the hypothalamus and subsequently reversibly inactivate the region by infusing procaine through it.


Reference Electrode
Hippocampal Cannula
Chemotrode
Bregma −4.16 mm
Bregma −3.30 mm

Recordings of hippocampal field activity were continuous throughout the stimulation protocol. As well, if any of the above mentioned anaesthetic arousal indicators were observed during a stimulation block in any condition of an experiment, supplemental doses of anaesthetic were administered (0.05 cc urethane) before the stimulation protocol resumed.

Following the baseline recordings of hippocampal theta field activity elicited by hypothalamic stimulation, 0.5-1.5 μl of carbachol was infused into the hippocampus at 0.5 μl/min through the hippocampal cannula. Typically, intrahippocampal carbachol theta was present in approximately two minutes from the start of the infusion. In some experiments seizure activity ensued. In that case, the experiment was postponed for approximately one hour until seizure activity subsided. Intrahippocampal carbachol theta was always prominent after this delay. A 10 second sample of carbachol theta was then recorded (baseline carbachol theta). Recordings of the effect of the second block of stimulation intensities delivered to the hypothalamic region were taken. A second baseline carbachol theta sample was recorded following the stimulation protocol.

To evaluate the effects of hypothalamic inactivation on carbachol theta activity, 4 μl of procaine hydrochloride was infused into the hypothalamus via the chemotrode at 1 μl/min. At the start of the infusion a stopwatch was activated. Eight minutes after the procaine infusion had started, a 10 second sample of intrahippocampal theta was recorded. At that time, the third block of stimulus intensities were delivered to the inactivated hypothalamus. The resulting affect that electrical stimulation of the hypothalamus had on intrahippocampal carbachol-induced field activity was recorded on tape. Lastly, a second sample of intrahippocampal carbachol-induced field activity was recorded. The same procedure as described above for the post 10 minute procaine interval was carried out for the post 30, 45, and 60 minute post procaine infusion intervals.

As discussed above, the stimulation block delivered via the chemotrode tested the efficacy of hypothalamic inactivation. Following procaine infusion into the hypothalamus, electrical stimulation at this site should no longer have an influence on

hippocampal field activity. One should also note that the stimulation block was started at 8 minutes post procaine rather than at 10 minutes. This was to ensure that the stimulation block was grouped around the post 10 minute procaine infusion interval. Since the stimulation block had a duration of approximately five minutes, starting the block at post 8 minutes ensured that the stimuli delivered to the hypothalamic region were grouped evenly around the post 10 minute interval.

Upon completion of an experiment, the rat was overdosed with urethane and perfused intracardially first with saline (0.9%) and then with a mixture of 10 % formalin and saline. Their brain was removed and stored for at least 24 hours in a formalin-sucrose mixture (30%) at 0-5° C. Later, the brain was sectioned into 40 μm coronal sections on a CO_2 freezing microtome, mounted on slides and stained with cresyl violet. Positioning of the reference electrode, chemotrode and hippocampal cannula were confirmed and recorded.

vii) ***Data Analysis***

All field activity samples, recorded by the hippocampal theta reference electrode in an experiment, were stored onto VHS tape and later analyzed off line. Individual experiments were played back on a TEAC XR-50, 14 Channel Cassette Data Recorder. Analog data segments on the tape were fed into a Brüel and Kjaer Dual Channel Signal Analyzer (Type 2032). The Brüel and Kjaer inputted and analyzed eight seconds of hippocampal field activity in a band pass range of 0.125 - 50 Hz.

The analysis was triggered so that any selected sample of data from the VHS tape could be quantified by the Brüel and Kjaer. After a selected sample of data was triggered, the following eight seconds of hippocampal field activity were inputted to the Brüel and Kjaer and subjected to a Fourier transformation. The Fourier transformation of the hippocampal field activity produced an instantaneous spectrum of the eight second segment of hippocampal theta field activity. This spectrum was displayed on a screen and provided quantification of the peak frequency (Hz) and amplitude (0 dB = 1.00 mV RMS) of the signal. Analysis of eight seconds of field activity resulted in a spectral resolution of 0.125 Hz. A discussion of the

requirements needed to legitimate Fourier transformation and spectral analysis of hippocampal EEG are contained in Appendix I.

The peak frequency and amplitude were determined for each dependent and repeated measure in an experiment. For example, eight second samples of spontaneous theta, carbachol theta, and field activity during the varying intensities of hypothalamic stimulation were quantified. These values were later entered and systematized using a PC microcomputer and LOTUS 1-2-3 (ver. 3.0).

Statistical analysis of the data was done using a Macintosh SE and STATVIEW program. Graphics were done using a Macintosh SE and Cricket Graph program. All Macintosh computers and related software were provided by the Psychology Department at the University of Calgary.

Results - Experiment 1

i) *Histology*

Histological analysis enabled verification of intrahippocampal reference electrode and infusion cannula placements. In all five animals, the implant tracts into the hippocampus were obvious and the tips were confirmed to be located in either the left or right upper blade of the dentate. Chemotrode placements exhibited more damage than reference electrode implants due to their larger size which also resulted in difficulty localizing the chemotrode tip. In four rats, the tip was confirmed to be in the dorsomedial posterior hypothalamic region ipsilateral to the reference electrode. In one rat, the placement was slightly anterior (-2.8 - 3.0 mm AP). In this experiment, threshold electrical stimulation to elicit hippocampal theta field activity was slightly higher but subsequent procaine inactivation of the hypothalamus did not differ from other experiments.

ii) *PH-SUM Stimulation & Reversible Inactivation: Effects on the Frequency of Hippocampal Theta Field Activity*

Electrical stimulation of the PH-SUM complex was able to modulate hippocampal theta field activity elicited by the intrahippocampal carbachol infusion. Subsequent hypothalamic inactivation affected the ability of stimulation to modulate hippocampal theta field activity but did not abolish it. It was shown that following

hypothalamic inactivation, high intensities of electrical stimulation in the hypothalamus could no longer modulate carbachol theta. The frequency of hippocampal theta field activity elicited by hypothalamic stimulation recovered gradually. First, high stimulation intensities produced reduced frequencies compared to those prior to inactivation. These reduced frequencies recovered to baseline values over time.

Figure 6 shows the effects of these manipulations on the frequency and amplitude of hippocampal theta, providing analogs of two seconds of theta which occurred during selected conditions. The top row of analogs, from left to right are as follows: baseline spontaneous theta, carbachol theta - no stimulation, carbachol theta 10 minutes after procaine, and carbachol theta 60 minutes after procaine. The bottom row of analogs, from left to right depict the following: baseline theta during 0.9 mA hypothalamic stimulation, carbachol theta during 0.9 mA stimulation, carbachol theta 10 minutes after the procaine infusion during 0.9 mA stimulation, and carbachol theta 60 minutes after the procaine infusion during 0.9 mA stimulation. The second column of the figure demonstrates that intrahippocampal carbachol-induced theta could be modulated by electrical stimulation in the PH-SUM region. Note the third column of analogs which compares carbachol theta 10 minutes after hypothalamic inactivation without stimulation (top) and during 0.9 mA of stimulation (bottom). The frequencies are identical (approximately 5.65 Hz) demonstrating that hypothalamic stimulation is no longer effective. As well, the amplitude of the signals were unaffected. The effect of hypothalamic inactivation had recovered by 60 minutes post procaine infusion (last column). Equal volumes of saline infused into either hippocampus or hypothalamus has no affect on hippocampal field activity.

A 2-factor repeated measures ANOVA found the main effect of stimulation intensity on the frequency of hippocampal carbachol theta ($F_{(5,54)} = 7.675$, $p < .0001$), the repeated measure of inactivation on frequency ($F_{(5,54)} = 59.264$, $p < .0001$) and the intensity X inactivation interaction ($F_{(25,270)} = 36.563$, $p < .0001$) to be statistically significant.

Figure 6. Experimental analogs of hippocampal theta field activity during the pre-procaine measure (left column - spontaneous theta (top) and theta elicited by 0.9 mA hypothalamic stimulation (bottom); 2nd left column, carbachol theta (top) and carbachol theta modulated with 0.9 mA hypothalamic stimulation (bottom)), 10 minutes after hypothalamic inactivation (middle right column) without hypothalamic stimulation (top) and during 0.9 mA stimulation (bottom) and 60 minutes after hypothalamic inactivation (right column) without stimulation (top) and during 0.9 mA hypothalamic stimulation (bottom).

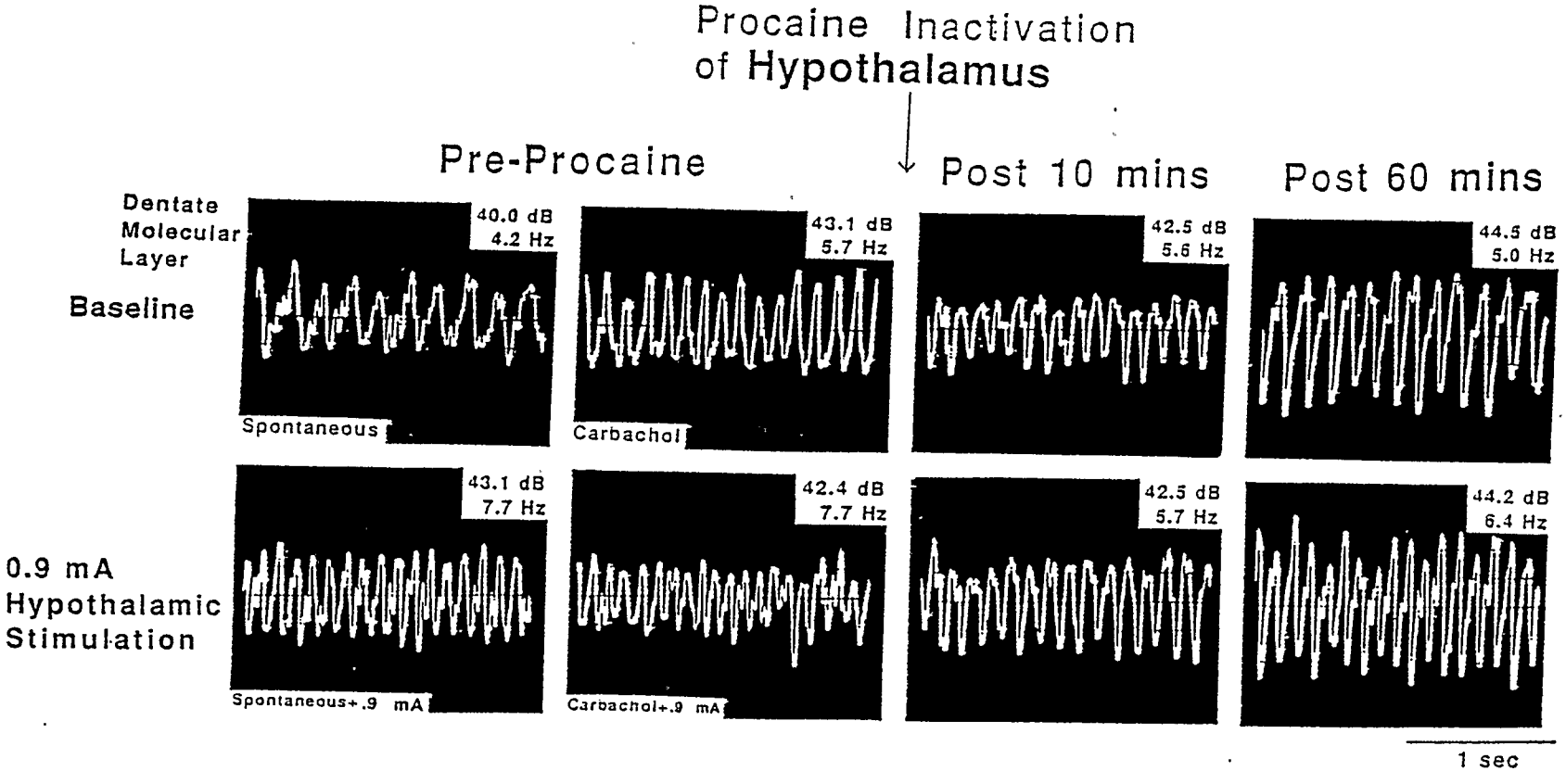
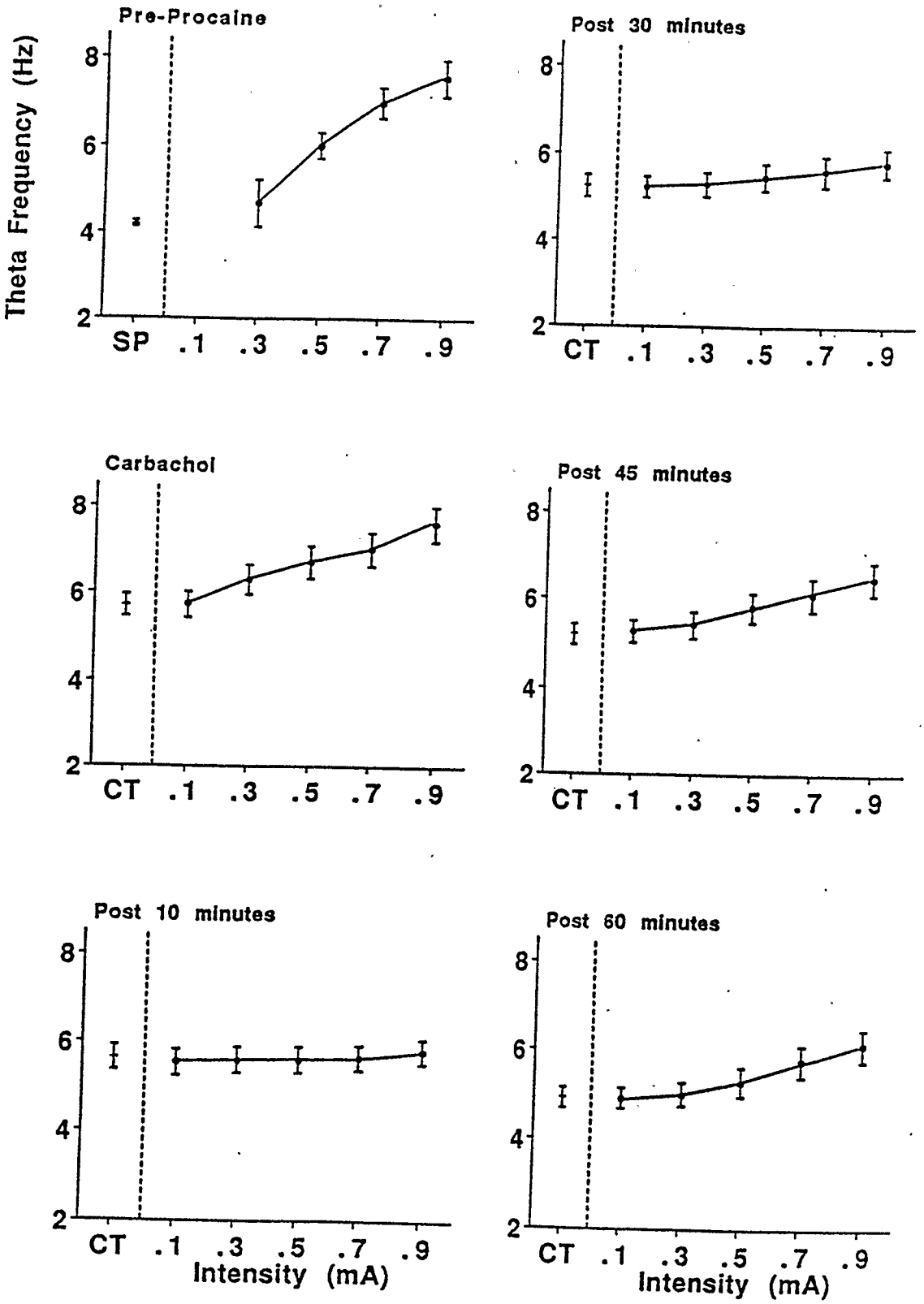

Procaine Inactivation
of Hypothalamus
Pre-Procaine
Post 10 mins
Post 60 mins
Dentate
Molecular
Layer
Baseline
40.0 dB
4.2 Hz
Spontaneous
43.1 dB
5.7 Hz
Carbachol
42.5 dB
5.6 Hz
44.5 dB
5.0 Hz
0.9 mA
Hypothalamic
Stimulation
43.1 dB
7.7 Hz
Spontaneous+.9 mA
42.4 dB
7.7 Hz
Carbachol+.9 mA
42.5 dB
5.7 Hz
44.2 dB
6.4 Hz
0.5 mV
1 sec

Figure 7 presents the peak frequency of the field activity during eight seconds of electrical stimulation delivered to the hypothalamus. The values are averaged across samples and animals and then plotted for the varying stimulation intensities delivered to the hypothalamus through the chemotrode. The figure contains six panels, each representing the results for the six conditions, 1) pre-procaine baseline values, 2) following a carbachol infusion into the hippocampus, 3) ten minutes after procaine infusion into the hypothalamus via the chemotrode, 4) post 30 minutes procaine, 5) post 45 minutes procaine and 6) post 60 minutes procaine.

Prior to the infusion of carbachol and procaine, electrical stimulation of the hypothalamus had an obvious influence on hippocampal field activity (upper left panel). The peak frequency of spontaneous theta was 4.188 $\pm$ .083 Hz ($\pm$SEM). Recall that spontaneous theta is not evoked with hypothalamic stimulation and was used as a stimulus condition for comparative purposes. This panel also reveals the increased theta frequencies found when increased stimulation intensities were delivered to the hypothalamus. Note, that in the hypothalamus, 0.1 mA stimulation was subthreshold and would not produce hippocampal theta. Electrical stimulation in the hypothalamus at intensities of 0.3, 0.5, 0.7 and 0.9 mA produced hippocampal theta frequencies which averaged 4.674 $\pm$ 0.56 Hz, 6.025 $\pm$ 0.31, 7.0 $\pm$ .335 and 7.575 $\pm$ .409 Hz, respectively. Throughout the thesis, all variance is reported as a standard error of the mean.

The middle left panel shows the effects following the infusion of the cholinergic agonist carbachol into the hippocampus. Note that the spontaneous theta frequency was increased to 5.688 $\pm$ 0.26 Hz and indicates the frequency of theta produced by the infusion of carbachol (indicated as carbachol theta, CT). It is also evident in this panel that frequencies below this carbachol frequency were not observed. Even the subthreshold stimulation intensity of 0.1 mA revealed an increased frequency of 5.713 $\pm$ 0.94 Hz, virtually the same frequency as the carbachol theta. Increased hypothalamic stimulation intensities produced increased theta frequencies of 6.287 $\pm$ 0.35 at 0.3 mA, 6.688 $\pm$ .37 at 0.5 mA, 6.988 $\pm$ .35

Figure 7. A diagram summarizing the results of experiment one on the peak frequency of hippocampal theta field activity. Each of the six panels present the peak frequency of spontaneous theta (SP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, carbachol theta (CT), post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI conditions respectively.


Pre-Procaine
Post 30 minutes
Carbachol
Post 45 minutes
Post 10 minutes
Post 60 minutes
Theta Frequency (Hz)
8
6
4
2
SP
CT
.1
.3
.5
.7
.9
Intensity (mA)

at 0.7 mA and 7.6 ± 0.41 at 0.9 mA. Follow-up analysis which examined the effect of stimulation intensity on hippocampal thetafrequency within each level of the repeated measure revealed significant results for the carbachol condition ($F_{(5,54)}$ = 4.617, p = .0014). A follow-up ANOVA also examined contrasts among the levels of the repeated measure. A significant difference was found between the frequency of theta before and after carbachol infusion (Scheffe $F_{(1,58)}=13.38$, $p<0.01$). This was probably due to increased theta frequencies only for the spontaneous, 0.1 mA and 0.3 mA stimulus conditions. I mention this to emphasize the point that theta frequencies for all stimulus intensities were not elevated. It was only the stimulation intensities that evoked hippocampal theta at frequencies below or around the spontaneous carbachol frequency which were elevated, namely the spontaneous, 0.1 and 0.3 mA conditions; those stimulation intensities which evoked theta at frequencies above the spontaneous carbachol frequency did so at frequencies almost identical to those produced before the carbachol infusion.

The bottom right panel of Figure 7 depicts the effect which procaine inactivation of the hypothalamus had on intrahippocampal carbachol-induced theta. It can be seen that 10 minutes after procaine was infused into the hypothalamus, stimulation at that site no longer modulated carbachol elicited hippocampal theta field activity. A follow-up ANOVA revealed that there were no significant differences in the frequency of hippocampal theta field activity elicited across the stimulus intensity condition for the post 10 minute procaine measure ($F_{(5,54)}=0.088$, p = .994). This demonstrated that the procaine inactivation of the hypothalamic region was effective. Despite the hypothalamic inactivation, the peak frequency of intrahippocampal carbachol-induced theta remained unchanged. Pre-procaine carbachol theta had a peak frequency of 5.688 ± 0.26 Hz, whereas post-procaine carbachol theta had a frequency of 5.637 ± 0.29 Hz, virtually no difference at all.

The right hand column of panels in Figure 7 demonstrates the recovery of the hypothalamic region from procaine inactivation at 30, 45 and 60 minute recovery intervals. The ability to modulate the frequency of carbachol theta gradually recovered, first at higher stimulus intensities and later at lower modulating stimulus

intensities. A theta frequency of 5.838 ± 0.35 Hz was evoked by 0.9 mA hypothalamic stimulation at 30 minutes after the procaine infusion, 6.488 ± 0.37 at 45 minutes after, and 6.125 ± 0.35 sixty minutes after inactivation. By 60 minutes post procaine, the frequency of theta elicited by hypothalamic stimulation was no longer significantly different from baseline values demonstrating that the hypothalamus had recovered (comparison of pre vs. post 60, F = 0.927, p = not significant).

iii) ***PH-SUM Stimulation & Reversible Inactivation: Effects on the Amplitude of Hippocampal Theta Field Activity***

Stimulation of the hypothalamus had no significant effects on the amplitude of spontaneous hippocampal theta or carbachol theta. A 2-factor repeated measures ANOVA found the main effect of stimulation intensity on the amplitude of theta to be nonsignificant. However, significant results were found for the repeated measure of inactivation on amplitude ($F_{(5,25)} = 32.061$, $p < .0001$) as well as the intensity X inactivation interaction ($F_{(25,270)} = 14.021$, $p < .0001$). This effect was due to the elevated amplitude of the signal following carbachol infusion into the hippocampus (pre vs. carbachol, $F_{(1,58)} = 6.788$; pre vs. post 10, $F_{(1,58)} = 4.666$; pre vs. post 30, $F_{(1,58)} = 5.061$; pre vs. post 45, $F_{(1,58)} = 11.96$; pre vs. post 60, $F_{(1,58)} = 10.469$, all $p<0.01$. The mean amplitude value, collapsed across stimulation intensities, for the pre-carbachol (baseline) condition was 39.679 ± 0.86 dB, whereas following the carbachol infusion it was elevated to 43.268 ± 0.42 dB and maintained that amplitude throughout the remainder of the experiment.

No further changes in amplitude were evident following procaine inactivation of the hypothalamus. The amplitude of the signal, collapsed across the stimulation intensity factor, 10 minutes after the procaine infusion was maintained at 42.655 ± 0.36 dB, 42.778 ± 0.40 dB at post 30 minutes, 44.443 ± 0.57 dB at post 45 minutes and 44.137 ± 0.63 dB at 60 minutes post procaine. A follow-up repeated measures ANOVA which collapsed across the stimulation intensity factor, revealed only pre versus post carbachol differences. There were no significant changes in the amplitude of the signal post-procaine.

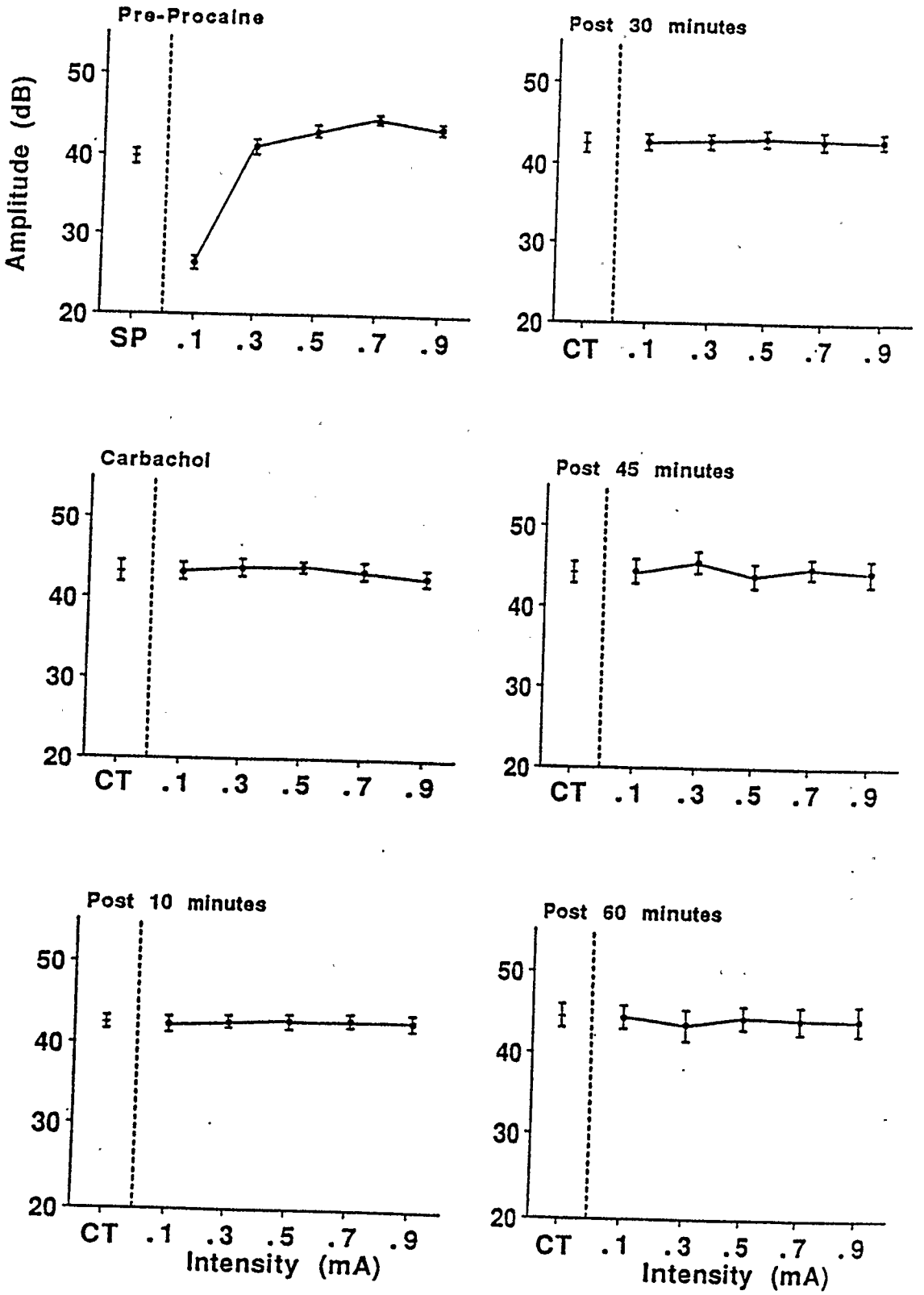
Figure 8 summarizes the results of the experiment on the amplitude of the hippocampal signal. The values depicted in each panel of Figure 8 provide the mean amplitude of hippocampal field activity samples used to determine the peak frequency measurements of Figure 7. Thus, amplitude and frequency measurements are from the same sample of hippocampal field activity. The upper left panel of figure 8 shows the baseline amplitude measurements for spontaneously occurring theta and for each stimulation intensity. It is shown here that when hypothalamic stimulation fails to elicit theta in the hippocampal formation, as in the 0.1 mA sub threshold condition, the amplitude is diminished. The amplitude level was measured at the same peak frequency as spontaneous theta. That is, during 0.1 mA stimulation, which did not produce hippocampal theta, the amplitude measure was taken at the same frequency that spontaneous theta had previously occurred. Note that there are no significant amplitude increases with increasing levels of hypothalamic stimulation.

The middle left panel of Figure 8 relates the effect of the carbachol infusion into the hippocampus. The carbachol-induced theta amplitude had increased, and where there was no theta previously produced by the sub threshold stimulation intensity, carbachol theta now predominated. Carbachol-induced theta during all intensities of stimulation had the same amplitude as the theta induced by carbachol alone. The lower left panel and following right column of panels in figure 8 demonstrate that the amplitude following procaine inactivation of the hypothalamus was unaffected.

Discussion - Experiment 1

Experiment 1 revealed that hippocampal theta field activity, elicited by an intrahippocampal infusion of carbachol, could be modulated by hypothalamic stimulation. At first inspection this may seem to be a trivial finding, however, it demonstrated that even though intrinsic circuitry within the hippocampal formation was cholinergically activated, resulting in hippocampal synchrony, ascending extrinsic influences such as hypothalamic stimulation were still able to modulate the synchronized field activity. This also provided some evidence in the acute preparation for what has already been demonstrated chronically in the behaving rat.

Figure 8. A diagram summarizing the results of experiment one on the amplitude of hippocampal theta field activity. Each of the six panels present the peak frequency of spontaneous theta (SP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, carbachol theta (CT), post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI conditions respectively.


Pre-Procaine
Post 30 minutes
Carbachol
Post 45 minutes
Post 10 minutes
Post 60 minutes
Amplitude (dB)
50
40
30
20
SP
CT
.1
.3
.5
.7
.9
Intensity (mA)

Hypothalamic stimulation has been shown to modulate hippocampal theta activity as well as behaviour (Bland, 1971; Bland *et al.*, 1972). During ongoing behaviour cholinergic activation is prominent during voluntary motor behaviour (Bland, 1986) and during the processing of relevant sensory stimuli (Day, Damsma and Fibiger, 1990). Cholinergic activation of type II theta would correspond to a given frequency and amplitude of hippocampal theta field activity. As the intensity of sensory stimulation increases in the environment, increases in the activation of the synchronizing circuitry have to be possible to accommodate for appropriate adjustments in the animal's behaviour. As well, if increasing intensities of hypothalamic stimulation produce increased velocities of wheel running, as Bland *et al.*, (1972) have shown, one would have predicted this finding in the acute preparation. Taken together, this supports the role of the hippocampal formation in sensory-motor integration.

Another important finding was that following hypothalamic inactivation, theta elicited by intrahippocampal carbachol infusion was maintained with no change in the frequency or amplitude of hippocampal theta field activity. In this case, theta was evoked by direct cholinergic activation of the hippocampal formation's intrinsic circuitry. Following hypothalamic inactivation, there was no change in intrinsic activation since the effect of intrahippocampal carbachol was still predominant. Expectedly, only the ability to modulate this elicited form of theta was abolished. This is in direct contrast to **septal** inactivation (Smythe et. al., 1991) where theta evoked by carbachol infusion directly into hippocampus was totally abolished. In the same study, the influence of hypothalamic stimulation on hippocampal field activity was blocked by septal inactivation. The explanation for this hypothesized by Smythe, *et al.* (1992) to be due to removal of the septal GABA-ergic pathway, thus removing the disinhibitory influence on hippocampal interneurons and allowing inhibition to predominate. Inactivation of the hypothalamus apparently did not have this effect.

Finally, examination of the recovery of the hypothalamic region, and its ability to modulate hippocampal theta field activity induced by carbachol, provided some

interesting findings. The stimulation's ability to modulate hippocampal theta recovered first at high intensities, as would be expected, but evoked frequencies lower than baseline values. First, let us assume that as the hypothalamus recovers from procaine anaesthesia, greater numbers of previously inactivated cells recover as time passes. Although the exact number or proportion of cells which have recovered 30 minutes after the procaine infusion is not known, we can assume that it will be substantially less than the number of cells recovered 45 minutes after infusion. The 15 minute difference in recovery time allows more cells to recover from the affects of the anaesthetic. It was shown that 0.9 mA of hypothalamic stimulation modulated carbachol theta at 5.8 Hz, 30 minutes after the procaine infusion and at 6.5 Hz, 45 minutes after the infusion. Taken together, this suggests that greater levels of tonic activation in the hypothalamus results in higher frequencies of hippocampal theta activity. That is, if one operationally defines greater levels of tonic activation to be larger proportions of activated cells in that region, this may be a plausible explanation. However, an equally likely explanation would be that the effects are due to the effect of procaine on axons passing through the PH-SUM region. The present results cannot be used to distinguish between these two possibilities.

In Experiment 1, theta was induced at the level of the hippocampal formation. In Experiment 2, the idea that the PH-SUM receives inputs ascending from the pontine brainstem region was tested by examining the effects of reversible activation of this region on both spontaneous theta and sensory induced (tail pinch) theta. In other words, Experiment 2 was designed to test the effects of hypothalamic inactivation and recovery on hippocampal theta produced by activation of the ascending brainstem pathways below the level of the hypothalamus.

Objectives - Experiment 2

Experiment 2 attempted to evaluate the effect of hypothalamic inactivation on hippocampal field activity spontaneously cycling between LIA and theta and on theta induced by sensory stimulation (pinching the tail of the rat). Hypothalamic stimulation at varying intensities was used as a test input to assess both the effectiveness of the procaine block on the hypothalamus and its subsequent recovery.

Methods - Experiment 2

i) ***Subjects***

Five male Long Evans rats weighing between 300 and 550 g served as subjects. The animals were maintained as previously outlined in experiment one.

ii) ***Apparatus***

The experimental apparatus used was identical to that used in experiment one with the exception that a hippocampal infusion cannula was not used.

iii) ***Surgery***

Surgical preparation followed a protocol similar to that outlined in experiment one. A lighter level of anaesthesia was used in an attempt to promote spontaneous cycling between theta and LIA. Once samples of these dependent measures were collected, then the animal was deeply anaesthetized to obtain the results of hypothalamic stimulation.

iv) ***Drugs Administered***

In experiment 2, urethane and procaine hydrochloride were used at the same concentrations as previously noted.

v) ***Experimental Procedure***

Experiment 2 examined the effect of procaine inactivation of the hypothalamus on spontaneous field activity and theta induced by sensory stimulation. It implemented a procedure identical to the first with the exception that carbachol was not used to evoke hippocampal theta activity. However, sensory stimulation in the form of a tail pinch was used to elicit hippocampal theta, referred to as tail pinch theta. As well, the affect of hypothalamic inactivation on LIA, spontaneous theta and tail pinch theta was investigated. Therefore, the design examined the dependent measures LIA, spontaneous theta, tail pinch theta and theta evoked by the varying intensities of hypothalamic stimulation, across the same repeated inactivation condition as outlined in experiment one.

Five blocks of stimulus intensities were randomly generated, one for each of the baseline, post-10, 30, 45, and 60 minute inactivation conditions. Following surgical preparation of the rat an appropriate level of anaesthesia was attained which

allowed the rat to involuntarily cycle between theta and LIA. Two 10 second samples of spontaneous theta and LIA were recorded, along with two 10 second samples of tail pinch theta, and then the animal was given supplemental doses of urethane to deepen the level of anaesthesia. A recording of hippocampal field activity was obtained during the first block of stimulus intensities. Following this there was a time out period initiated in order to allow the level of anaesthesia to lighten until spontaneous and tail-pinch theta had returned. This procedure was necessary in order that the affect of hypothalamic inactivation on spontaneous field activity and tail pinch theta could be evaluated.

Upon collection of baseline recordings, 4 μl of procaine was infused into the hypothalamic region through the chemotrode at 1 μl/min and a stopwatch was started. At each condition interval following the infusion (i.e. 10, 30, 45 and 60 minutes post infusion), samples of spontaneous field activity as well as field activity during tail pinch were collected. Electrical stimulation blocks were delivered to the hypothalamus at their respective condition intervals and also recorded on tape.

At the completion of an experiment, the rat was overdosed with urethane and the brain prepared for histological analysis as described in experiment one.

Results - Experiment 2

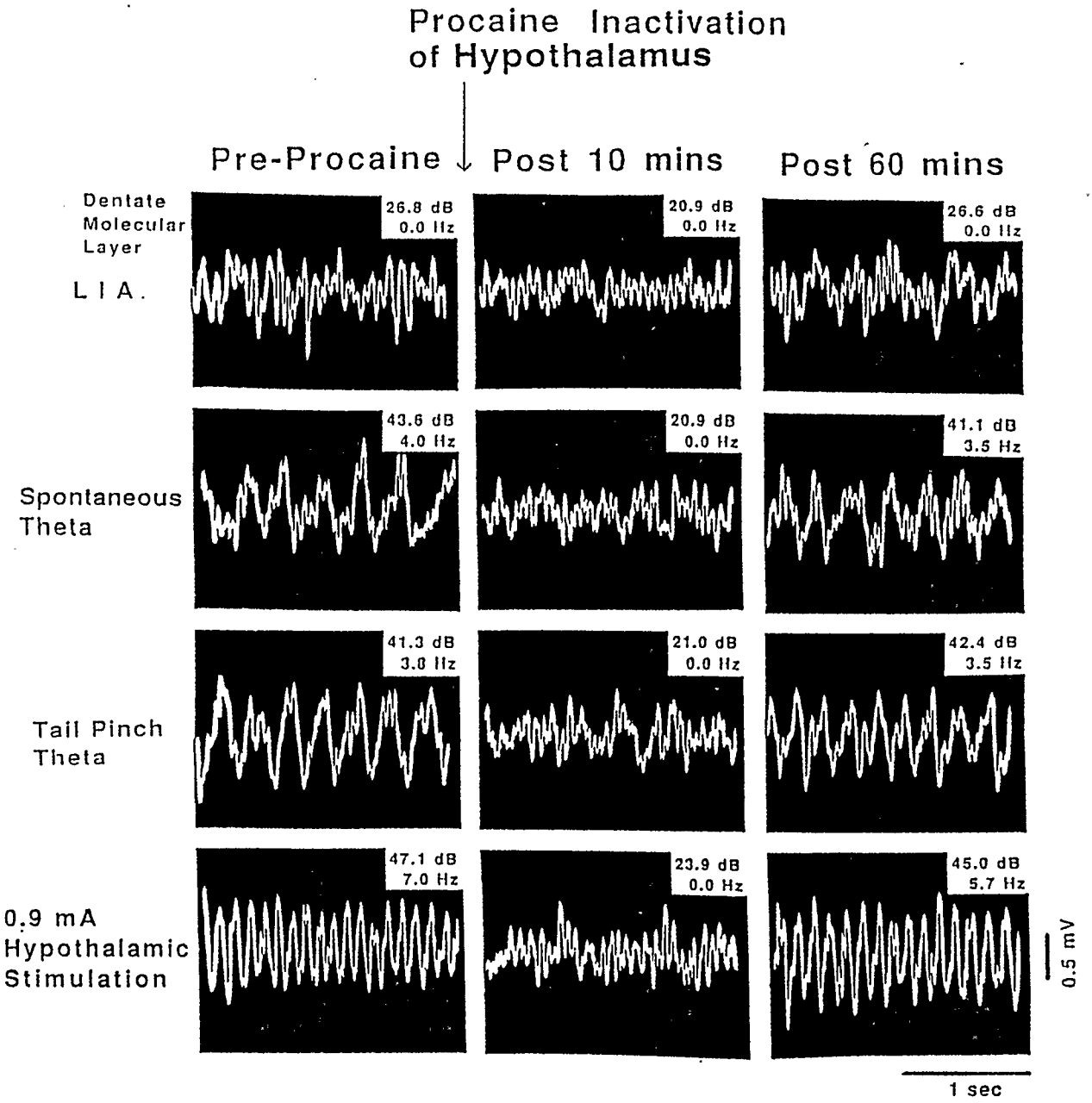
i) *Histology*

Histological analysis revealed the chemotrode tip to be positioned in the posterior hypothalamus of all five animals. Hippocampal reference electrode tracts were found in either the left or right hippocampal formation, the tip found in the region of the upper blade of the dentate gyrus.

ii) *PH-SUM Stimulation & Reversible Inactivation: Effects on the Frequency of Hippocampal Field Activity*

The second experiment revealed interesting results with respect to the contributions of the hypothalamus to the amplitude and frequency of sensory related hippocampal theta field activity. Figure 9 illustrates the effect of hypothalamic inactivation on the amplitude and frequency of hippocampal field activity recorded during the different experimental conditions (LIA, spontaneous theta, tail pinch theta,

Figure 9. Experimental analogs revealing the effect of hypothalamic inactivation on hippocampal LIA, spontaneously occurring theta field activity, theta elicited by pinching the tail of the rat, and theta activity elicited by 0.9 mA hypothalamic stimulation. Following infusion of procaine into the hypothalamus, LIA and spontaneous theta were abolished in the hippocampal field activity as well as theta elicited by tail pinch and 0.9 mA stimulation.


Procaine Inactivation
of Hypothalamus
Pre-Procaine
Post 10 mins
Post 60 mins
Dentate
Molecular
Layer
L I A.
26.8 dB
0.0 Hz
20.9 dB
0.0 Hz
26.6 dB
0.0 Hz
Spontaneous
Theta
43.6 dB
4.0 Hz
20.9 dB
0.0 Hz
41.1 dB
3.5 Hz
Tail Pinch
Theta
41.3 dB
3.8 Hz
21.0 dB
0.0 Hz
42.4 dB
3.5 Hz
0.9 mA
Hypothalamic
Stimulation
47.1 dB
7.0 Hz
23.9 dB
0.0 Hz
45.0 dB
5.7 Hz
0.5 mV
1 sec

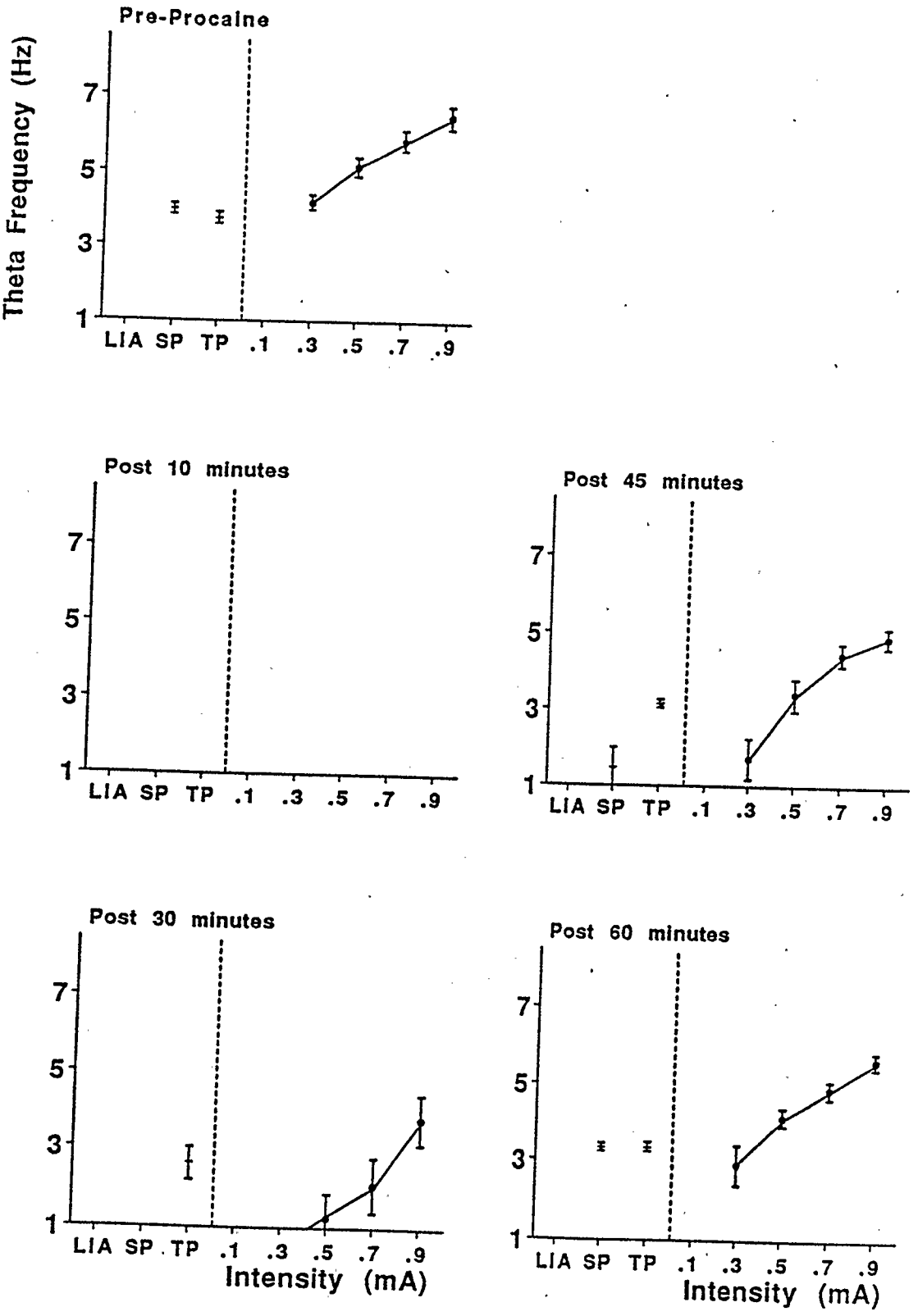
and theta evoked by 0.9 mA hypothalamic stimulation for pre-procaine, 10 minutes post-procaine and 60 minutes post-procaine conditions). Note the decrease in the amplitude of LIA 10 minutes after procaine infusion in the hypothalamus. Also note that following procaine (post 10 mins), there was no theta present for any stimulus condition, including during 0.9 mA hypothalamic stimulation. The numeric values at the top right of each panel provide the amplitude and frequency measurements from the spectral analysis of the individual analog samples shown.

Statistical analysis found the main effect of stimulation intensity on the frequency of hippocampal field activity to be statistically significant ($F_{(7,72)} = 59.219$, $p < .0001$). The repeated measure procaine inactivation on the frequency of hippocampal field activity and the intensity X inactivation interaction were also found to be statistically significant ($F_{(4,72)} = 267.556$, $p < .0001$; $F_{(28,288)} = 18.264$, $p < .0001$, respectively).

The results of experiment two on the peak frequency of hippocampal field activity are presented in Figure 10. The five panels represent the pre-procaine (baseline), 10 minutes, 30 minutes, 45 minutes, and 60 minutes post infusion conditions. The graphed values were averaged across samples and animals and include spontaneously occurring LIA and theta (SP), theta induced by pinching the tail of the animal (TP), and the stimulation intensity measures (0.1-0.9 mA).

The upper right panel of Figure 10 presents the peak baseline frequencies. Spontaneously occurring theta had a mean frequency of 3.975 $\pm$ 0.16 Hz ($\pm$SEM) whereas theta induced by tail pinch had a mean frequency of 3.737 $\pm$ 0.17. Hypothalamic stimulation at 0.1 mA failed to elicit hippocampal theta where 0.3 mA elicited hippocampal theta field activity with a frequency of 4.175 $\pm$ 0.22 Hz, 5.125 $\pm$ 0.26 Hz at 0.5 mA, 5.825 $\pm$ 0.30 Hz at 0.7 mA and 6.438 $\pm$ 0.32 at 0.9 mA of hypothalamic stimulation. Note that although LIA had an amplitude across a spectra of frequencies, there was no predominant peak frequency at which it occurred and thus no values were plotted. However, LIA was presented for the sake of consistency with respect to the next figure illustrating amplitudes.

Figure 10. A diagram summarizing the results of experiment two on the peak frequency of hippocampal theta field activity. Each of the five panels represents the peak frequency of spontaneous theta (SP), tail pinch theta (TP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI conditions respectively. As the hypothalamus recovered from inactivation, the frequency recovered slowly, eliciting frequencies significantly lower than preprocaine values which slowly returned to baseline.


Pre-Procaine
Theta Frequency (Hz)
7
5
3
1
LIA SP TP .1 .3 .5 .7 .9
Post 10 minutes
Post 45 minutes
Post 30 minutes
Post 60 minutes
Intensity (mA)

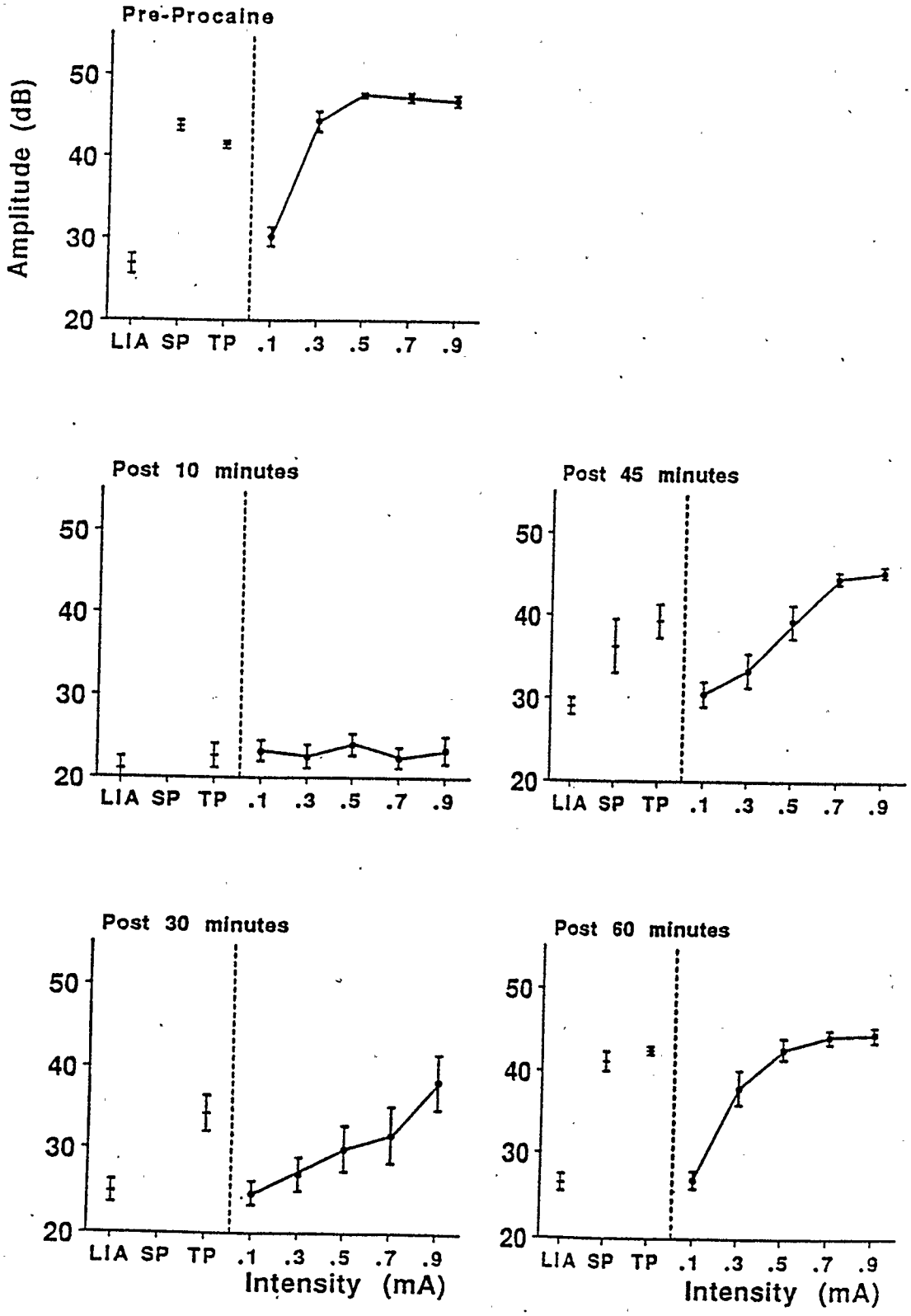
The middle left panel of Figure 10 reveals the dramatic effect of procaine inactivation on the peak frequency of hippocampal field activity. The procaine infusion in the hypothalamus eliminated spontaneously occurring theta, theta produced by tail pinch, and theta elicited by electrically stimulating the hypothalamus at all intensities. The latter demonstrated that an effective block of hypothalamic function was attained since even the highest level of hypothalamic stimulation produced no theta.

The bottom left panel and the following right hand column of panels in Figure 10 show the time course of recovery from hypothalamic inactivation. The ability of hypothalamic stimulation to evoke hippocampal theta field activity recovered gradually, first at the highest intensities of stimulation (0.7 and 0.9 mA) and later at the lower intensities (0.3 and 0.5 mA). For the baseline condition, 0.9 mA of hypothalamic stimulation evoked theta at 6.438 $\pm$ .32 Hz. Following procaine this level of stimulation no longer evoked theta, 30 minutes after the procaine infusion the same intensity elicited hippocampal theta field activity with a peak frequency of 3.763 $\pm$ .67 Hz, 4.875 $\pm$ .25 Hz at 45 minutes post infusion, and finally 5.625 $\pm$ .22 Hz 60 minutes after the procaine inactivation. Follow-up analysis revealed that pre-procaine frequencies were significantly different from the post 10 minute, 30 minute, and 45 minute conditions (all $p < .001$). However, this was no longer significant for the post 60 minute condition suggesting that the hypothalamus had recovered from the effects of the procaine.

iii) ***PH-SUM Stimulation & Reversible Inactivation: Effects on the Amplitude of Hippocampal Field Activity***

The results of hypothalamic inactivation on the amplitude of hippocampal field activity are presented in Figure 11. Statistical analysis revealed significant results for the main effect of stimulation intensity on the amplitude of hippocampal field activity ($F_{(7,72)} = 33.413$, $p < .0001$). The repeated effect of procaine inactivation on the amplitude of hippocampal field activity ($F_{(4,72)} = 313.186$, $p < .0001$) and the intensity X inactivation interaction ($F_{(28,288)} = 21.007$, $p < .0001$) were also statistically significant. The panels presented in Figure 11 depict the amplitude of the

Figure 11. A diagram summarizing the results of experiment two on the amplitude of hippocampal theta field activity. Each of the five panels represents the amplitude of hippocampal LIA, spontaneous theta (SP), tail pinch theta (TP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI. As the hypothalamus recovered from inactivation, the amplitude recovered rapidly.


Pre-Procaine
Amplitude (dB)
50
40
30
20
LIA SP TP .1 .3 .5 .7 .9
Post 10 minutes
Post 45 minutes
Post 30 minutes
Post 60 minutes
Intensity (mA)

example, in an experiment, if spontaneous hippocampal theta field activity had a peak hippocampal field activity samples which were used to determine peak frequency values in Figure 10.

The upper left panel of figure 11 provides the baseline amplitude for LIA (taken at the frequency of spontaneous theta), spontaneous theta (SP), theta induced by tail pinch (TP) and for each of the stimulus intensities. The amplitude for LIA was measured at the peak frequency at which spontaneous theta occurred. For frequency of 4.125 Hz, then during hippocampal LIA, an amplitude measure was recorded at 4.125 Hz. This provided a consistent reference point at which amplitude measurements for LIA could be obtained. LIA had a mean amplitude of 26.81 $\pm$ 1.33 dB at $\approx$4 Hz. Spontaneously occurring theta field activity and theta field activity induced by pinching the rat's tail had amplitudes of 43.62 $\pm$ 0.73 dB and 41.37 $\pm$ 0.46 dB, respectively. During 0.1 mA hypothalamic stimulation, an intensity unable to elicit theta, the hippocampal field activity had an amplitude of 30.2 $\pm$ 1.27 dB, similar to the amplitude of LIA, which was the field activity recorded during stimulation at this intensity. Hypothalamic stimulation with an intensity of 0.3 mA produced a hippocampal theta amplitude of 44.24 $\pm$ 1.32 dB, 47.44 $\pm$ 0.37 dB at 0.5 mA, 47.29 $\pm$ 0.62 dB at 0.7 mA and 46.8 $\pm$ 0.72 at 0.9 mA. Although the main effect of stimulation intensity on the amplitude of theta was found to be significant, follow-up analysis comparing stimulation intensities found that 0.3 mA of stimulation was not significantly different from 0.5, 0.7, or 0.9 mA; that 0.5 mA was not different from 0.7 or 0.9 mA; and that 0.7 was not significantly different from 0.9 mA. This finding is consistent with the results of the first experiment which found that stimulation intensity did not affect the amplitude of hippocampal theta. The above significant result then was due to the obvious difference in the amplitude of LIA compared to the theta amplitudes.

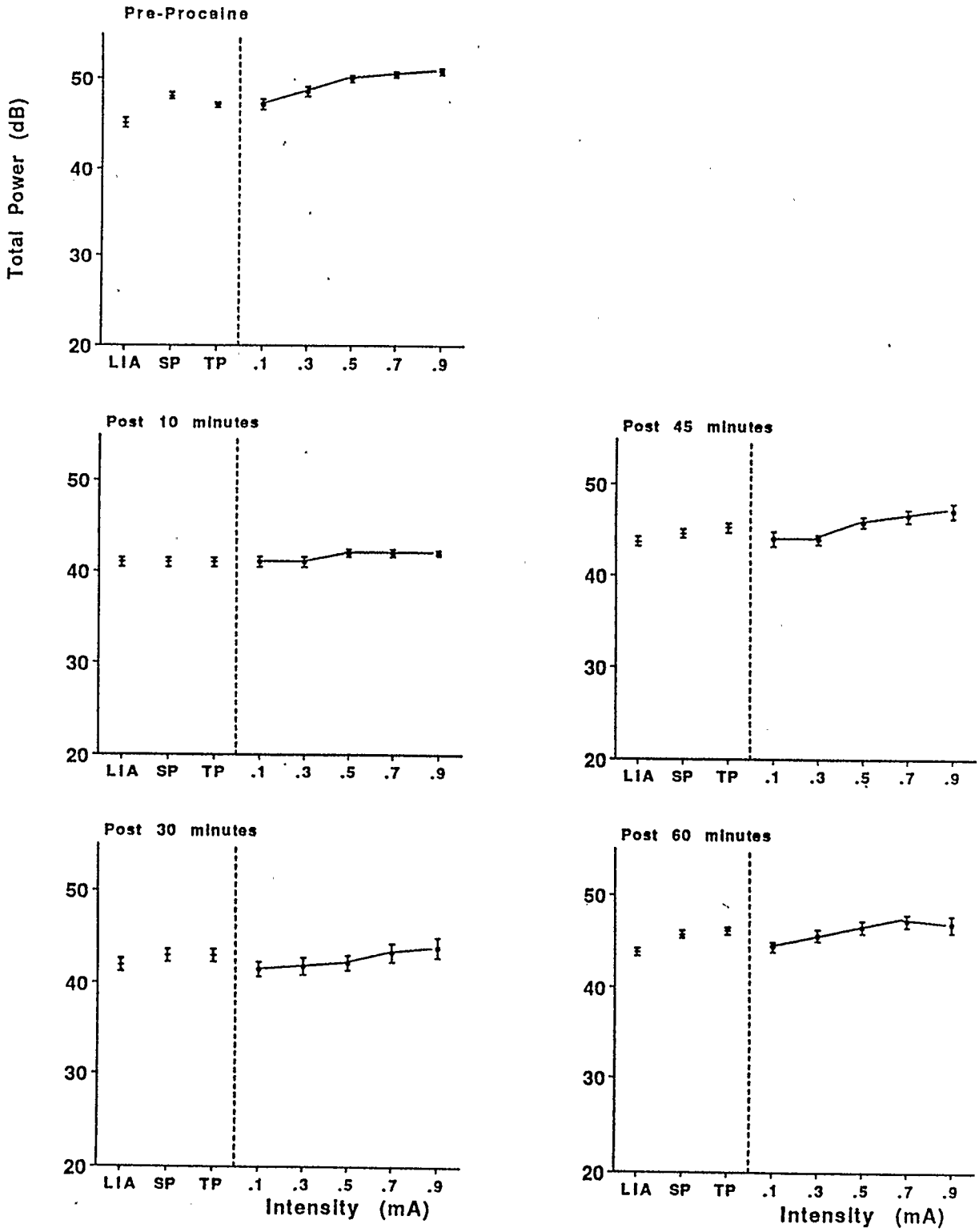
The middle left panel of Figure 11 presents the effect of hypothalamic inactivation on hippocampal field activity. The amplitude of the signal in all stimulus conditions decreased. Even the amplitude of LIA was decreased from 26.81 $\pm$ 1.33 dB to 20.94 $\pm$ 1.74 dB. There was no spontaneous theta and the amplitude of the

field activity during tail pinch was 22.72 ± 1.63 dB. The mean amplitude of hippocampal field activity during 0.1 mA stimulation was 23.22 ± 1.29 dB, 22.54 ± 1.48 dB at 0.3 mA, 24.04 ± 1.46 dB at 0.5 mA, 22.41 ± 1.32 at 0.7 mA and 23.32 ± 1.77 dB at 0.9 mA of hypothalamic stimulation. With the exception of spontaneous theta, there were no significant differences in the amplitude of the field activity compared across the stimulus intensity conditions at the post 10 minute procaine interval.

The bottom left panel and right column of panels in Figure 11 depict hippocampal field amplitude as the hypothalamus recovers from procaine inactivation. The amplitude of hippocampal theta at the highest intensity of stimulation for the 30, 45, and 60 minute post infusion intervals was 38.37 ± 0.34 dB, 45.29 ± 0.72 dB and 44.38 ± 0.94 dB, respectively. Follow-up analysis of the repeated measure revealed that although the amplitude of the hippocampal theta pre-procaine was significantly greater than the post 10 and 30 minute conditions, it was no longer significantly different when compared to the post 45 and 60 minute recovery intervals. This comparison demonstrates that the amplitude of the theta signal recovered to baseline values by the post 45 minute infusion interval.

Measurements of the total power in the spectrum are presented in Figure 12. The results were similar to those found for the amplitude measurements. The main effect of stimulation intensity on the total power in the spectrum was significant ($F_{7,72}$= 5.345, $p < .0001$), however, follow-up analysis which removed measures for LIA, spontaneous theta, tail pinch theta, and during 0.1 mA stimulation from the analysis found no significant effect of stimulation intensity on the total power. Analysis revealed that the repeated measure of procaine inactivation on the total power was significant ($F_{4,72}$= 190.477, $p<.0001$). The mean total power in the spectrum prior to procaine inactivation was 48.5 ± 0.3 dB yet after procaine infusion into the hypothalamus the power was reduced to 41.9 ± 0.2 dB. As the hypothalamus recovered from the procaine infusion, post recovery measurements of the total power in the spectrum were 42.5 ± 0.3 dB, 45.2 ± 0.3 and 45.9 ± 0.2 dB

Figure 12. A figure depicting the total power in the spectrum at the varying stimulation intensities for the pre-and post-procaine conditions.


Pre-Procaine
Post 10 minutes
Post 45 minutes
Post 30 minutes
Post 60 minutes
Total Power (dB)
50
40
30
20
LIA SP TP .1 .3 .5 .7 .9
Intensity (mA)

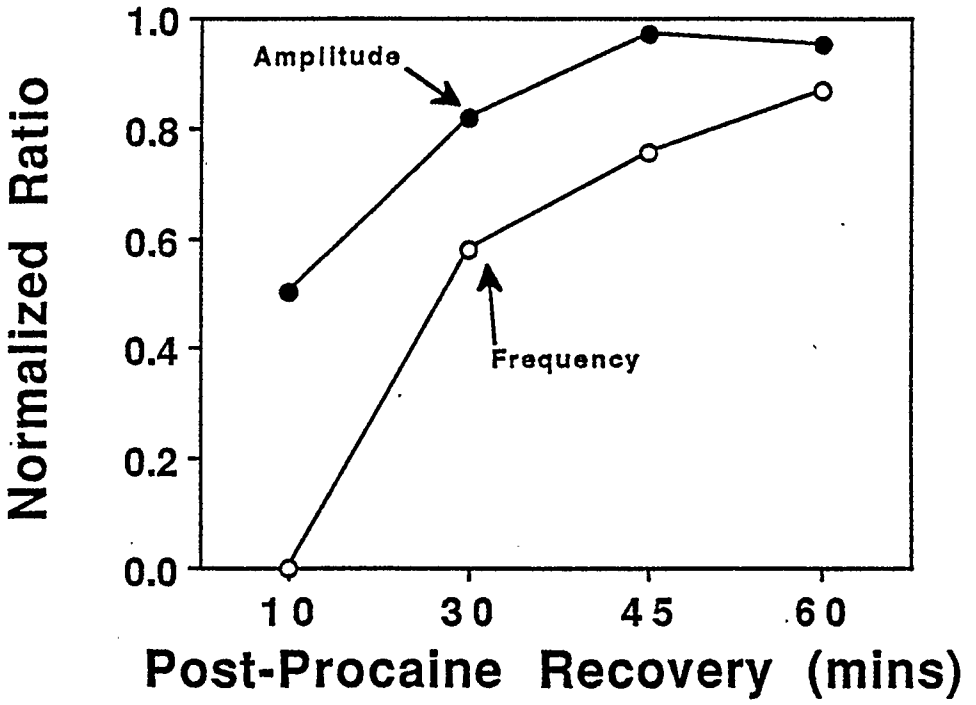
for the post 30, 45 and 60 minute recovery intervals, respectively. Recovery of the total power was faster than that of the frequency of the hippocampal field activity.

Discussion - Experiment 2

The significant finding of the second experiment was that spontaneous hippocampal theta field activity and tail pinch theta (the latter, a sensory induced form of theta field activity) were abolished by hypothalamic inactivation. This suggested that the PH-SUM region forms a critical part of the synchronizing system ascending from the brainstem to the hippocampal formation. It also suggests that an ascending desynchronizing system may pass through this region since the amplitude of hippocampal LIA was also depressed following hypothalamic inactivation. This finding supports the suggestion purported by Vertes (1988) that a desynchronizing input ascends from the brainstem raphe region which desynchronizes hippocampal EEG. It appears as though extrinsic synchronizing and desynchronizing influences ascend via the hypothalamic region investigated here.

The recovery of hypothalamic stimulation's ability to influence hippocampal field activity reaffirmed the findings of the first experiment. As well, it is important to note that the recovery of frequency, to pre-procaine levels, was gradual. This was in contrast to the rapid time course of amplitude recovery. To better interpret the differential recovery of the signal's frequency and amplitude, the data were normalized and presented in Figure 13. Normalizing involved taking the mean amplitude or frequency value for hippocampal theta during 0.9 mA stimulation at the 10, 30, 45, and 60 minute recovery intervals, and dividing these values by the mean pre-procaine amplitude or frequency value. The normalized scores reveal that the amplitude of theta recovered more rapidly than did the frequency. This suggested that the hypothalamus had differential contributions to the theta with respect to amplitude and frequency. It appeared as though its role in determining and maintaining theta frequency was more predominant than its role in maintaining the integrity of theta amplitude, although, it had affects on both. This is in contrast to the conclusions of Smythe et.al., (1991) who found that the amplitude recovered gradually whereas the frequency recovered rapidly after **septal** inactivation. Taken collectively, the septum

Figure 13. A graph of normalized values for amplitude and frequency demonstrating that the amplitude of the signal recovered to pre-procaine values more rapidly than did the peak frequency of the signal.


Normalized Ratio
1.0
0.8
0.6
0.4
0.2
0.0
Amplitude
Frequency
10
30
45
60
Post-Procaine Recovery (mins)

and hypothalamus, two integral components of the ascending hippocampal synchronizing system, have very different roles in generating and maintaining the integrity of hippocampal theta field activity. More on this view will follow.

It was of interest to examine the total power in the spectrum to determine if its decline was responsible for attenuated theta frequency peaks. However, since the amplitude of the hippocampal theta field activity recovered rapidly, and it was the frequency of the prominent peaks in the spectrum which recovered slowly, the effects seen here could not be attributable to a reduction in the total power of the spectrum. Therefore it may be concluded that the effect on the theta frequency was greater than the effect on theta amplitude. Following confirmation that the influence of procaine inactivation on the amplitude of the predominant frequency peaks in the hippocampal field signal was not due to reductions in the total power of the spectrum, further presentations of this type are not needed and are limited to this experiment only.

The third experiment which follows examined the effect of intraseptal infusions of carbachol on hippocampal field activity. A portion of the afferent fibers from the septum which synapse on hippocampal circuitry are cholinergic in nature (Freund and Antal, 1988). The fact that theta is evoked by intrahippocampal infusions of carbachol, a cholinergic agonist, reinforces this notion. Likewise, carbachol infusions into the septal region would not only determine if the region was cholinoceptive, but would also imply that hypothalamic afferents to septum are cholinergic. The analysis would also clarify the relative importance of septal afferents, and the nature of its extrinsic influence, on the hippocampus.

Consider the critical nature of the septal influence on hippocampal field activity. Could hypothalamic stimulation modulate theta evoked by septal infusion of carbachol? Recall that septal inactivation abolished all hippocampal theta (Smythe et.al., 1991). If the septum is such a critical nodal point, can ascending activation from the hypothalamus modify enhanced cholinergic activity previously conferred on the septum by carbachol infusion? Experiment 3 addressed these questions by examining the effect of hypothalamic stimulation and subsequent inactivation on hippocampal field activity elicited by the microinfusion of carbachol into the medial

septum. This was done in an attempt to further clarify the interactions taking place between the various components of the ascending hippocampal synchronizing system.

Objectives - Experiment 3

Experiment three examined hippocampal field activity resulting from carbachol infusion into the septum. It further attempted to evaluate the effect of hypothalamic stimulation and subsequent inactivation on hippocampal theta evoked by this type of infusion. Hypothalamic stimulation at varying intensities was examined prior to and after hypothalamic inactivation in an attempt to clarify the contributions made by this region to hippocampal field activity.

Methods - Experiment 3

i) ***Subjects***

Five male Long Evans rats weighing between 300 and 550 g served as subjects. The animals were maintained as previously outlined in experiment one.

ii) ***Apparatus***

The experimental apparatus used was identical to that used in experiment one and two with the following exception. An infusion cannula was used to deliver carbachol to the septum. Carbachol infusion into the septum was performed using a Harvard Apparatus Infusion pump (model 22). First, a 10 μl Hamilton Microliter/Gas tight syringe (#701) was connected via PE 30 intramedic tubing to a cannula placed in the septum. The cannula was constructed from 26 g needles (Chromatographic Specialties Inc.). The 10 μl syringe was placed onto the pump and the flow rate set to 0.5 μl/min.

iii) ***Surgery***

Surgical preparation followed a protocol similar to that outlined in experiment one. However, for the third experiment, a hole was drilled above the septum and carbachol was infused through the cannula positioned at + 0.5 mm A-P, 0 mm L, and 5.5 mm D-V to bregma. Again a deep level of anaesthesia was used as outlined in experiment one.

iv) *Drugs Administered*

In experiment three, urethane, carbachol and procaine hydrochloride were used at the same concentrations as previously noted in experiment one.

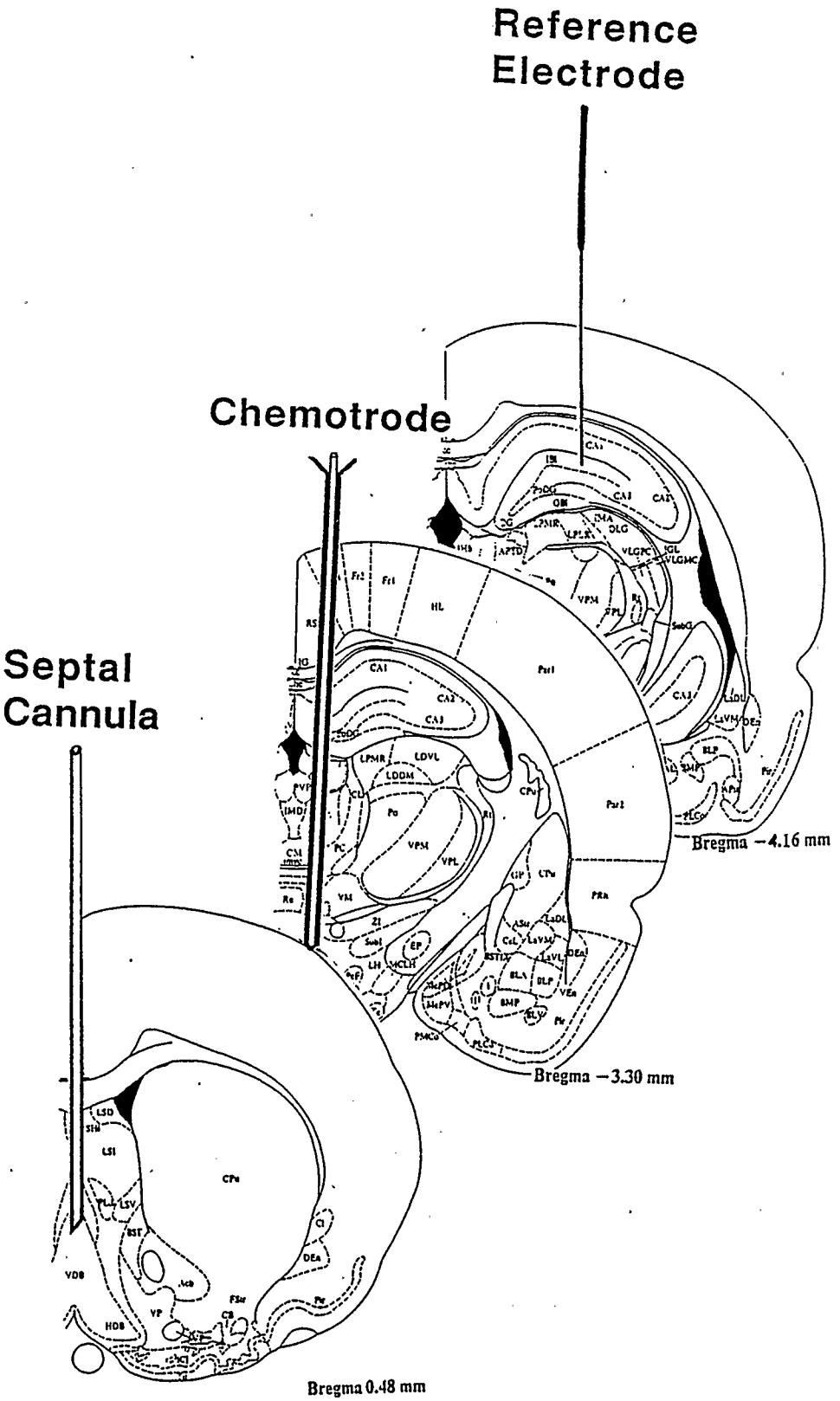
v) *Experimental Procedure*

The third experiment examined the effect of hypothalamic stimulation and inactivation on theta elicited by intraseptal infusions of carbachol. Again, this is referred to as carbachol-induced theta even though it was evoked by intraseptal infusions of carbachol rather than intrahippocampal infusions. The design employed mimicked that used in the first experiment. Figure 14 provides a diagram of the experimental stimulation, infusion, and recording apparatus used in the experiment. The infusion cannula was placed in medial septum in the region of the vertical limb of the diagonal band of Broca. Carbachol was infused into the septum through this cannula. The chemotrode was placed in the PH-SUM region and infused procaine to achieve hypothalamic inactivation. Again, a reference electrode was placed in the upper blade of the dentate close to the *stratum moleculare* for field recordings.

Following surgical preparation of the animal, which included the placement of a septal cannula, an experiment was started by obtaining two samples of spontaneous theta. The animal was then deeply anaesthetized and a recording was procured of the first stimulation block for the baseline condition. Following the baseline recordings of hippocampal field activity, 0.5-1.5 μl of carbachol was infused into the medial septum at a rate of 0.5 μl/min through the septal cannula. If seizure activity ensued, an appropriate delay was allowed to let any such activity subside. Two samples of carbachol-theta and the resulting field activity from the stimulus array delivered to the hypothalamus were recorded on tape.

Following the carbachol condition recordings, hypothalamic inactivation proceeded by injecting 4 μl of procaine via the hypothalamic chemotrode. Two samples of intrahippocampal field activity and the stimulation block delivered to the hypothalamus for the post 10 minute condition were recorded on tape. Identical recordings were taken for the post 30, 45, and 60 minute inactivation conditions.

Figure 14. A diagram which illustrates the placement of the recording electrode used to record hippocampal theta field activity (reference electrode), the cannula used to infuse carbachol into the medial septum to elicit theta field activity, and the chemotrode used to electrically stimulate the hypothalamus and subsequently reversibly inactivate the region by infusing procaine through it.


Reference Electrode
Chemotrode
Septal Cannula
Bregma −4.16 mm
Bregma −3.30 mm
Bregma 0.48 mm

At the end of the experiment, the rat was then overdosed with urethane and the brain prepared for histological analysis.

Results - Experiment 3

i) *Histology*

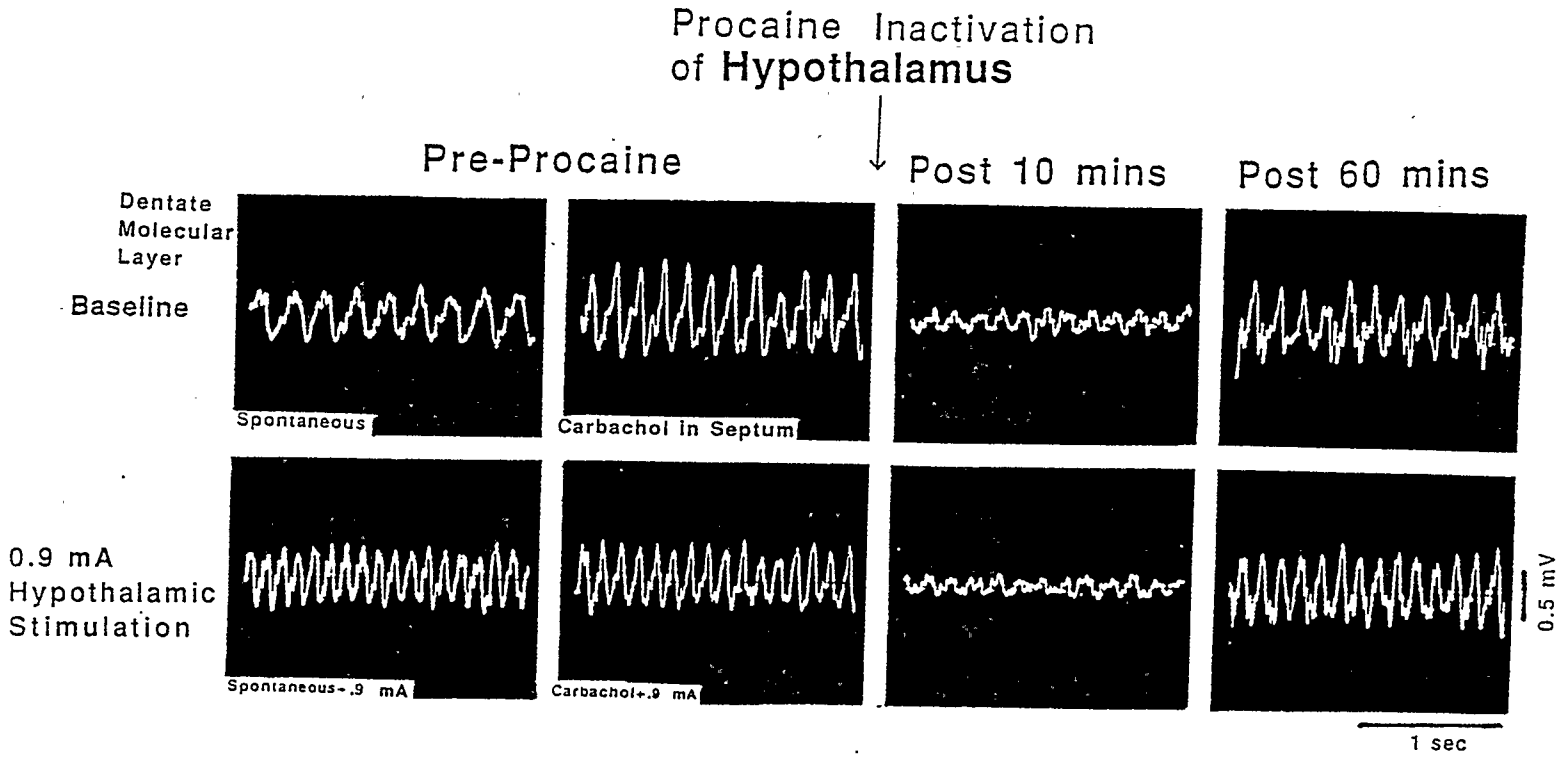
Reference electrode implants were localized to the dentate. Tracts left by infusion cannulae coursed ventrally through the midline, the tip found in the region of the medial septum - vertical limb of the diagonal band of Broca. Chemotrode implants were localized to the posterior hypothalamus.

ii) *PH-SUM Complex Stimulation & Reversible Inactivation: Effects on the Frequency of Hippocampal Field Activity*

The results of experiment three revealed that hippocampal theta could be evoked by septal infusions of carbachol and that this carbachol theta could be modulated with hypothalamic stimulation. The amplitude of theta following hypothalamic inactivation was significantly attenuated. Figure 15 presents analogs of field activity for baseline and during 0.9 mA stimulation. The top row of analogs show spontaneous field activity for the baseline, carbachol, 10 minutes after hypothalamic inactivation and 60 minutes after inactivation conditions. The bottom row show analogs of field activity during 0.9 mA of hypothalamic stimulation at the same conditions as noted above. Note that carbachol infusion into septum produces hippocampal theta (upper panel, second column). Also note that the theta produced by intraseptal carbachol infusion could be modulated with hypothalamic stimulation (bottom panel, same column). Next, note the prominent affect of hypothalamic inactivation on the carbachol theta (upper and lower panels of third column). Although the frequency of the signal was maintained, the amplitude of theta was significantly reduced. This was also the case during 0.9 mA of hypothalamic stimulation suggesting that inactivation was complete. Finally, the ability to modulate carbachol theta with hypothalamic stimulation and the amplitude of theta had recovered 60 minutes after inactivation.

Following procaine inactivation of the hypothalamus, carbachol theta persisted at the pre-procaine frequency yet eliminated hypothalamic stimulation's ability to

Figure 15. Experimental analogs of hippocampal theta field activity during the pre-procaine measure (left column - left, spontaneous theta (top) and theta elicited by 0.9 mA hypothalamic stimulation (bottom); 2nd left column, carbachol theta (top) and carbachol theta modulated with 0.9 mA hypothalamic stimulation (bottom)), 10 minutes after hypothalamic inactivation (middle right column) without hypothalamic stimulation (top) and during 0.9 mA stimulation (bottom) and 60 minutes after hypothalamic inactivation (right column) without stimulation (top) and during 0.9 mA hypothalamic stimulation (bottom). Although hypothalamic inactivation did not completely abolish hippocampal theta field activity elicited by intraseptal infusion of carbachol, there was a significant attenuation of the signal's amplitude.


Procaine Inactivation
of Hypothalamus
Pre-Procaine
Post 10 mins
Post 60 mins
Dentate
Molecular
Layer
Baseline
Spontaneous
Carbachol in Septum
0.9 mA
Hypothalamic
Stimulation
Spontaneous+.9 mA
Carbachol+.9 mA
0.5 mV
1 sec

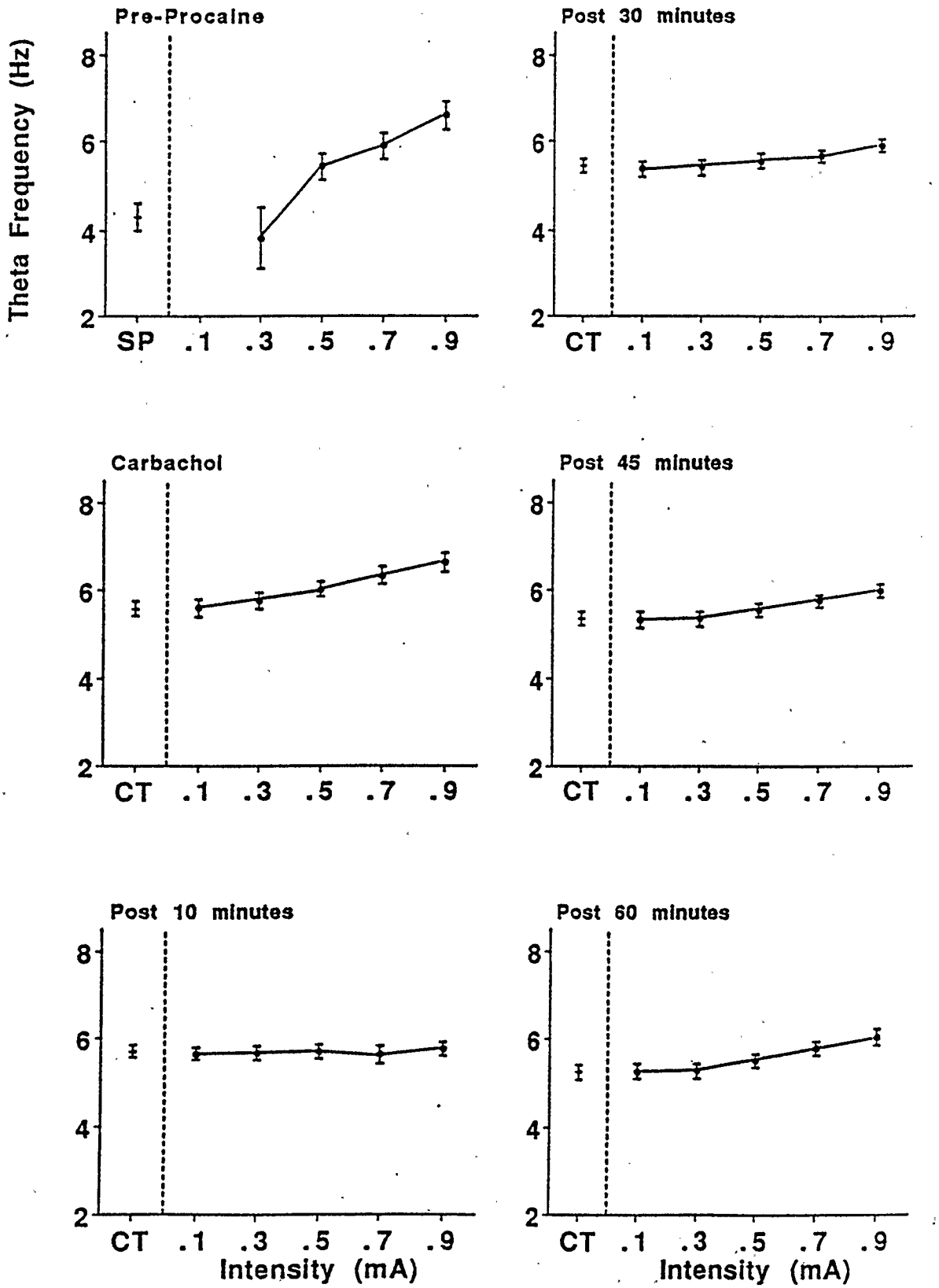
modulate theta activity. A 2-factor repeated measures ANOVA found the main effect of stimulation intensity on the frequency of hippocampal theta ($F_{(5,54)} = 10.486$, $p < .0001$), the repeated measure inactivation on frequency ($F_{(5,54)} = 91.609$, $p < .0001$), and the intensity X inactivation interaction ($F_{(25,270)} = 38.595$, $p < .0001$) all to be statistically significant.

Figure 16 summarizes the results of the experiment on the peak frequency of hippocampal field activity. The upper left panel illustrates the average baseline peak frequencies for each of the stimulation intensity measures. Spontaneous theta had an average peak frequency of 4.313 $\pm$ 0.31 Hz. Hypothalamic stimulation at 0.1 mA was not sufficient to evoke hippocampal theta whereas 0.3 mA of stimulation evoked a peak theta frequency of 3.812 $\pm$ 0.70 Hz, 5.438 $\pm$ 0.28 Hz at 0.5 mA, 5.912 $\pm$ 0.30 Hz at 0.7 mA and 6.588 $\pm$ 0.33 Hz at 0.9 mA.

The middle left panel of Figure 16 provides the peak frequency of theta evoked after carbachol had been infused into the medial septum. Carbachol theta (CT) had an average peak frequency of 5.588 $\pm$ 0.18 Hz. This panel also demonstrates the ability of hypothalamic stimulation to significantly modulate the carbachol theta (follow-up ANOVA within the carbachol condition, $F_{(5,54)} = 4.993$, $p = .0008$). Subthreshold stimulation (0.1 mA) had no influence on the carbachol theta and had a frequency of 5.6 $\pm$ 0.20 Hz, whereas 0.3 mA evoked a frequency of 5.775 $\pm$ 0.19 Hz, 6.025 $\pm$ 0.17 Hz at 0.5 mA, 6.338 $\pm$ 0.19 Hz at 0.7 mA and 6.637 $\pm$ 0.21 Hz at 0.9 mA of hypothalamic stimulation.

The bottom left panel and right column of panels in Figure 16 provide the results of hypothalamic inactivation and subsequent recovery of the region after the procaine infusion. The results appear similar to the first experiment where the frequency of intrahippocampal theta was maintained at the pre-procaine carbachol theta value (in this case, 5.713 $\pm$ 0.15 Hz). Hypothalamic stimulation no longer was able to modulate the carbachol theta which maintained a frequency around 5.7 Hz for all stimulation intensities. This suggested that hypothalamic inactivation was complete. The remaining panels show the recovery of hypothalamic stimulation as

Figure 16. A diagram summarizing the results of hypothalamic inactivation on the peak frequency of hippocampal theta field activity elicited by intraseptal infusion of carbachol. Each of the six panels present the peak frequency of spontaneous theta (SP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, carbachol theta (CT), post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI conditions respectively.


Pre-Procaine
Post 30 minutes
Carbachol
Post 45 minutes
Post 10 minutes
Post 60 minutes
Theta Frequency (Hz)
8
6
4
2
SP
CT
.1
.3
.5
.7
.9
Intensity (mA)

procaine anaesthesia of the area dissipated. Although the frequency of carbachol theta was maintained after procaine infusion, theta amplitude was dramatically reduced.

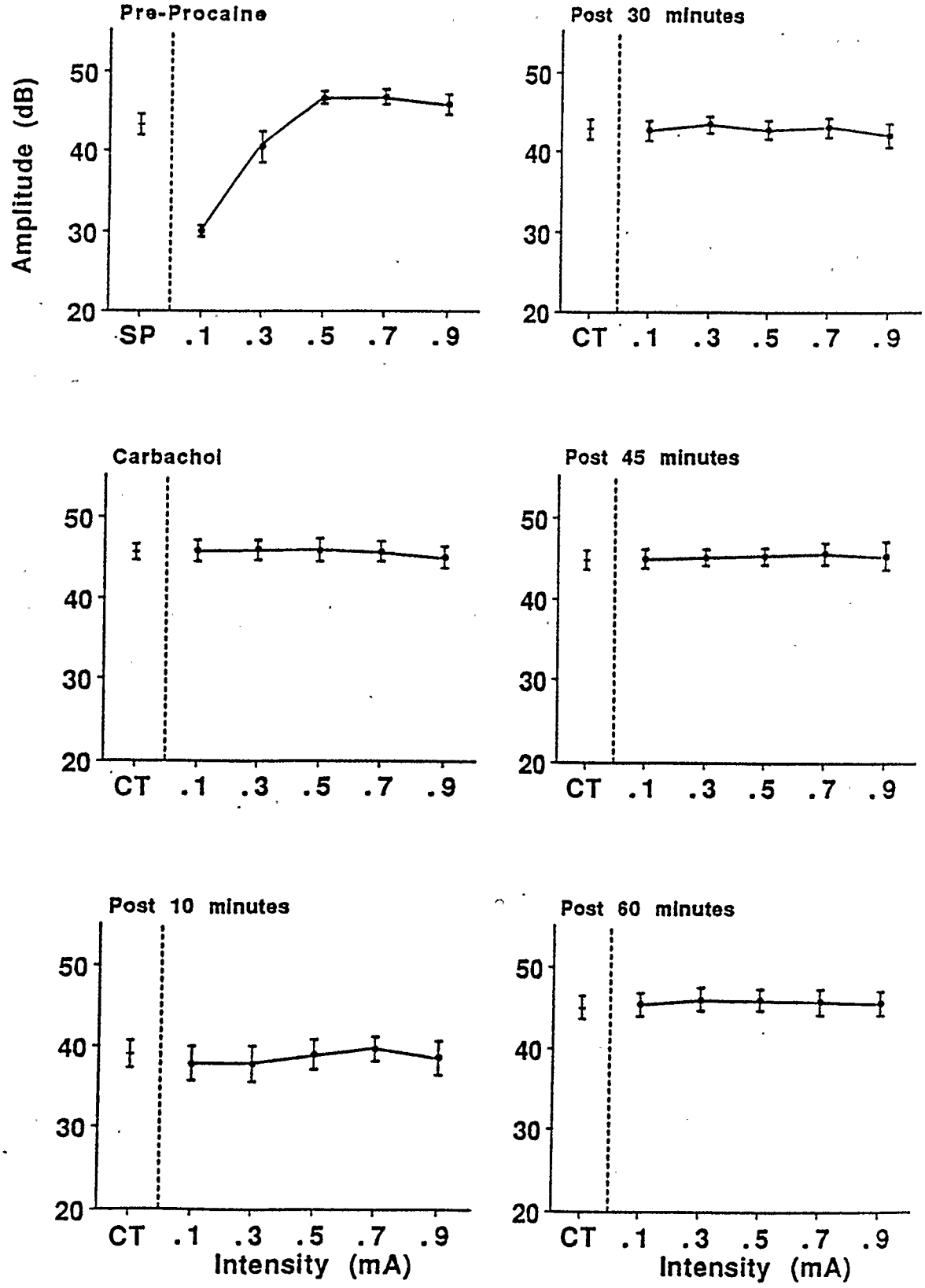
iii) ***PH-SUM Complex Stimulation & Reversible Inactivation: Effects on the Amplitude of Hippocampal Field Activity***

As stated above, the amplitude of theta was significantly attenuated following hypothalamic inactivation. The main effect of stimulation intensity on the amplitude of theta was not significant, however, the repeated measure of procaine inactivation on amplitude of hippocampal field activity ($F_{(5,54)} = 45.373$, $p < .0001$) and the intensity X inactivation interaction ($F_{(25,270)} = 6.636$, $p < .0001$) were significant. Figure 17 summarizes the results of the experiment on the amplitude of hippocampal theta.

The upper left panel of Figure 17 provides the mean amplitude of the field activity for the pre-procaine (baseline) condition. Spontaneous theta had a mean amplitude of 43.16 $\pm$ 1.32 dB. Subthreshold stimulation (0.1 mA) had an average amplitude of 30.09 $\pm$ 0.66 dB which was measured at the frequency of spontaneous theta. Hypothalamic stimulation at 0.3 mA produced an amplitude of 40.41 $\pm$ 1.93 dB, 46.64 $\pm$ 0.89 dB at 0.5 mA, 46.8 $\pm$ 1.03 dB at 0.7 mA and 45.8 $\pm$ 1.29 dB at 0.9 mA. Following carbachol infusion into the septum, the amplitude of the signal did not change as shown in the middle left panel.

The bottom left panel of Figure 17 shows that following procaine infusion into hypothalamus, amplitude of the signal dropped from pre-procaine levels. The mean amplitudes, collapsed across all intensities of the stimulation intensity effect were as follows: the pre-procaine carbachol theta amplitude was 45.627 $\pm$ 0.50 dB and following procaine infusion it was reduced to 38.618 $\pm$ 0.75 dB. This comparison was found to be statistically significant (carbachol vs. post 10 minutes procaine, $F_{(1,58)} = 19.882$, $p < .001$). As is evident from the upper right panel of Figure 17, the amplitude of hippocampal theta had recovered 30 minutes after inactivation, and the effect was no longer statistically significant. No further changes were evident.

Figure 17. A diagram summarizing the results of hypothalamic inactivation on the amplitude of hippocampal theta field activity elicited by intraseptal infusion of carbachol. Each of the six panels present the peak frequency of spontaneous theta (SP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, carbachol theta (CT), post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI conditions respectively.


Pre-Procaine
Post 30 minutes
Carbachol
Post 45 minutes
Post 10 minutes
Post 60 minutes
Amplitude (dB)
50
40
30
20
SP
CT
.1
.3
.5
.7
.9
Intensity (mA)

Discussion - Experiment 3

These findings demonstrated that carbachol infusion into the septum resulted in hippocampal synchrony. This suggested that a population of cells in this region were cholinoceptive, corroborating the findings of Lawson *et al.*, (1991a) and Monmaur and Breton (1991). It was also determined that hypothalamic stimulation was able to modulate theta evoked by intraseptal infusions of carbachol. This demonstrated that the extrinsic influence of the hypothalamus on septal action was an important part of the ascending circuitry. Like the previous experiments of this thesis, the current findings emphasized the role of the hypothalamic region in regulating hippocampal field activity.

Hypothalamic inactivation had quantitatively different effects on the amplitude and frequency of hippocampal theta field activity. It appeared as though these influences were integrated by the septum and then relayed on to the hippocampal formation. In experiment two, varying degrees of hypothalamic inactivation resulted in correspondingly varying frequencies of hippocampal theta. In experiment three, inactivation seemed to influence not only the frequency of theta, as shown in experiment two, but also the amplitude of theta. It appears that the affect of inactivation on the amplitude of hippocampal theta field activity recovers much more quickly than the influence of inactivation on the frequency of theta. This was seen for the amplitude of carbachol-induced theta when it was compared before and after procaine inactivation of the hypothalamus. Following carbachol infusion into the septum, the amplitude of the signal was not significantly greater than pre-carbachol values. Following procaine inactivation of the hypothalamus, the amplitude dropped below pre-carbachol and pre-procaine values. This was not the case in the first experiment when carbachol was infused into hippocampus, and following inactivation, the amplitude of theta was maintained. One possibility was that the septal cells were integrating the level of tonic activation provided by the hypothalamus and conferring these changes on hippocampal field activity. Even though a population of septal cells was cholinergically activated, loss of basal tonic levels of hypothalamic input reduced the amplitude of the signal. These changes were not evident in the first experiment

since the intrinsic circuitry of the hippocampus was cholinergically activated and the septo-hippocampal projections were also intact.

The findings taken together to this point suggest that the PH-SUM region of the hypothalamus sends a tonic level of activation to the septum. The greater this level of tonic activation, the greater number of septal cells which are incorporated. This increases both the frequency and amplitude of the resulting theta field activity. It may be the case that the tonic extrinsic influence which the hypothalamus has on rhythmic and non-rhythmic septal cells determine these changes in hippocampal theta field activity. Greater levels of tonic activation may incorporate greater numbers of rhythmic septal cells, ultimately responsible for increasing the frequency of hippocampal theta field activity via its rhythmic extrinsic influence on hippocampal circuitry. As well, greater levels of activation from the hypothalamus would incorporate the activity of greater numbers of nonrhythmic septal cells responsible for increasing the amplitude of hippocampal theta. Since the amplitude recovers much more rapidly than does the frequency however, it appears that large regional changes of tonic activation from hypothalamus are needed to change the frequency of hippocampal theta field activity.

Utilizing the conclusions of the experiments to this point, it's possible to draw some inferences about the role of the ascending synchronizing system. First, if carbachol is infused into the hypothalamus, and if the region is cholinoceptive, one would predict that theta would be evoked in the hippocampal formation. Next, the amplitude of the theta might be expected to increase due to the increased level of tonic activation provided by the carbachol infusion. Furthermore, if the microinfusion of carbachol into the PH-SUM produced effects on hippocampal field activity which were similar to those produced by electrical stimulation of this area, then an argument could be made in favour of cells versus axons of passage. That is, carbachol activates receptors, not axons. Finally, if the PH-SUM region forms part of the ascending synchronizing pathway, procaine inactivation of this region should abolish the ability of electrical stimulation of the nuclei in the pons region to produce

theta in the hippocampal formation. Examination of these hypotheses became the focus of the fourth experiment.

Objectives - Experiment 4

The objectives were the following: 1) to determine the effect of electrical stimulation of the pontis oralis on hippocampal field activity; 2) to determine the effect of microinfusion of carbachol into the PH-SUM region on hippocampal field activity; 3) to determine the effect of microinfusing carbachol into the PH-SUM on the electrical stimulation of pontis oralis; 4) to determine the effect of a subsequent microinfusion of procaine into the PH-SUM following an initial microinfusion of carbachol, on the carbachol-induced theta.

Method - Experiment 4

i) *Subjects*

Five male Long Evans rats weighing between 300 and 550 g served as subjects. The animals were maintained as previously outlined in experiment one.

ii) *Apparatus*

The experimental apparatus used was identical to that used in experiment one with the following exceptions. Electrical stimulation of the pons was delivered using a bipolar electrode made by twisting together two lengths of 0.011" teflon coated stainless steel wire (A-M Systems, Inc.). Each end of wire had male Winchester subminiature connector soldered to it which connected the stimulation electrode to a second Grass SIU 4678 stimulation isolation unit and Grass CC UIA constant current unit. A second Grass S44 stimulator provided a voltage output with parameters that mimicked that presented to the hypothalamus.

iii) *Surgery*

Surgical preparation followed a protocol similar to that outlined in experiment one but added the following. Prior to placement of the hypothalamic chemotrode, a pons stimulation electrode was positioned ipsilateral to the reference electrode at -1.0 mm A-P (from lambda), $\pm$ 0.8 mm L (from lambda), and $\approx$ 5.5 mm ventral the dural surface. As outlined for hypothalamic stimulation in experiment one, the final site

chosen for pons stimulation was that site which evoked optimal driving of theta with a stimulus amplitude of 0.1 mA.

iv) ***Drugs Administered***

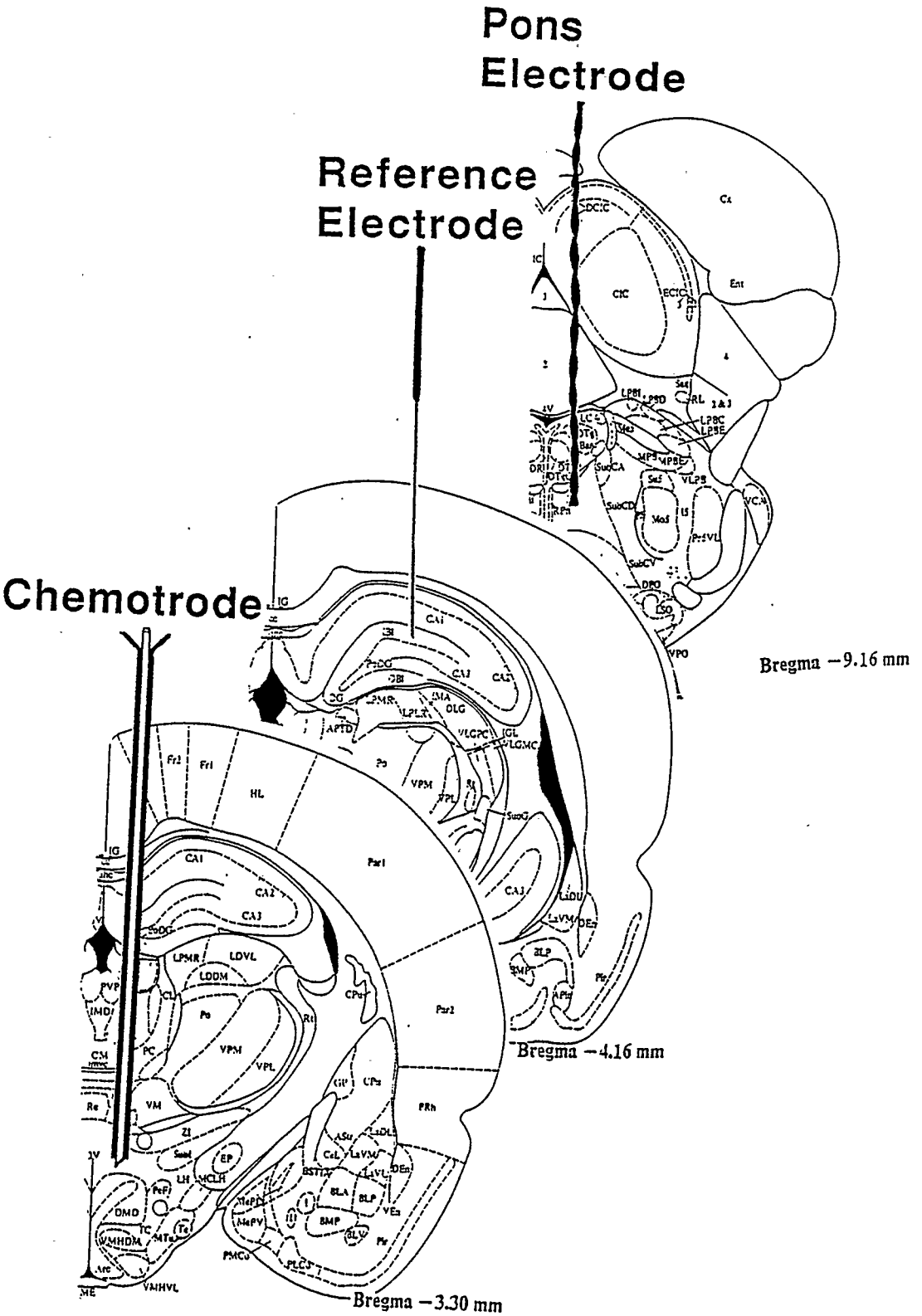
In experiment four, urethane, carbachol and procaine hydrochloride were used at the same concentrations as previously noted in section four of experiment one.

v) ***Experimental Procedure***

Prior to implanting the chemotrode, it was prepared to deliver two drugs into the hypothalamus. First, 4 μl of procaine were drawn into the chemotrode by pulling back on the gas tight syringe. Then a small air bubble was drawn into the chemotrode to separate the procaine from the next drug, carbachol. Following that, 2 μl of carbachol were drawn into the chemotrode. The chemotrode then contained procaine and carbachol separated by an extremely small air bubble. The chemotrode was positioned in the hypothalamus as previously outlined. In this preparation then, once the pump was turned on, the chemotrode first infused carbachol into the hypothalamus, the infusion was stopped and recordings following carbachol infusion could be made. The pump was then reactivated and procaine was infused via the same chemotrode into the same site, and the effect of inactivation was evaluated.

A design similar to those previously mentioned was implemented for the fourth experiment. The design examined the following dependent variables: spontaneous theta, field activity evoked by intrahypothalamic carbachol infusion, theta evoked from 0.9 mA hypothalamic stimulation, as well as theta evoked via an electrical stimulation block delivered to the pons region. As was the case with a hypothalamic stimulation block, a pons stimulation block used 0.1, 0.3, 0.5, 0.7, and 0.9 mA intensities which were randomly generated. These dependent measures were compared across the repeated measure procaine inactivation which included the following conditions: baseline, carbachol, post 10, 30, 45, and 60 minutes following hypothalamic inactivation. Figure 18 presents a diagrammatic representation of the experimental apparatus used in obtaining recordings for the fourth experiment. From this figure it can be seen that the chemotrode was positioned in the posterior hypothalamus. The chemotrode delivered carbachol into the hypothalamus, then

Figure 18. This diagram illustrates the placement of the recording electrode used to record hippocampal theta field activity (reference electrode), the chemotrode used to infuse carbachol into the hypothalamus, electrically stimulate the same region and then reversibly inactivate the hypothalamus by infusing procaine through it. The site of a bipolar stimulation electrode in the pons used to elicit hippocampal theta field activity and test hypothalamic inactivation is also indicated in the figure.


Pons
Electrode
Reference
Electrode
Chemotrode
Bregma −9.16 mm
Bregma −4.16 mm
Bregma −3.30 mm

procaine. Figure 18 also shows the placement of the stimulating electrode in the posterior pons region.

After the necessary preparations, the ability of electrical stimulation in the hypothalamic and pons regions to optimally drive hippocampal theta was established and the amplitude had to be deemed acceptable. Once the preparation was successfully completed, two samples of spontaneous theta were recorded on tape. The animal was then deeply anaesthetized and a 10-second sample of hippocampal theta evoked by a 0.9 mA hypothalamic stimulation bout delivered via the chemotrode was recorded on tape. A stimulation block was delivered to the pons via the stimulation electrode and the resulting hippocampal field activity throughout the block was recorded on tape. Finally, a second sample of theta evoked by 0.9 mA hypothalamic stimulation was obtained.

Following the baseline recordings, 2 μl of carbachol was infused through the chemotrode into the hypothalamus at 0.5 μl/min. As in previous experiments, if seizure activity was evident a period of time was allowed to pass until only clear theta activity was being generated. One 10 second sample of field activity post carbachol and a 10 second sample of hippocampal theta evoked by 0.9 mA hypothalamic stimulation was recorded. Then a stimulation block was delivered via the pons electrode and recorded on to tape. Finally, a second post carbachol field activity sample and a hypothalamic stimulation sample were obtained.

After recording the carbachol condition, the procaine which remained in the chemotrode (4 μl) was injected into the hypothalamus at a rate of 1 μl/min. Again, identical sets of samples as those outlined immediately above were obtained for the post 10 minute inactivation condition. The same sample sets were then recorded for the remaining post hypothalamic inactivation conditions.

After an experiment was complete, the animal was overdosed and the brain prepared for histological analysis.

Results - Experiment 4

i) *Histology*

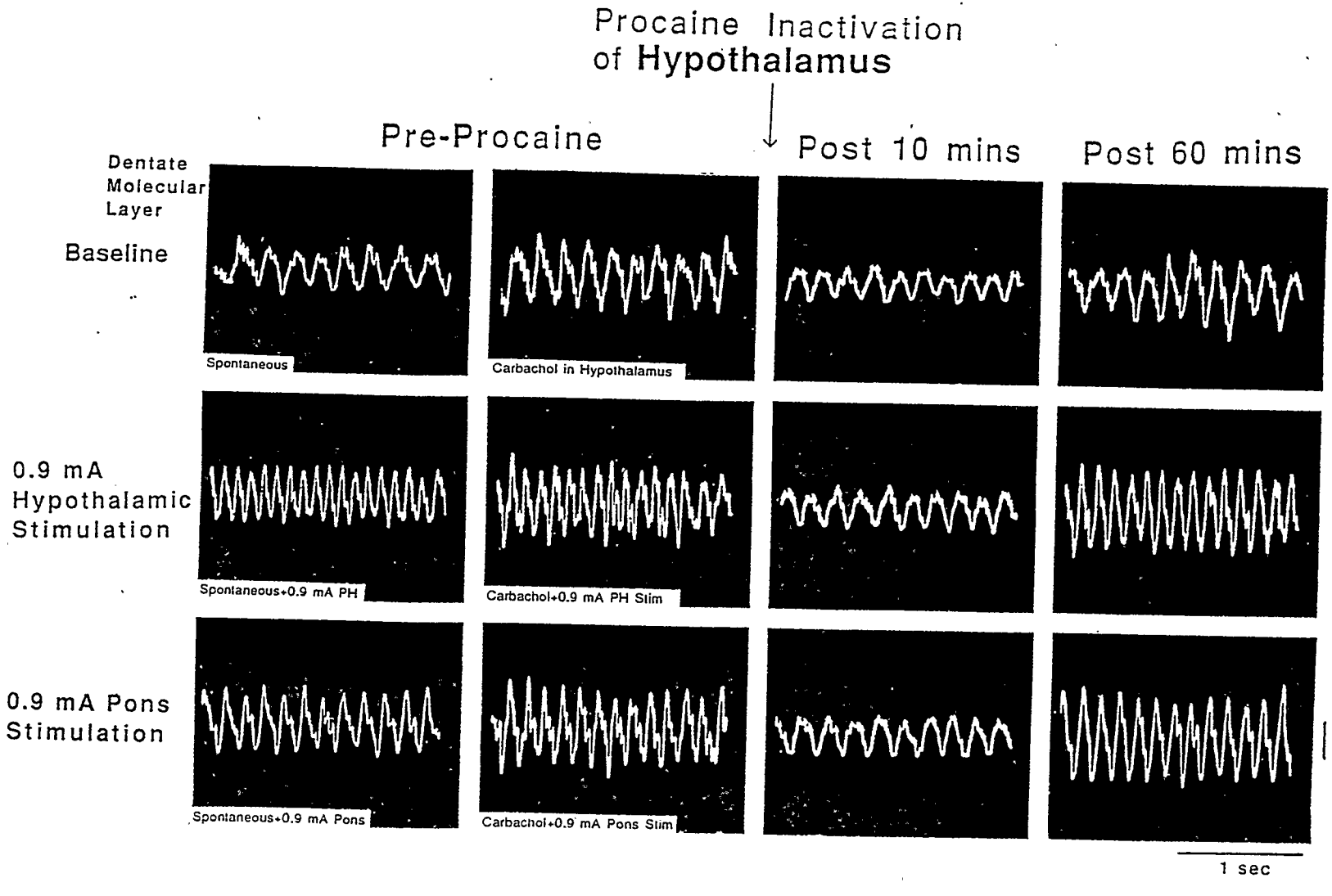
The reference electrode tract endings were found in the upper blade of the dentate in all five experiments. The chemotrode was found to be positioned off of the midline in the posterior hypothalamus in all animals. The pons electrode was located in only four of the five animals due to a poor brain extraction in the third preparation. The four remaining tracts and tips were localized to the dorsal pontis oralis region of the brainstem.

ii) *Pons Stimulation and the Reversible Inactivation of the PH-SUM: Effects on the Frequency of Hippocampal Field Activity*

In the fourth experiment it was found that carbachol infusions into the hypothalamus produced hippocampal theta. This was a profound effect because it produced pronounced seizure activity in the hippocampal formation prior to evoking theta, suggesting a large degree of cholinoceptive activity. It was then determined that pons stimulation, as well as hypothalamic stimulation, could modulate the theta activity. However, contrary to what was hypothesized, procaine infusion into the hypothalamus was not sufficient to abolish the hippocampal theta induced by carbachol infusion into the hypothalamus.

Figure 19 presents analogs of field activity recorded during the experiment. The first column of analogs show baseline spontaneous activity (top), and theta evoked by 0.9 mA hypothalamic and pons stimulation (middle and bottom, respectively). In the second column of figure 19 it can be seen that carbachol produced hippocampal theta which could be modulated with both types of stimulation. The third column shows that hippocampal theta persisted after procaine was infused into the hypothalamus yet at a reduced amplitude. This column also reveals that both hypothalamic and pons stimulation no longer influenced the hippocampal field activity demonstrating an effective inactivation. Finally, the fourth column of figure 19 shows the recovery of hippocampal field activity 60 minutes after the procaine infusion into the hypothalamus. Both the amplitude and frequency evoked by hypothalamic and pons stimulation had recovered by this time interval.

Figure 19. Experimental analogs of hippocampal theta field activity during the pre-procaine measure (left column - spontaneous theta (top), theta elicited by 0.9 mA hypothalamic stimulation (middle), and theta elicited by 0.9 mA pons stimulation (bottom); 2nd left column, carbachol theta (top), carbachol theta modulated with 0.9 mA hypothalamic stimulation (middle), and carbachol theta modulated by 0.9 mA pons stimulation), 10 minutes after hypothalamic inactivation (middle right column) without hypothalamic stimulation (top) and during 0.9 mA stimulation (bottom) and 60 minutes after hypothalamic inactivation (right column) without stimulation (top) and during 0.9 mA hypothalamic stimulation (bottom). Although hypothalamic inactivation did not completely abolish hippocampal theta field activity elicited by intraseptal infusion of carbachol, there was a significant attenuation of the signal's amplitude.

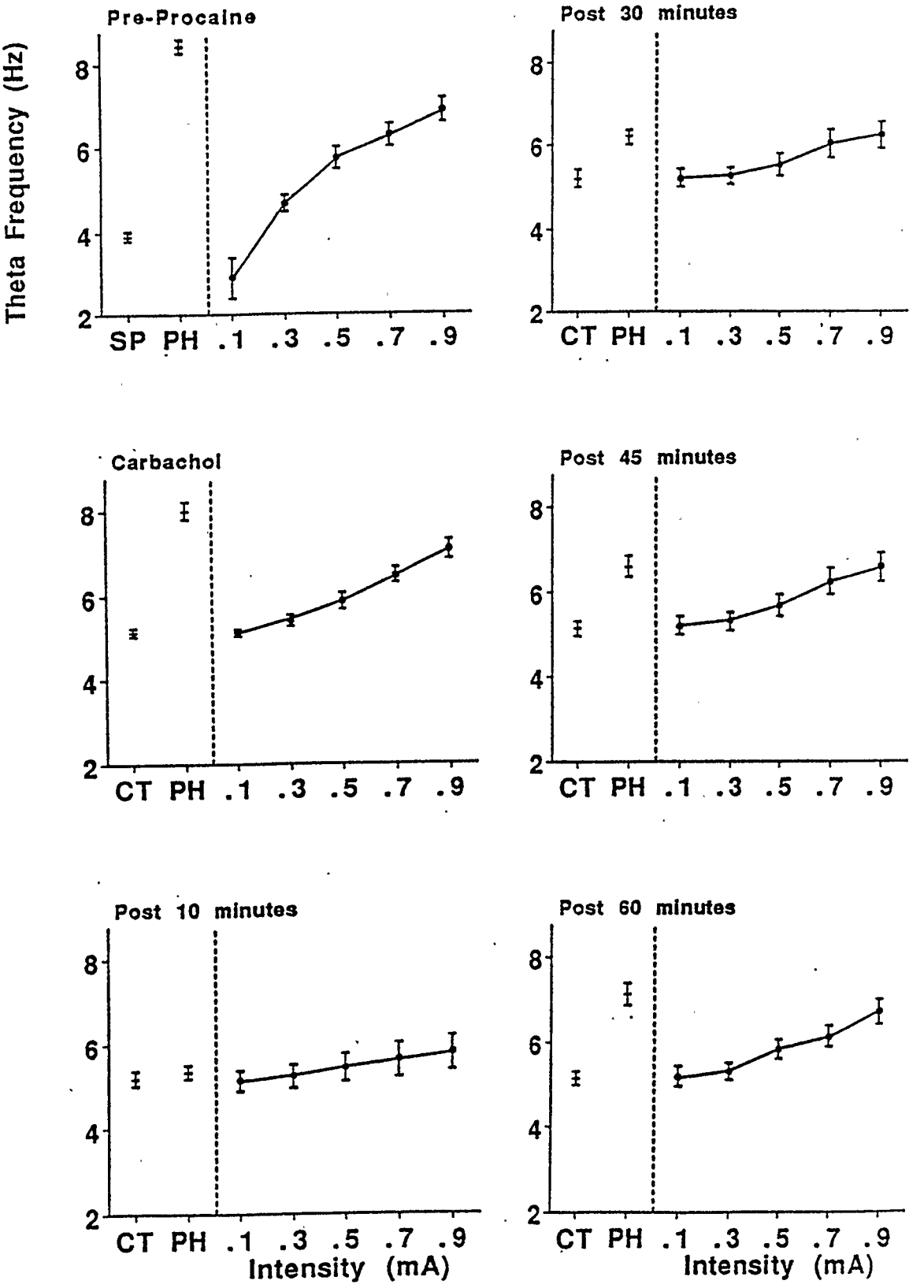

Procaine Inactivation
of Hypothalamus
Pre-Procaine
Post 10 mins
Post 60 mins
Dentate
Molecular
Layer
Baseline
Spontaneous
Carbachol in Hypothalamus
0.9 mA
Hypothalamic
Stimulation
Spontaneous+0.9 mA PH
Carbachol+0.9 mA PH Stim
0.9 mA Pons
Stimulation
Spontaneous+0.9 mA Pons
Carbachol+0.9 mA Pons Stim
0.5 mV
1 sec

A 2-factor repeated measures ANOVA found a significant main effect of pons stimulation intensity on the frequency of hippocampal theta ($F_{(6,63)} = 13.414$, $p < .0001$), a significant repeated measure effect of inactivation on the frequency of hippocampal field activity ($F_{(5,63)} = 33.061$, $p < .0001$) and a significant intensity X inactivation interaction ($F_{(30,315)} = 25.215$, $p < .0001$).

Figure 20 summarizes the results of the experiment on the peak frequency of hippocampal field activity. The abscissa, from left to right, now refers to spontaneous theta (SP), 0.9 mA of posterior hypothalamic stimulation (PH) and the varying intensities of pons stimulation. The upper left panel of Figure 20 presents the pre-procaine baseline frequencies for each condition of the stimulus intensity measure. Spontaneous theta had an average frequency of 3.918 $\pm$ 0.12 Hz. Hypothalamic stimulation with an intensity of 0.9 mA produced an average frequency of 8.452 $\pm$ 0.17 Hz. Electrical stimulation of the pons region at an intensity of 0.1 mA produced an average peak frequency of 2.9 $\pm$ 0.5 Hz, 4.688 $\pm$ 0.19 Hz at 0.3 mA, 5.75 $\pm$ 0.25 Hz at 0.5 mA, 6.287 $\pm$ 0.28 Hz at 0.7 mA and 6.875 $\pm$ 0.29 Hz at 0.9 mA.

The middle left panel of Figure 20 provides a summary of the results of carbachol infusion into the hypothalamus. The infusion produced hippocampal theta activity with an average peak frequency of 5.15 $\pm$ 0.11 Hz. This carbachol-induced theta (CT) was significantly modulated with hypothalamic stimulation at 0.9 mA (carbachol vs. 0.9 mA PH-SUM, $F_{(1,68)} = 23.734$, $p<.001$), producing an average peak frequency of 8.012 $\pm$ 0.22 Hz, and with pons stimulation (carbachol vs. 0.9 mA pons, $F_{(1,68)} = 10.595$, $p<.001$). Electrical stimulation in pons at an intensity of 0.1 mA resulted in an average frequency similar to the carbachol theta frequency, 5.137 $\pm$ 0.09 Hz. Electrical stimulation in the pons region with an intensity of 0.3 mA evoked a peak frequency of 5.412 $\pm$ 0.13 Hz, 5.85 $\pm$ 0.19 Hz at 0.5 mA, 6.45 $\pm$ 0.18 Hz at 0.7 mA and 7.062 $\pm$ 0.22 Hz at 0.9 mA (.1 vs. .9, $p<.001$; .3 vs. .7 and .9, $p<.001$). This demonstrates that pons stimulation was able to modulate carbachol-induced theta to frequencies higher than its baseline carbachol frequency of about 5 Hz. The frequency at which stimulation modulated theta was typically the same frequency evoked in the baseline condition.

Figure 20. A diagram summarizing the results of hypothalamic inactivation on the peak frequency of hippocampal theta field activity elicited by intrahypothalamic infusion of carbachol. Each of the six panels present the peak frequency of spontaneous theta (SP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, carbachol theta (CT), post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI conditions respectively.


Pre-Procaine
Post 30 minutes
Carbachol
Post 45 minutes
Post 10 minutes
Post 60 minutes
Theta Frequency (Hz)
8
6
4
2
SP PH .1 .3 .5 .7 .9
CT PH .1 .3 .5 .7 .9
Intensity (mA)

The bottom left panel of Figure 20 shows the results of procaine infusion into the hypothalamus. Interestingly, the infusion was not sufficient to abolish theta activity but did block the effects of 0.9 mA hypothalamic stimulation as well as stimulation of the pons region. Spontaneous carbachol theta (CT) remained at a frequency of 5.213 $\pm$ 0.19 Hz, similar to the frequency during hypothalamic stimulation post procaine, 5.357 $\pm$ 0.16 Hz. The average frequency during 0.1 mA pons stimulation was 5.15 $\pm$ 0.26 Hz, 5.25 $\pm$ 0.27 Hz at 0.3 mA, 5.45 $\pm$ 0.32 at 0.5 mA, 5.637 $\pm$ 0.40 Hz at 0.7 mA and 5.8 $\pm$ 0.41 Hz at 0.9 mA. Although the frequency of theta appeared to increase as the intensity of pons stimulation increased, a follow-up analysis found the effect to be nonsignificant ($p = .7023$). This indicates that the afferent pathway from the pons region which modulates hippocampal field activity passes through the hypothalamus within the region of inactivation and that the pathway was effectively inactivated following procaine infusion into the hypothalamus.

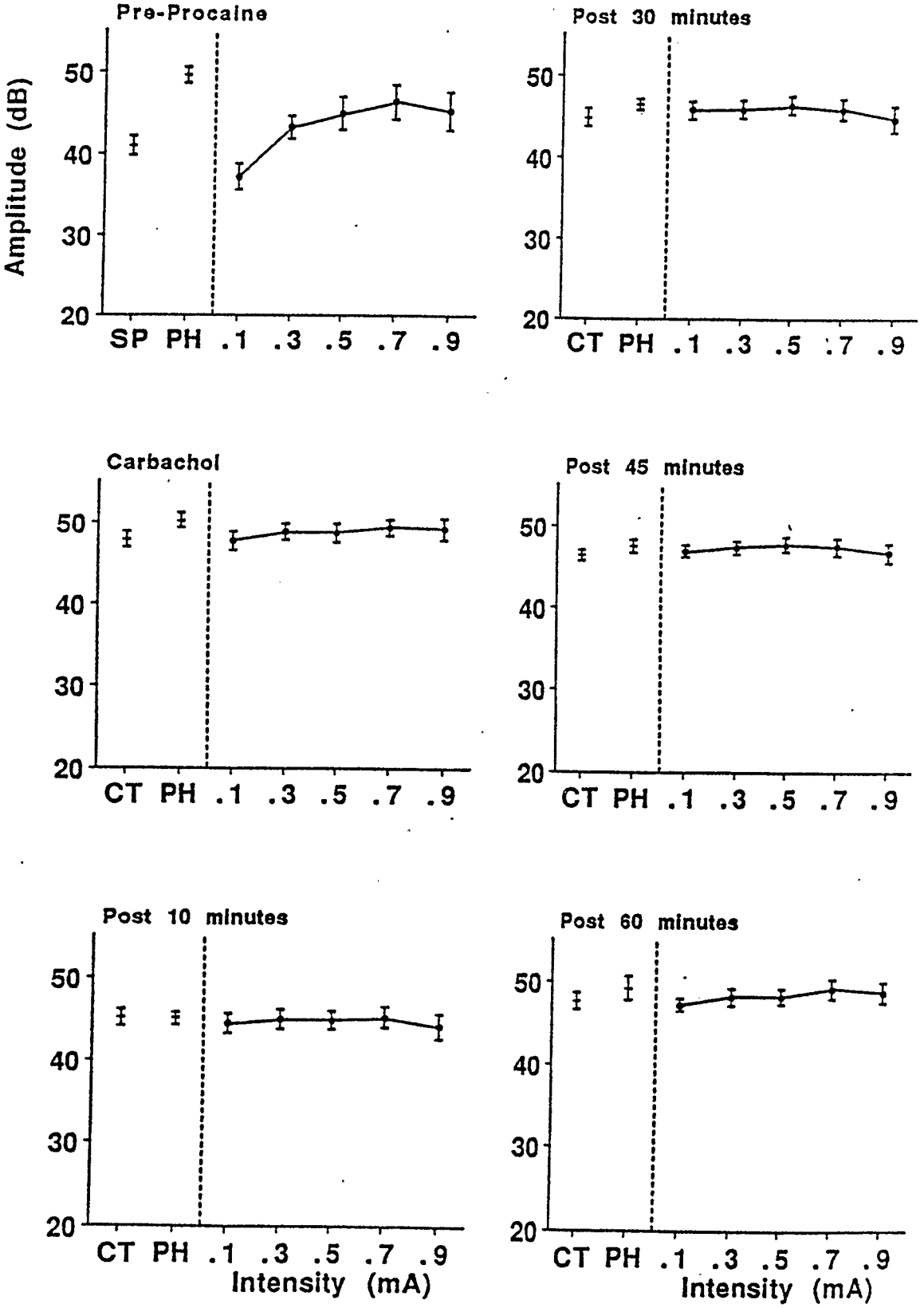
The final panels of Figure 20 show the recovery of the ascending system from hypothalamic inactivation. Both hypothalamic and pons stimulation's ability to modulate theta recovered gradually across the repeated time intervals in a similar manner to the findings outlined in previous experiments.

iii) ***Pons Stimulation and the Reversible Inactivation of the PH-SUM: Effects on the Amplitude of Hippocampal Field Activity***

The results of the experiment on the amplitude of hippocampal field activity are presented in Figure 21. A 2-factor repeated measures ANOVA did not find the main effect of pons stimulation intensity on amplitude to be significant. The repeated measure of inactivation on amplitude as well as the intensity X inactivation interaction were found to be significant ($F_{(5.63)} = 46.406$, $p < .0001$; $F_{(30,315)} = 4.134$, $p < .0001$, respectively).

The average amplitude for the pre-procaine condition, collapsed across levels of the stimulation intensity effect, was 43.943 $\pm$ 0.78 dB. Following carbachol infusion into the hypothalamus, the amplitude increased to 48.796 $\pm$ 0.41 dB. Following procaine infusion into the hypothalamus, the amplitude fell back to 44.82

Figure 21. A diagram summarizing the results of hypothalamic inactivation on the amplitude of hippocampal theta field activity elicited by intrahypothalamic infusion of carbachol. Each of the six panels present the peak frequency of spontaneous theta (SP), during 0.1, 0.3, 0.5, 0.7 and 0.9 mA hypothalamic stimulation for the pre-procaine, carbachol theta (CT), post 10 minutes hypothalamic inactivation (HI), post 30 minutes HI, post 45 minutes HI, and post 60 minutes HI conditions respectively.


Pre-Procaine
Post 30 minutes
Carbachol
Post 45 minutes
Post 10 minutes
Post 60 minutes
Amplitude (dB)
50
40
30
20
SP PH .1 .3 .5 .7 .9
CT PH .1 .3 .5 .7 .9
Intensity (mA)

$\pm$ 0.42 dB. The amplitude recovered to pre-procaine carbachol levels over the time course (45.681 $\pm$ 0.42 dB at 30 minutes, 47.097 $\pm$ 0.34 dB at 45 minutes and 48.11 $\pm$ 0.41 dB at 60 minutes post procaine infusion). Follow-up analysis providing pairwise comparisons revealed that pre-procaine, the amplitude of the signal was significantly lower than the amplitude following carbachol ($F_{(1,70)} = 22.675$, $p < .001$). The comparisons also revealed that the amplitude of carbachol theta was significantly higher than both the post 10 and 30 minute recovery interval amplitudes ($F_{(1,70)} = 15.219$ and 9.338, $p < .001$, respectively), but not significantly different from the post 45 and 60 minute recovery intervals ($F_{(1,70)} = 2.778$ and 0.714). This suggested that recovery of the hypothalamus was not complete until the post 45 minute interval.

Discussion - Experiment 4

The fourth experiment revealed that carbachol microinfusion into the PH-SUM region elicited marked synchrony in the hippocampal formation. Not only did carbachol elicit long continuous trains of theta, the amplitude was larger than that which occurred spontaneously. This was not the case when carbachol was infused into the medial septum. Taken together these findings demonstrated that a population of cells in the PH-SUM region were cholinoceptive and that such activation was sufficient to elicit hippocampal synchrony. Furthermore, the experiment revealed that PH-SUM stimulation could modulate intrahypothalamic carbachol-induced theta. This appeared to be an inherent aspect of the ascending system since PH-SUM region stimulation could modulate carbachol-induced theta, no matter which level of the system carbachol was microinfused. This finding proved to be consistent for pons stimulation as well.

Electrical stimulation of the pons region was a potent method of producing hippocampal theta field activity over a wide range of frequencies. This was consistent with the findings of Vertes (1981, 1982, 1988) who has suggested that the ascending synchronizing system originates in the brainstem region. Pons stimulation also modulated intrahypothalamic carbachol-induced theta. Nuñez *et al.*, (1991), Bland and Colom (1991), and Colom, Bland, and Oddie (1991) have shown that carbachol

infused into the pons region produced hippocampal theta field activity. Thus, it appeared that a significant component of the ascending hippocampal synchronizing system was mediated by cholinergic synapses.

Hypothalamic inactivation rendered both PH-SUM and pons stimulation ineffective. This supported the hypothesis that the PH-SUM region was a critical part of the ascending synchronizing system. It was an interesting finding that the hippocampal theta induced by intrahypothalamic infusion of carbachol, could not be abolished by inactivation of the same region with procaine. Recall that up to two microlitres of carbachol was sufficient to activate the ascending system to elicit hippocampal theta field activity, yet four microlitres of procaine, a volume twice that used to activate the region, was not able to inactivate the system to a degree which abolished theta. This finding could be attributed to a large degree of septal activation provided by the intrahypothalamic carbachol infusion. The microinfusion could have provided cholinergic activation of the septum, a plausible explanation since following hypothalamic inactivation, the results seen were identical to the third experiment. That is, the cholinergic activation of the septum maintained hippocampal theta following hypothalamic inactivation, yet at a reduced amplitude. In one experiment not reported in this thesis, procaine microinfusion into the septum abolished intrahypothalamic carbachol-induced theta.

Taken collectively, the findings reaffirmed the view that the septal and hypothalamic regions processed ascending inputs in different manners which resulted in distinct influences on hippocampal theta field activity.

General Discussion

I. Overview

One of the initial findings of this thesis was that electrical stimulation in the PH-SUM region was able to modulate intrahippocampal carbachol-induced theta field activity. This was important since it demonstrated that hippocampal synchrony previously conferred on the intrinsic circuitry of the hippocampus by cholinergic activation, could be modulated by activation of extrinsic ascending inputs. As will be discussed shortly, the evidence supported the hypothesis that a function of the

hippocampal formation is to serve as a sensorimotor integrator. In order for the sensorimotor integration theory to be a viable one, this would have to be a general characteristic of the ascending synchronizing system, thus providing an animal with the ability to appropriatly integrate sensory-motor information. That is, during ongoing behaviour accompanied by cholinergic activation and hippocampal synchrony, ascending activation within the system has to be able to enhance the activity of the hippocampal formation to appropriately change an animal's behaviour in the face of relevant sensory stimulation. Furthermore, intraseptal and intrahypothalamic infusions of carbachol also produced marked synchrony in the hippocampal formation which could be modulated by electrical stimulation of the PH-SUM region. This reaffirmed the hypothesis that the PH-SUM region was a critical extrinsic influence and that cells in this nucleus formed part of the ascending hippocampal synchronizing system. The notion that it was the cells in the PH-SUM region and not axons of passage which were responsible for producing hippocampal synchrony, was confirmed in the fourth experiment. This also demonstrated the cholinoceptive nature of cells in this region.

Another major finding was that the PH-SUM region was shown to play a greater role in providing synchronizing influences on the hippocampal formation than was previously thought. Hypothalamic inactivation abolished spontaneously occurring theta and theta induced by pinching the tail of the rat. This revealed that sensory information ascended via the circuitry examined here, namely via the PH-SUM region to the septum and then to hippocampus where it could be appropriately integrated. The finding that hippocampal synchrony was abolished after the suppression of the PH-SUM region supports the work of Gottesman (1992) who showed in the anaesthetized rat that in posterior hypothalamic transected animals no theta rhythm could be recorded. Gottesman further suggested that the posterior hypothalamus was the trigger zone for hippocampal synchrony and that tonic increases in the frequency of theta could be the consequence of ascending brainstem activating influences which originated in the nucleus pontis oralis. This activation triggered the posterior hypothalamus to induce hippocampal synchronization. Originally proposed by Bland

(1971), the notion that the medial posterior hypothalamus was a trigger zone was later advanced by Wilson, Motter and Lindsley (1976). Until recently, however, the concept had not been a significant one in terms of directing the investigation of hippocampal synchronization. Finally, these early investigations were not able to determine if the extrinsic influencing properties ascended directly to the hippocampal formation, or exerted their influence via the septum, as was demonstrated in the present thesis.

Recently, Vertes, Colom, Bland and Oddie (unpublished) have provided anatomical evidence that the afferents of cells in the PH-SUM region terminate mainly in the medial septum. Using the anterograde tracing technique *phaseolus vulgarus* agglutinogen (*phal*), they showed dense labelling of the medial septal nucleus following injections of *phal* in the PH-SUM complex. I previously discussed the retrograde labelling Vertes found in the PH-SUM and pons following HRP injections in the medial septum as well. This provided strong anatomical evidence for monosynaptic inputs from the hypothalamus to the medial septum and then to the hippocampal formation. The anatomical evidence just stated taken together with the findings reported here that hypothalamic inactivation significantly reduced the amplitude of intraseptal carbachol-induced theta, suggested that a significant proportion of the hypothalamic extrinsic influence on hippocampal theta was mediated via transynaptic inputs it sends to the medial septum.

Not only did hypothalamic inactivation block ascending sensory inputs in the form of tail pinch and high intensity electrical stimulation within the same region, but it was also able to block the potent affect of electrical stimulation in the pons region. This was an important finding demonstrating that the brainstem inputs must ascend via the region of the hypothalamus inactivated in these studies. I am not aware of any studies which have evaluated the extent of synaptic termination of pontis oralis afferents in the PH-SUM region. However, though the present findings do not provide conclusive evidence that the projections from the pons region terminate in the hypothalamus, they do provide support for this hypothesis. Recall that HRP infusions into the septum revealed primarily PH-SUM staining and not pons staining. That

suggested that there were few direct inputs to the septal region from the pons and that the intervening region was the PH-SUM (since this area was labelled). Intrahypothalamic and intrapontine injections of carbachol produced hippocampal synchrony suggesting the presence of cholinergic connections between the two structures. Furthermore, Bland, Colom and Oddie (1991) demonstrated that hypothalamic inactivation abolished spontaneously occurring theta, tail pinch theta and theta elicited by high intensity stimulation in the pons region further corroborating the results reported here. Taken collectively, the evidence suggested that the robust extrinsic influence which the pons region has on hippocampal theta field activity was mediated via cholinergic synaptic inputs synapsing in the PH-SUM region.

In addition to confirming that the PH-SUM region and medial septum formed a critical part of the ascending hippocampal synchronizing pathway, the results of the present thesis provided evidence that the hypothalamus and septum also have distinct roles in the generation and maintenance of hippocampal theta field activity. **Septal** inactivation had previously been shown to abolish hippocampal theta field activity including intrahippocampal carbachol-induced theta (Smythe *et al.*, 1991). Further, as the septum recovered from procaine inactivation the frequency of hippocampal theta recovered rapidly to pre-procaine values whereas the amplitude of theta recovered slowly. The results of **hypothalamic** inactivation were completely the reverse. Hypothalamic inactivation was not able to abolish intrahippocampal carbachol-induced theta but did abolish spontaneously occurring theta and theta induced by pinching the tail of the rat. In comparison to septal inactivation, as the hypothalamic region recovered from the procaine infusion, different time courses of recovery for theta amplitude and theta frequency were observed. The frequency of hippocampal theta field activity took much more time to recover to pre-procaine values than did the amplitude of hippocampal theta field activity which recovered rapidly to pre-procaine values. These results demonstrated that the hypothalamic and septal components of the hippocampal synchronizing system made different contributions to the mechanisms underlying the amplitude and frequency of hippocampal theta field activity. The present data, along with previous findings,

allow some speculation as to the nature of these contributions. Recall that within the septum there were populations of phasic and tonic theta-on and theta-off cells. Bland and Colom (1992) have suggested that inputs from phasic and tonic cells in the septum to the hippocampal formation are needed to maintain as well as produce changes in the frequency of hippocampal field activity. Tonic theta-on cells in the medial septum translate the degree of activation ascending in the hippocampal synchronizing system relayed through the PH-SUM. This tonic level of depolarization is projected on to hippocampal phasic theta-on cells which serve to synchronize hippocampal theta-on cells. A large proportion of these tonic septal inputs on to hippocampal phasic theta-on cells are probably cholinergic. The ascending activation relayed through the PH-SUM also provides inputs to GABA-ergic septal cells which in turn project to large numbers of hippocampal phasic theta-off cells which also aid in producing synchronized activity. Varying levels of tonic depolarization provided by the septal cells, and a reflection of increases in the amount of ascending brainstem activity relayed via the PH-SUM, can serve to drive different frequencies of hippocampal synchrony. Further, an important characteristic of the system is its ability to reflect moment-to-moment changes in the levels of ascending brainstem activity. These changes are coded by the rapid frequency changes of hippocampal theta field activity and the varying discharge rates of phasic hippocampal theta-on and theta-off cells. Phasic septal cells can reset the synchronous activity of phasic hippocampal theta cells to reflect changes in the level of ascending excitation. These changes also include general state changes from theta to LIA. This important task is achieved by a population of phasic septal cells that receive a common input from cells in the PH-SUM region of the hypothalamus.

Variations in the amplitude of hippocampal theta field activity are most likely related to the level of tonic depolarizing inputs from tonic theta-on cells in the medial septum which is dependent upon increases in the amount of ascending brainstem activity relayed via the PH-SUM. The results reported by Smythe *et al.*, (1991) indicated that relatively few phasic theta-on cells in the septum had to be recruited in order to produce upward shifts in theta field frequency compared to the number of

tonic theta-on cells in the medial septum needed to produce increases in theta field amplitude. The results reported here give an indication of the amount of ascending activation needed to activate the recruitment stated above. It was shown that less activation from PH-SUM cells was needed to maintain the amplitude of theta than that needed to maintain the frequency of hippocampal theta activity. Thus, lower levels of ascending activation from the PH-SUM to tonic cells in the septum is sufficient to maintain the amplitude of hippocampal field activity. Although few phasic septal cells are needed to produce frequency shifts, the amount of ascending activation needed to produce those shifts appeared to be extensive.

The system must also be able to confer desynchrony on hippocampal field activity. This was indicated in the findings reported here since decreased amplitudes of hippocampal LIA were seen after hypothalamic inactivation. Vertes (1988) maintains that a desynchronizing input originates in the median raphe of the brainstem which when activated, desynchronizes hippocampal field activity. The desynchronizing system is thought to ascend along the same pathway as the synchronizing inputs. Bland and Colom (1992) suggest that desynchronized field activity also evolves from decreased activation ascending in the synchronizing system, relayed via the PH-SUM region, which would decrease the activity of GABAergic cells in the septum. The GABAergic septal cells inhibit hippocampal inhibitory interneurons on to which they project. Thus decreased ascending activation in the system results in a functional disinhibition of inhibitory interneurons in the hippocampus which would increase their inhibitory influence on hippocampal theta-on cells resulting in desynchronized field activity.

The mechanisms hypothesized above could be tested by recording septal unit activity before and after hypothalamic inactivation and monitor the timecourse of recovery of the septal units. It was demonstrated in the present thesis that the amplitude of hippocampal theta recovered rapidly after hypothalamic activation whereas the frequency was slow to recovery. If tonic cells in the septum translate amplitude information and septal phasic cells translate the frequency changes of theta, then tonic theta-on cell activity should recover before phasic theta-on cell activity

following hypothalamic inactivation. There is some evidence which suggests that this may be the case. Colom and Bland (1991b) have demonstrated that the unit activity of septal pairs are correlated not only during theta but also during hippocampal LIA. Following hypothalamic inactivation the correlation between cells was abolished. Further, there was some indication that tonic cells recovered before phasic cells (personal communication).

How might changes in the amplitude and frequency of hippocampal theta activity affect an animal's behaviour? Bland's sensory-motor integration theory of the hippocampal formation provides some initial propositions as to how that could be determined. There are many examples which suggest that inappropriate behaviour results following the abolision of hippocampal synchrony. Hippocampal lesions have been shown to disrupt pup retrieval and food hoarding behaviour in rats (Kenyon, Cronin and Keeble, 1981; Wishart, Brohman and Mogenson, 1969; Wallace and Tigner, 1972; Whishaw, Nicholson and Oddie, 1989; Whishaw, Oddie, McNamara, Harris and Perry, 1990). Maternal rats are able to locate displaced pups (primarily via olfactory and vibrissae input) and return them to the nest. However, following hippocampal lesions, mothers are able to find the pups, pick them up, move around the environment while carrying the pups, but are not able to return them to the nest. The pups are dropped randomly in the test environment. All the components of pup retrieval behaviour are present but they cannot be appropriately integrated; a disruption of sensory-motor integration caused by the hippocampal damage. Rats which find large pieces of food while foraging in their environment will delay consumption and move it to a safe location (hoarding behaviour). Following hippocampal damage or cholinergic blockade, rats still forage for food but when presented with large pieces food, are not able to hoard them but place them randomly around the test environment demonstrating disrupted sensory-motor integration. Most deficits commonly attributed to learning and memory could be explained in a similar fashion.

Evidence which suggest that changes in amplitude and frequency of hippocampal theta govern appropriate behaviour are scarce, however, there is

evidence to suggest that the sensory and motor correlates of theta are utilized to adjust appropriate behaviour. Balleine and Curthoys (1991) tested three groups of rats. One group of rats was subjected to escapable footshock and another to inescapable footshock and the third was a no shock control. The escapable group could terminate the shock by performing a one-way shuttle response. The inescapable group was yolked to the escapable animals yet could not terminate the shock. The animals received 80 shock trials prior to testing. Twenty-four hours later, the animals were returned to the recording apparatus where records of hippocampal field activity were taken. Then a five-second probe shock was given and their hippocampal field activity was again recorded. In the escapable group, sustained trains of theta activity, accompanied by immobility, were observed, whereas no theta activity was observed in the inescapable group despite the same initial degree of immobility. The finding reveals that to the escapable footshock group, the five-second probe shock was a meaningful sensory stimulus. The animals elicited long trains of sensory related theta in response to the probe shock (a sensory input) which prepared the animal for an appropriate motor response (a one-way shuttle). In the inescapable footshock group, the probe shock was meaningless. There was no motor response which the animal could engage in which would avoid the shock thus the significance of the sensory input (the probe shock) was not relevant. There was no type II theta elicited because there was no sensory-motor integration component to the task. Caine, Geyer and Swerdlow (1991) have demonstrated that carbachol infusions into the dentate gyrus, at concentrations large enough to produce continuous trains of theta field activity, disrupted sensorimotor gating of startle response in rats. The study revealed that following the infusion of carbachol, rats showed reduced startle amplitudes compared to pre-carbachol values to acoustic and tactile startle stimulation. The authors conclude that sensory inputs were not being integrated correctly to produce an appropriate startle response. Taken together, the studies provide evidence for the hypothesis that sensory and motor related forms of theta are integrated by the hippocampus to govern appropriate behaviour for the animal.

II. Summary and Conclusions

The results of this thesis have revealed a number of interesting aspects of synchrony in limbic cortex. Microinfusion of carbachol, a cholinergic agonist, into varying levels of the ascending hippocampal synchronizing system, produced marked synchrony in the hippocampal formation which demonstrated that the ascending circuitry was mediated by a cholinergic component. Electrical stimulation in the PH-SUM and pontis oralis modulated carbachol-induced theta revealing the strong extrinsic influence these nuclei have on hippocampal theta field activity. Subsequent procaine inactivation of the PH-SUM revealed that the PH-SUM region has distinct affects on the amplitude and frequency of hippocampal theta field activity, influences which were different than medial septal influences previously shown to exist. This suggested that the medial septum and PH-SUM have distinct roles in the ascending hippocampal synchronizing system; the PH-SUM relays the level of ascending brainstem activation to septal cells which contribute to the amplitude and frequency changes in hippocampal field activity. Furthermore, hypothalamic inactivation demonstrated that the hypothalamus also has synchronizing properties on hippocampal field activity in that it abolished spontaneously occurring theta and theta elicited by pinching the tail of the rat. Hypothalamic inactivation also blocked the affects of high intensity electrical stimulation in the PH-SUM and pons region demonstrating that the system ascends via the PH-SUM region. The findings suggested that transynaptic connections were predominant within the ascending circuitry and that the pons afferents have extrinsic influences on the hypothalamus and its function, which in turn sends afferents to the medial septum where it conveys its extrinsic influences on septal function, which in turn has afferent extrinsic influences on hippocampal synchrony. The results emphasize the idea that the integrity of the ascending circuitry as a whole is necessary for hippocampal synchrony; not the current ideology that the septum is the critical nuclei or "pacemaker" of hippocampal theta. Since these findings revealed that the extrinsic influence which the hypothalamus has on the septum was as necessary as the septal influence on hippocampal circuitry, this was a more appropriate proposition.

The findings here support a functional role for the limbic circuitry examined here in providing ascending activation which results in the generation and maintenance of hippocampal synchrony and desynchrony. The results support the hypothesis that an important function of the hippocampal formation and its ascending synchronizing circuitry is to monitor and process changing sensory conditions in the environment and integrate that information with motor information which ultimately results in appropriate voluntary motor behaviour in the animal.

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Appendix I

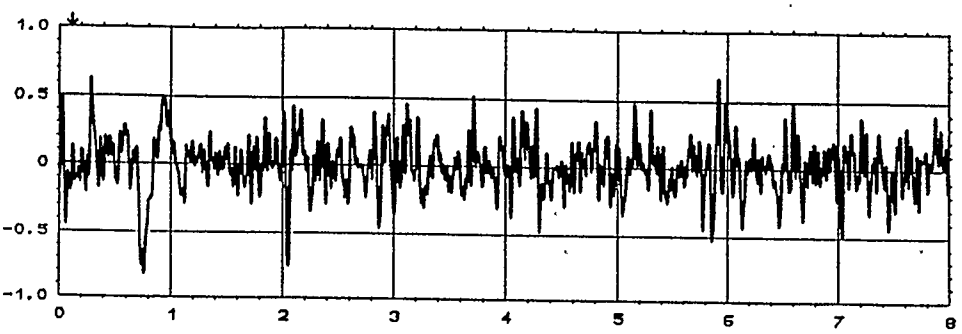
In 1672, Isaac Newton used the term spectrum to describe continuous bands of colours produced by a prism. We know that each colour corresponds to a specific frequency of the visible spectrum. The analysis of light into colours was a form of frequency analysis. Frequency analysis of a signal involves the resolution of the signal into its frequency (sinusoidal) components. A representation of the relative strength of all frequencies present in a signal is called the spectrum of the signal. Instead of light, the signal examined in these experiments is hippocampal field activity, a signal which is a function of time. Fourier transformation is a mathematical operation which transforms data from the time domain to the frequency domain. Continuing the analogy from above, the prism performs a Fourier transformation on the light signal and Fourier transformation of hippocampal field activity yields similar results, breakdown of the hippocampal field activity into its sinusoidal components.

The basic mathematical representation of periodic signals, like hippocampal theta, is the Fourier series, which is a linear weighted sum of harmonically related sinusoids. The sum of these components would result in the original signal. Thus, Fourier transformation of a recorded sample of hippocampal field activity will allow us to determine the predominant peak frequencies which make up the signal and also determine the amplitude at any chosen frequency in the spectrum. Figure 22 illustrates the spectral analysis of eight seconds of hippocampal LIA (top panel, real time signal; upper middle panel, instantaneous spectrum) and theta field activity (bottom middle panel, real time signal; bottom panel, instantaneous spectrum). The spectrum plots amplitude (dB) on the y-ordinate and the varying frequencies of sinusoids which make up the signal on the abscissa. The instantaneous spectrum of LIA revealed no predominant peak frequency whereas the spectrum of spontaneous theta showed a predominant peak frequency at 3.75 Hz with an amplitude of 44.7 dB at that peak frequency.

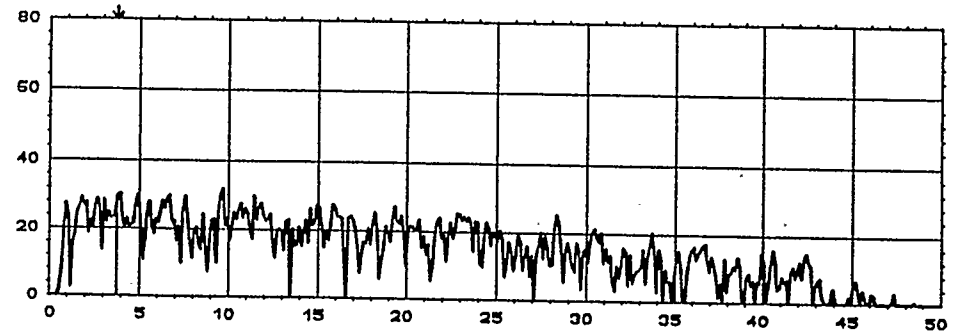
To appropriately interpret the results of Fourier or spectral analysis, a certain basic characteristic must be exhibited by the data, namely stationarity of the signal.

Figure 22. A figure which presents examples of instantaneous spectral analysis on eight seconds of hippocampal LIA (top panel, real time signal; upper middle panel, instantaneous spectrum of LIA real time signal above) and theta field activity (lower middle panel, real time signal; bottom panel, instantaneous spectrum of theta real time signal above). Note the absence of any prominent peak frequencies in the spectrum of the LIA signal whereas there is a prominent peak frequency of 3.75 Hz in the spontaneous theta field activity spectrum. The amplitude of that peak is 44.7 dB whereas at that frequency in the spectral analysis of the LIA signal (indicated by an arrow), the amplitude is 23.8 dB.

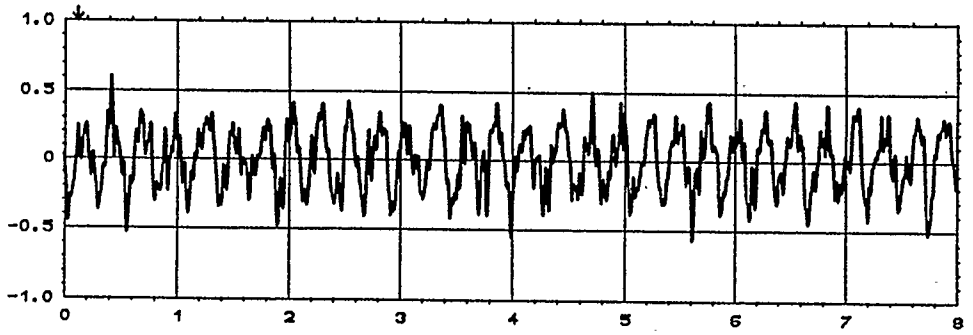
W9 TIME CH.A REAL INPUT MAIN Y: -158mV
Y: 1.00V X: 117.1ms
X: 0.0ms + 8s



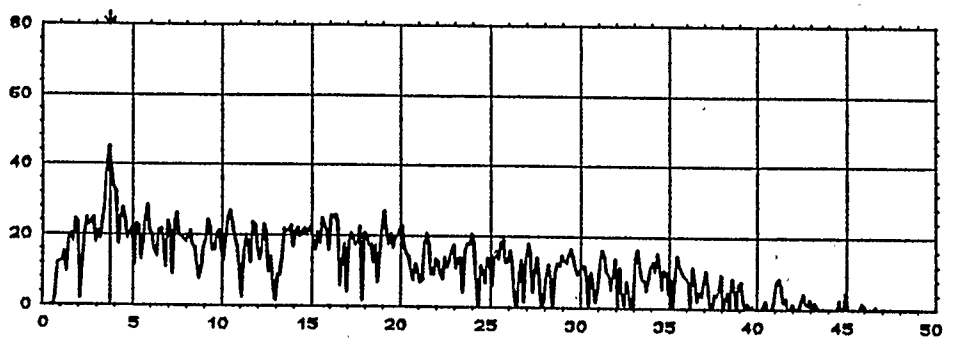
9 INST SPEC CH.A MAG INPUT MAIN Y: 23.8dB
Y: 80.0dB /1.00mV RMS 80dB X: 3.750Hz
X: 0.000Hz + 50Hz LIN



W9 TIME CH.A REAL STORED MAIN Y: 147mV
Y: 1.00V X: 117.1ms
X: 0.0ms + 8s



9 INST SPEC CH.A MAG STORED MAIN Y: 44.7dB
Y: 80.0dB /1.00mV RMS 80dB X: 3.750Hz
X: 0.000Hz + 50Hz LIN

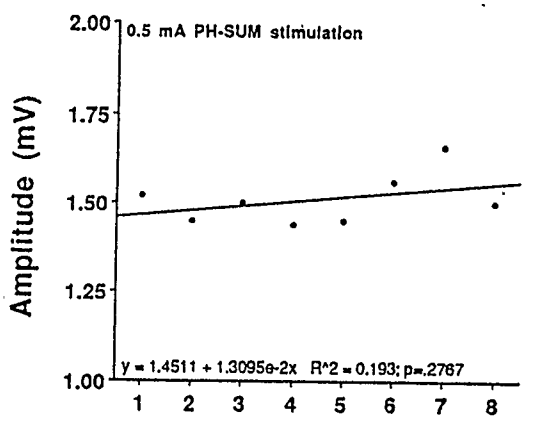


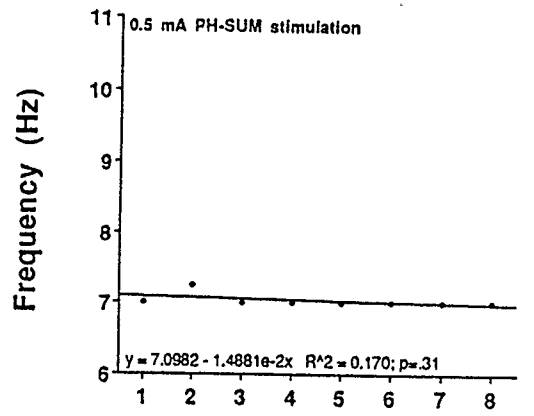
Typically, if the signal is time invariant, then stationarity is accepted. For example, for hippocampal theta field activity elicited by hypothalamic stimulation, the following would be indicative of nonstationarity: across the eight seconds of data sampled during stimulation, the frequency of theta during the first second was 4 Hz, then 4.5 Hz, 5 Hz, 5.5 Hz, 6 Hz and so on. The signal increases its frequency across the eight seconds which would violate the notion of time invariancy. As well, if the amplitude of the signal increased as stimulation duration increased, this would also violate assumptions of stationarity.

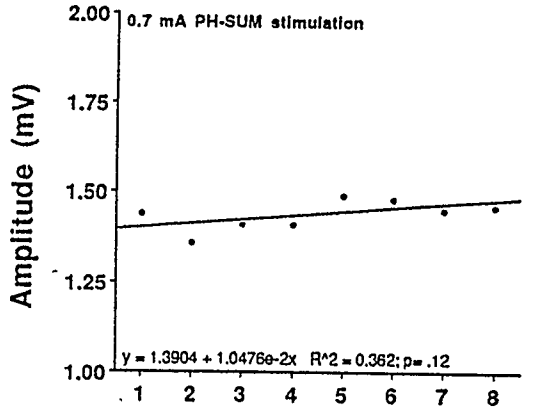
The stationarity of a signal may be tested in a number of ways. Firstly, a visual inspection of the signal usually provides some information as to the signal's stationarity. However, Bendat and Piersol (1971) provide a means of investigating the stationarity of data. They recommend analysis of a single record in time as follows: 1) divide the sample record into *N* equal time intervals; 2) compute a mean value for each interval and align these sample values in a ordered time sequence; 3) test the sequence for underlying trends. The results of this type of analysis which was done on the hippocampal theta field activity elicited by hypothalamic stimulation are presented in figure 23.

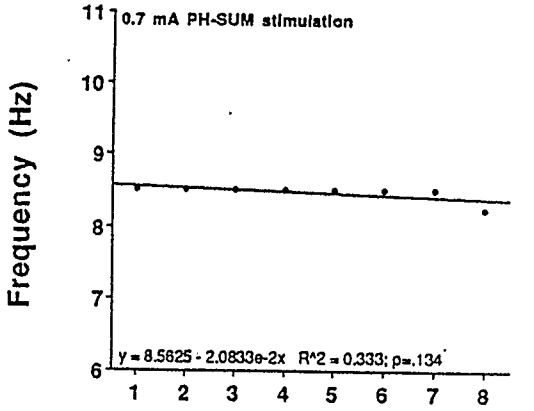
Analogs of hippocampal theta evoked by electrical stimulation of the hypothalamus were plotted on paper and measurements of the average amplitude (left column) and frequency (right column) of each cycle in an eight second sample were quantified. Mean values were determined for each second of the sample and these values were plotted and regressed on the independent measure time (secs) and tested for significance. This analysis was done for 0.5 mA (top row), 0.7 mA (middle row) and 0.9 mA (bottom row) intensities of stimulation. If nonstationarity of the sample was predominant then significant trends would be evident in the signal. As is indicated however, none of the regression lines are significantly different from zero indicating stationarity.

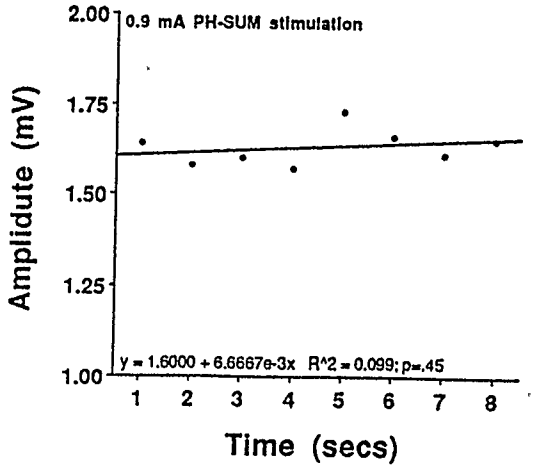
Figure 23. Tests of stationarity on the amplitude (left column) and peak frequency (right column) of eight second samples of hippocampal field activity elicited by 0.5 mA (top row), 0.7 mA (middle row), and 0.9 mA hypothalamic stimulation (bottom row). Measures of the mean cycle amplitude and frequency for each second of field activity were determined and then those values were plotted across time. Linear trends significantly different from horizontal would indicate non-stationarity however none of the tests were significant. Trends other than linear also proved to be nonsignificant.

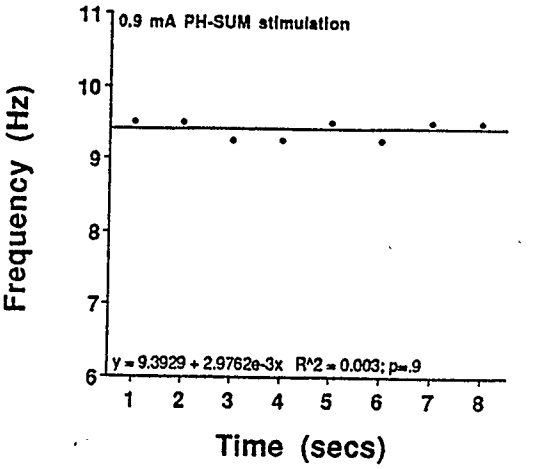

0.5 mA PH-SUM stimulation
Amplitude (mV)
2.00
1.75
1.50
1.25
1.00
y = 1.4511 + 1.3095e-2x R^2 = 0.193; p=.2767
1 2 3 4 5 6 7 8


0.5 mA PH-SUM stimulation
Frequency (Hz)
11
10
9
8
7
6
y = 7.0982 - 1.4881e-2x R^2 = 0.170; p=.31
1 2 3 4 5 6 7 8


0.7 mA PH-SUM stimulation
Amplitude (mV)
2.00
1.75
1.50
1.25
1.00
y = 1.3904 + 1.0476e-2x R^2 = 0.362; p= .12
1 2 3 4 5 6 7 8


0.7 mA PH-SUM stimulation
Frequency (Hz)
11
10
9
8
7
6
y = 8.5625 - 2.0833e-2x R^2 = 0.333; p=.134
1 2 3 4 5 6 7 8


0.9 mA PH-SUM stimulation
Amplidute (mV)
2.00
1.75
1.50
1.25
1.00
y = 1.6000 + 6.6667e-3x R^2 = 0.099; p=.45
1 2 3 4 5 6 7 8
Time (secs)


0.9 mA PH-SUM stimulation
Frequency (Hz)
11
10
9
8
7
6
y = 9.3929 + 2.9762e-3x R^2 = 0.003; p=.9
1 2 3 4 5 6 7 8
Time (secs)