THE UNIVERSITY OF CALGARY

Noradrenergic Modulation of Type Two Theta in The Urethane Anesthetized Rat

By

Arnold J. Heynen

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PSYCHOLOGY

CALGARY, ALBERTA

SEPTEMBER, 1989

ISBN 0-315-54241-1

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Noradrenergic Modulation of Type Two Theta in The Urethane Anesthetized Rat", submitted by Arnold J. Heynen in partial fulfillment of the requirements for the degree of Master of Science.

Dr. R. S. Sainsbury (Supervisor)

Department of Psychology

Dr. B. H. Bland

Department of Psychology

Dr. H. J. Stam

Department of Psychology

Dr. M. Olson

Department of Biology

DATE: Sept 25/89

ABSTRACT

The purpose of the present study was to determine the role of norepinephrine in the generation of hippocampal type 2 theta activity. Experiments were performed on urethane anesthetized rats, implanted with recording electrodes in the dentate gyrus and stimulating electrodes in the dorso-medial posterior hypothalamus. The effects of norepinephrine on hippocampal theta activity was studied by directly infusing norepinephrine and other noradrenergic agents into the hippocampus. Norepinephrine microinfusion produced a decrease in the amplitude of theta activity as observed in the polygraph chart record. Subsequent spectral analyses demonstrated a decrease in power at peak theta frequencies, as well as a decrease in power at frequencies between 20-25 Hz (noise). The inhibitory effect of norepinephrine on hippocampal type 2 theta activity was found to be mediated by $alpha_2$ adrenergic receptors. Microinfusions of an $alpha_2$ agonist (detomidine) mimicked the effects produced by norepinephrine, whereas $alpha_1$ and beta agonists were ineffective. The inhibitory effect of detomidine was blocked by microinfusions of an $alpha_2$ antagonist (tolazoline), which indicates that the site of action was specific to the noradrenergic $alpha_2$ receptor. Results were discussed in terms of a modulatory role for norepinephrine, rather than as a primary drive, in the production of type 2 theta activity.

ACKNOWLEDGMENTS

Firstly, I would like to thank my committee members, Dr. Stam, Dr. Bland, Dr. Olson, and Dr. Sainsbury, for their time and interest. In particular, I would like to thank Dr. Sainsbury for support and confidence in me as both an undergraduate and graduate student under his supervision. Thanks for always going to bat for me. In addition, I would like to thank the following people: Dr Bland, "Big Dude", for the use of his laboratory, and his guidance during the running of these experiments; Brian Christie, "Little Dude", for teaching me the techniques used herein, and getting me hooked on Coke Big Gulps - I'm on my way!; Jim Smythe, "Jimbo", for the long talks on pharmacology, for proofing of this manuscript, and for unending support during its preparation; Jos Eggermont and Geoff Smith, for helping me discard my clear plastic ruler and apply a more systematic technique to analyze "chaos"; Peter Faris and Randy McIntosh, for taking my data and giving it meaning; Sue Crawford and Jack Moxness, for helping me out in the crunch; Merle Olson, for being my drug supplier (for the experiments of course); Gen Thurlow, for sharing her expertise in histology and photography.

Also not forgotten are Dave and Jack Heynen, for helping me out during financial dire straits; as well as Mom and Dad, for support, encouragement, and the never-ending stream of care packages.

Most importantly, I would like to thank Bob, not only as my supervisor but as my friend - the long talks, advice, and humor makes leaving here very difficult. Thanks for "being there" over the course of my studies here.

TABLE OF CONTENTS

Table of Contents (Cont'd) **PAGE**

Table of Contents (Cont'd) **PAGE**

LIST OF FIGURES

List of Figures (Cont'd)

INTRODUCTION

A primary aim of Physiological Psychology is to study the relationship between brain and behavior. The hippocampal formation is a structure where the relationship between brain and behavior has been intensively studied. As a result (in humans), this structure has come to be associated with such concepts as learning, memory, emotion, arousal, and attention.

One approach employed to study hippocampal function has been to record electrophysiological activity from within it and correlate behavior with such activity. From within the hippocampus of a number of different mammals, a large quasi-sinusoidal waveform can be recorded. This waveform, termed theta or rhythmic slow wave activity, has been found to vary in a very consistent manner with ongoing animal behavior. On the basis of electrophysiological, pharmacological, and anatomical studies, theta has been divided into type 1 and type 2 components. The former is associated with behaviours such as walking, running, and rearing; the latter is associated with more psychological concepts such as attention, arousal, and information processing.

It is the purpose of the present research to further investigate the pharmacological characteristics of type 2 theta activity, and show how this relates to electrophysiological activity. The introduction to follow provides a review of the area and develops the logic from which this research approach arose.

HIPPOCAMPAL FORMATION ANATOMY

The hippocampal formation is a telencephalic structure which borders the lateral ventricles, forming a semi-circle around the thalamus of each hemisphere. It is often divided into dorsal and ventral components; the former lying over top of the thalamus, and the latter found posterior and ventral to the thalamus, running into the posterior horn of the lateral ventricles.

The hippocampal formation is made up of two interlocking gyri- the hippocampus proper (Ammon's horn) and the dentate gyrus (fascia dentata). The subiculum, a zone leading into the hippocampus proper from the entorhinal cortex, is often included in anatomical descriptions of the hippocampal formation. Based on the morphology of cells in the area, the anatomist Lorente de No (1934) subdivided the hippocampus proper into four fields. Each field is labelled with the letters CA (cornu ammonis) and the numbers one through four. The CA1 region is found adjacent to the subiculum and contains a double row of tightly packed pyramidal cells. This area has been designated as Regio Superior by Blackstad (1956). The CA2 region is considered a transition zone between CA1 and CA3, and also contains large pyramidal cells, although they are arranged in loosely packed rows. The CA3 region also contains rows of loosely packed pyramidal cells which receive mossy fiber projections from the dentate granule cells, and project, via the Schaffer collaterals, to the CA1 pyramidal cells. The CA2 and CA3 regions are collectively known as the Regio Inferior. The CA4 region is the transition zone from the hippocampus proper to

the dentate gyrus, this layer contains rows of loosely arranged pyramidal cells. The CA4 region is often referred to as the hilus of the fascia dentata (Blackstad, 1956).

The subiculum lies between the CA1 region of the hippocampus proper and the medial entorhinal cortex; it is composed of three layers (Bayer, 1985; Lorente de No, 1934). Lying adjacent to the medial entorhinal cortex, containing a layer of medium sized cells, is the parasubiculum. Lying adjacent to the parasubiculum and abutting the hippocampal fissure is the presubiculum. This region contains a superficial layer of relatively small sized cells. The subiculum proper is located proximal to the CA1 region and contains large, loosely arranged pyramidal cells, as well as a superficial layer of granule cells. The transition area between the subiculum and CA1 region of the hippocampus proper is known as the prosubiculum (Bayer, 1985). The top portion of Figure 1 shows a schematic representation of the hippocampus proper, dentate gyrus, and subiculum.

The hippocampus proper and dentate gyrus are both laminated structures, with the layers oriented perpendicular to the principal cells in the area. In the hippocampus proper the principal cells are the pyramidal cells. The most superficial layer in the hippocampus proper, the stratum lacunosum-moleculare, lies adjacent to the hippocampal fissure and contains the distal portions of pyramidal cell apical dendrites. This layer is relatively void of cells, although fibers entering the hippocampus proper from the subicular region are found here. The stratum radiatum contains the main shafts of the apical dendrites arising from the pyramidal cells. The stratum

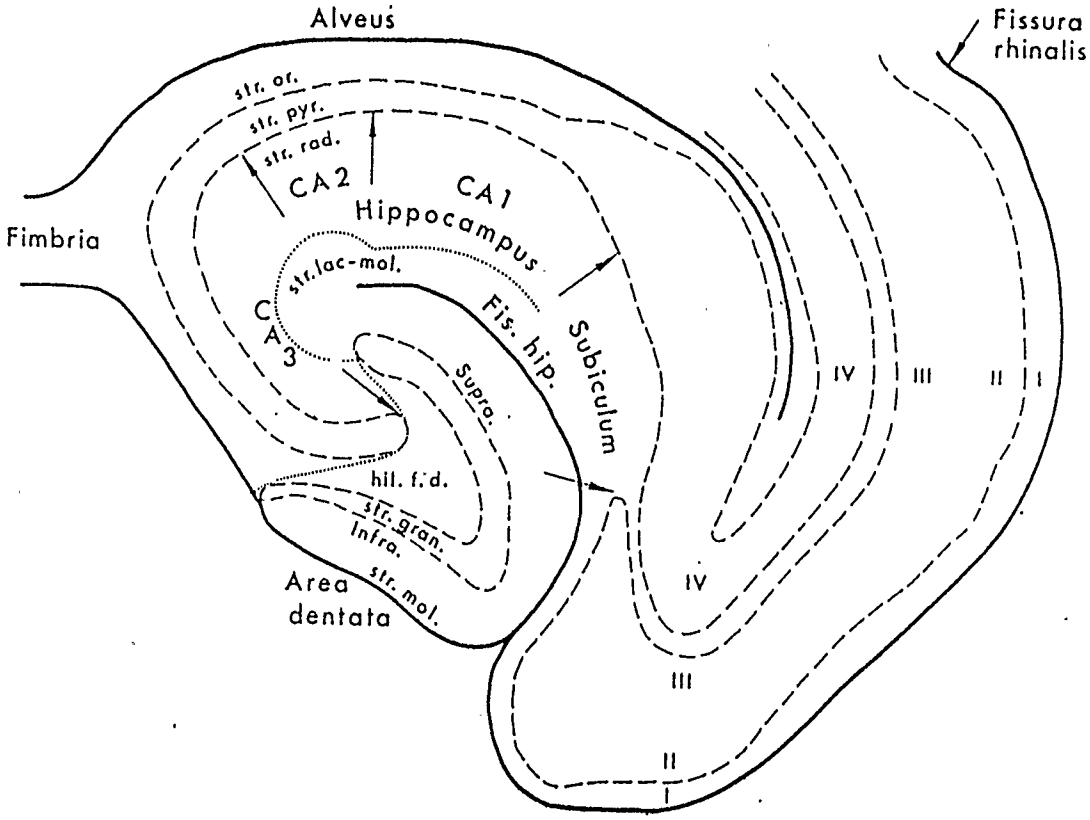
pyramidal is composed of a tightly packed layer of pyramidal cells. Unlike the pyramidal cells of the neocortex, which have only one dendritic process, these cells have two dendritic processes-the basal and apical dendrites. Also found within the pyramidal cell layer are interneurons, mostly basket cells, which extend their axonic processes around the somas of the pyramidal cells creating a basket-like plexus. Basket cells receive afferents from the medial septum-diagonal band complex (Lewis and Shute, 1967), and are thought to have an inhibitory effect on pyramidal cell activity (Andersen, Eccles, and Loyning, 1964). Lying below the pyramidal cell layer is the stratum oriens which contains the basal dendrites of the pyramidal cells. Also found within this layer are the proximal segments of pyramidal cell axons which enter into the alveus, as well as a number of interneurons. The alveus contains axonic fibers arising from the pyramidal cells, and projects to the fimbria, fornix, and subiculum.

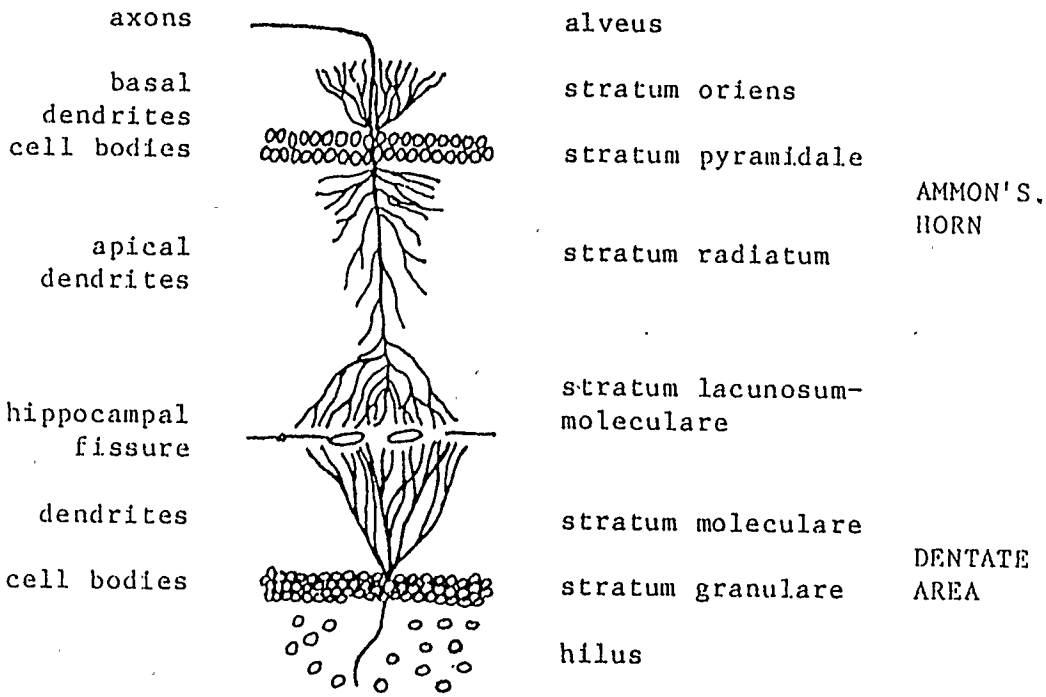
Found at the end of the moleculare layer of the hippocampus proper is the dentate gyrus. The dentate region's principal cell type is the granule cell. The most superficial layer of the dentate gyrus is the stratum moleculare. This layer contains the apical dendrites arising from cells in the granular layer and receives perforant path projections from the entorhinal cortex. The stratum granulare contains several rows of densely packed granule cells. Unlike the pyramidal cells of the hippocampus proper these cells have very few basal dendrites, but like pyramidal cells they are surrounded by interneurons. The third layer of the dentate gyrus is the polymorph layer which is located within the hilus and abutts the CA4 region of

the hippocampus proper. This layer contains granule cell axons which travel through the polymorph layer and end as mossy fibers above the pyramidal cells of the CA2 - CA4 regions. Also found in this layer are a number of basket cells. The lower portion of Figure 1 shows the relationship between the various layers and the principal cells of the hippocampus proper (Ammon's horn) and the dentate gyrus.

Figure 1 **Hippocampal Formation Anatomy**

The upper portion of this figure is a horizontal section through the hippocampal formation. Arrows indicate the boundaries between each cornu ammonis (CA) field, as well as the dentate region and subiculum. The lower portion of the figure shows the various hippocampal layers and their relationship to the pyramidal cells of the hippocampus proper (Ammon's horn) and the granule cells of the dentate area.


Alveus
Fissura rhinalis
str. or.
str. pyr.
str. rad.
CA2
CA1
Hippocampus
Fimbria
str.lac-mol.
CA3
Fis. hip.
Subiculum
Supra.
hil. f.d.
str. gran.
Infra.
str. mol.
Area dentata
IV
III
II
I
IV
III
II
I


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

PHYSIOLOGY OF THETA ACTIVITY

The first comprehensive study of theta activity, also known as rhythmic slow activity (RSA), was done by Green and Arduini (1954). These researchers demonstrated that theta activity could be elicited from the hippocampi of cats, rabbits, and monkeys by a number of sensory stimuli, as well as by stimulation of the reticular activating system. Lesions of the fornix and septum were found to abolish theta. These researchers concluded that theta activity reflected an alerting reaction in the hippocampus. Since that time numerous studies have been done which further elaborate on the initial descriptions provided by Green and Arduini (1954). Hippocampal theta activity can be recorded from a number of mammals including the rat (Bland and Vanderwolf, 1972; Bland and Whishaw, 1976; Vanderwolf, 1971), rabbit (Bland, Andersen, and Ganes, 1976; Andersen, Bland, Myhrer, and Schwartzkroin, 1979; Bland and Bland, 1986; Creery and Bland, 1980; Kramis, Vanderwolf and Bland, 1975), cat (Bland, Sainsbury, and Creery, 1979; Whishaw and Vanderwolf, 1973), gerbils, (Kramis and Routtenberg, 1969; Whishaw, 1972) and guinea pigs (Montoya and Sainsbury, 1985; Sainsbury, 1970; Sainsbury and Montoya, 1984).

Pharmacological and behavioural findings have demonstrated that two types of theta activity are present in the majority of these species. Type 1 theta (movement related RSA) occurs during what is considered operant or voluntary motor behaviours such as walking, running, jumping, swimming, manipulating objects with forelimbs, head movements, and postural shifts. Vanderwolf (1969) refers to these

behaviours as type 1 behaviours. The frequency range of this type of theta is between 6-12 Hz and varies with the speed with which movements are initiated (Vanderwolf, 1969; Whishaw, 1982). Type 1 theta, as well as its behavioural correlates are abolished by a number of anesthetics such as alcohol, ether, urethane, and sodium pentobarbital, but is resistant to large doses of atropine sulphate (a cholinergic blocker).

The second form of theta activity, type 2 theta, occurs during complete immobility and the concomitant presentation of sensory stimuli such as hand claps, tones, owl vocalizations, and touching the animals fur (Bland, Sainsbury, Seto, Sinclair, and Whishaw, 1981; Bland, Seto, Sinclair, and Fraser, 1981; Sainsbury, Heynen, and Montoya, 1987; Sainsbury, and Montoya, 1984). Sainsbury (1985) suggests that immobility related, type 2 theta activity occurs in response to sensory stimuli when the animal is in an aroused state. He notes that the level of arousal, or ability of sensory stimuli to elicit type 2 theta, may vary between species as well as individuals within a species. Other conditions where type 2 theta is also present include: (1) during conditioning paradigms where electrical shock results in freezing behaviour (Sainsbury, Harris, and Rowland, 1987; Vanderwolf, 1969; Whishaw, 1972); (2) after brain stem and hypothalamic lesions (Kolb and Whishaw, 1977; Robinson and Whishaw, 1974); and (3) following treatment with a number of drugs such as physostigmine, urethane, and reserpine (Robinson, Vanderwolf, and Pappas, 1977; Vanderwolf, 1975; Vanderwolf, Kramis, Gillespie, and Bland, 1975). This type of theta activity is believed to be

cholinergically mediated, as administration of drugs which interfere with central cholinergic transmission (eg. atropine sulphate, scopolamine, hemicholinium-3) block its occurrence (Robinson and Green, 1980; Kramis, Vanderwolf, and Bland 1975; Vanderwolf, Kramis, Gillespie, and Bland, 1975). Atropine methylnitrate, a drug which mimics the peripheral effects of atropine sulphate, but is unable to cross the blood brain barrier, has no effect (Vanderwolf et al, 1975). The overall frequency range of type 2 theta (4-9 Hz) is lower than that found for type 1 theta activity.

Two other wave forms which can be recorded from the hippocampus are large amplitude irregular activity (LIA), and small amplitude irregular activity (SIA). Large amplitude irregular activity is found during immobility and the occurrence of type 2 behaviours (Vanderwolf, 1971). These behaviours are considered to be automatic or reflexive and include: chewing, grooming, teeth chattering, licking, drinking, urinating, and pelvic thrusting (see Vanderwolf et al, 1975 for review). Small amplitude irregular activity occurs in short 1-2 second segments and appears during sudden pauses in ongoing behaviour (Vanderwolf, 1971).

Leung, Lopes Da Silva, and Wadman (1982) characterized the hippocampal field activity using spectral analysis and found that theta activity had a sharp, narrow band peak which was often accompanied by second and third harmonics. The harmonics were found to be most prominent during walking when the power of the fundamental frequency (first harmonic) was largest. During LIA and related behaviours a power peak in the theta frequency range (6-8 Hz) was

still present, but was flattened and reduced in size. Subsequent studies by Leung (1984, 1985) have demonstrated that the power of the second and third harmonics increase with the frequency of theta. Administration of cholinergic agonists induced a low frequency (3-6 Hz) power peak during immobility, while subsequent atropine sulphate injections abolished this power peak.

HIPPOCAMPAL THETA GENERATORS

Two theta amplitude maxima have been located in the hippocampal formation of rats (Bland and Whishaw, 1976; Winson, 1974), rabbits (Bland, Andersen, and Ganes, 1975), and cats (Bland, Sainsbury, and Creery, 1979). A dorsal amplitude maximum is found in the stratum oriens of the CA1 region. A second amplitude maximum is found ventral to the hippocampal fissure in the stratum moleculare of the dentate gyrus region (Bland et al., 1975; Winson, 1976a, 1976b). The dentate gyrus region typically shows larger amplitude field activity than that found in the CA1 region (Bland et al., 1975; Bland and Wishaw, 1976). Theta activity recorded from the CA1 region is found to be approximately 180 degrees out of phase with theta recorded in the dentate region. A null zone, where theta is absent, is located between the two regions in the stratum radiatum (Bland et al., 1975; Bland and Wishaw, 1976; Winson, 1974). Although the theta generators are considered closely coupled, studies suggest that they are able to generate theta activity independent of one another. Lesions of the lateral septum (Sainsbury and Bland, 1981), and entorhinal cortex

lesions (Vanderwolf and Leung, 1983), have been shown to eliminate theta occurring in the CA1 region while leaving theta in the dentate region intact. Bland et al. (1975) showed that manually stroking the surface of the CA1 region, as well as cooling it with Ringer's solution, selectively abolished CA1 theta while theta recorded in the dentate region remained unaltered. These researchers were unable to eliminate theta occurring in the dentate region without affecting CA1 theta. Rowntree and Bland (1986) found temporal differences in the responses of these generator regions to cholinergic agonists (carbachol and physostigmine), and antagonists (atropine sulphate) infused directly into the hippocampal formation. Theta activity could often be driven/abolished in the hippocampal region ipsilateral to the site of infusion with no effects seen in the contralateral hippocampus until several minutes after infusion. Recent work in the in vitro hippocampal slice preparation has shown that the two theta generator zones can produce theta activity that no longer has a 180 degree phase shift relationship as is found in the in vivo preparation (Konopacki, Bland, and Roth, 1987). Further, both generators have been found to be capable of generating theta following deafferentation from one another (Konopacki, Bland, MacIver, and Roth, 1987). Controversy surrounds the possibility that a third independent theta generator exists in the hippocampal CA3 region. Some researchers have recorded theta activity (Bland, and Whishaw, 1976; Konopacki, Bland and Roth, 1988a) and a phase reversal (Buzsaki, 1986) in this region, while others have found neither (Bland, Andersen, and Ganes, 1975). Disagreement in results are most likely due to differences in the

experimental procedures employed, and the species under study.

A number of researchers have found what appears to be theta outside of the hippocampal formation. Feenstra and Holsheimer (1979) reported a 180 degree phase reversal in layer five of the posterior cingulate cortex. Moreover, cells in this region were found to have periodic spike activity which was phase locked to the locally recorded theta rhythm (Holsheimer, 1982). In the freely moving rat, Leung and Borst (1987) recorded unit activity that was phase locked to the local theta rhythm, although these researchers were unable to find a phase reversal. A follow up study showed that some septal lesions caused a release of theta activity in the cingulate cortex while theta was abolished in the hippocampal formation. Recently, Colom, Christie, and Bland (1988) performed depth profiles through the posterior cingulate cortex and found no evidence of a phase reversal. Further, these researcher recorded from a number of neurons in this area and found, according to Colom and Bland's (1987) cell nosology, a predominance of tonic, non-rhythmic theta-on cells. They concluded that the lack of a phase reversal and an abundance of tonic, non-rhythmic theta-on cells suggests that the posterior cingulate cortex does not contain an independent theta generator, but rather monitors transitions from theta to LIA states.

Theta activity has also been recorded in the entorhinal cortex (Alonso and Garcia-Austt, 1987a, 1987b; Mitchell and Ranck, 1980), which provides a major afferent to the hippocampus. Depth profiles performed with moving electrodes have shown that theta activity recorded in the deeper layers of the entorhinal cortex is 180 degrees

out of phase with respect to theta recorded in superficial layers. It has been suggested that the medial septum may act as a pacemaker for the theta activity recorded in the entorhinal cortex, although no studies have been done to determine if lesions of the medial septum abolish theta recorded in this area.

Other areas of the brain where theta has been reported are: the thalamus (Lemoal and Cardo, 1975), hypothalamus (Bland and Whishaw, 1976; Petsche and Stumpf, 1960), ventral mesencephalic tegmentum (Le Moal and Cardo, 1975), and pontis oralis (Faris and Sainsbury, 1989). In this latter study, lesions of the pontis oralis had no effect on hippocampal theta or its behavioural correlates, suggesting that theta recorded in the hippocampus is not dependent upon the integrity of the pontis oralis. Lesions of the medial septum were found to abolish theta activity in both the hippocampus and pontis oralis. Cross correlations of field activity from both sites remained unaltered both before and after medial septal lesions - suggesting that both structures share a common input.

CELL ACTIVITY

Single cell activity within the hippocampal formation has been found to be related to locally recorded theta activity. Bland and colleagues have done a considerable amount of research in this area- both in the freely moving rabbit, and urethane anesthetized rat. Sinclair, Seto, and Bland (1982), in the freely moving rabbit, originally described cell firing patterns that were related to local

field activity and ongoing behaviour. During LIA and related behaviours (eg. immobility) theta related cells were arhythmic. During type 1 theta and related behaviours (eg. walking), these cells fired in a rhythmic manner. Similarly, these cells also displayed rhythmic activity during the occurrence of type 2 theta. The authors noted that there were fewer rhythmic discharges during type 2 as compared to type 1 theta, even though the frequency of both types of theta were often identical. The number of cell discharges during type 2 theta was found to be similar to the number during LIA, although in this latter case the discharges were in an arhythmic manner. Sinclair et al. (1982) suggested that the increase in the number of rhythmic cell discharges during type 1 theta activity could be evidence for: (1) both type 1 and 2 theta being present during type 1 behaviours; and (2) two anatomically distinct afferent drives for the production of theta activity, with both systems being active during type 1 theta. Bland, Seto, and Rowntree (1983) replicated the above study and also showed that theta related cells exhibited a linear increase in rhythmic discharge rates as the frequency of both type 1 and 2 theta increased. In a modification of Ranck's (1973) definition of theta cells, Bland et al. (1983) defined theta cells as cells that always fired in rhythmic, simple spike manner, locked to the negative phase of the locally recorded theta wave. During LIA, these cells were silent or discharged arhythmically, and as frequencies of type 1 and 2 theta increased, they linearly increased their discharge rates. A later study by Bland, Seto, Sinclair, and Fraser (1984) provided more evidence for the hypothesis that two

afferent systems mediate the production of type 1 and 2 theta activity. These researchers found that the administration of the cholinergic agonist, physostigmine, resulted in the production of type 2 theta and theta cell rhythmicity. Administration of atropine sulphate (a cholinergic antagonist) abolished type 2 theta and accompanying cell rhythmicity. Further, following atropine sulphate injections, a decrease in the number of cell discharges during type 1 theta and related behaviours was also found. This suggests that the type 2 theta system is operational during the production of type 1 theta and that following atropine sulphate administration, a rhythmic, non-cholinergic system remains. As is found for hippocampal theta activity, the integrity of the medial septum is required for normal theta cell activity. Lesions of this area have been shown to decrease theta cell discharge rates by approximately 50 percent. Lesions of this area have also been found to disrupt rhythmicity of theta cells in the freely moving rabbit (Bland and Bland, 1986).

Colom and Bland (1987), using the urethane anesthetized rat (type 2 theta only) developed a nosology to describe theta related cell activity, and its relationship to the theta and LIA states. Theta related cells were classified into theta-on and theta-off categories. Within each of these categories two subtypes - tonic and phasic, were also described. Tonic theta-on cells fired nonrhythmically while theta activity was present, and did not change their firing rate in response to frequency changes of theta activity. During LIA activity, these cells fired at low rates or were silent. Phasic theta-on cells were found to discharge rhythmically during

theta activity, and as the frequency of theta increased, these cells showed a linear increase in discharge rate. Discharges were arhythmic during LIA and the rate of discharge varied from cell to cell. Tonic theta-off cells never fired during theta activity, but discharged at a low, constant rate during LIA. Phasic theta-off cells were found to never fire during high frequency theta activity, but began to fire, sometimes rhythmically, at lower frequencies of theta. During LIA, phasic theta-off cells discharged continually at high rates. These researchers proposed that tonic cells code the changes from theta to LIA states, whereas phasic cells code changes within the LIA or theta state. Further investigation by Bland and colleagues have shown that phasic theta-on cells code increasing levels of activation in the septo-hippocampal cholinergic system, during both spontaneously occurring and stimulation induced (dorso-medial posterior hypothalamus) theta activity. Theta-off cells were shown to have an inverse relationship to such activation. Administration of atropine sulphate abolished theta-on cell rhythmicity, as well as their ability to code the level of dorso-medial posterior hypothalamus stimulation (DMPH) (Colom, Ford, and Bland, 1987). Muscarinic receptor activation appears to mediate tonic and phasic theta-on cell activity as systemic administration of eserine and carbachol excite both types of cells, whereas atropine sulphate adminstration antagonizes these effects. Activation of nicotinic receptors causes an inhibition of theta-on cell activity, although theta activity may still be present. It is likely that theta activity induced by nicotine is mediated outside of the hippocampus (Bland and Colom, 1988). Bland and Colom

(1989) have recently investigated theta-off cell activity using a number of physiological and pharmacological manipulations. Phasic linear theta-off cells were found to be inhibited during theta frequencies above 5.0 Hz irrespective of whether this frequency of theta occurred spontaneously, or was induced by tail pinches, physostigmine, or electrical stimulation of the DMPH . Tonic nonlinear theta-off cells were inhibited at all frequencies of theta produced with the above manipulations. Both cell types exhibited typical arhythmic firing patterns associated with LIA. Atropine sulphate abolished all theta activity in response to theta producing stimuli, but tail pinches and DMPH stimulation still resulted in inhibition of both types of cells. These researchers concluded that inhibition of theta-off cells is most likely due to a GABA-ergic pathway originating in the medial septum.

The morphology of theta cells is still under debate (see Bland 1986, for review). Based on anatomical and electrophysiological evidence, Fox and Ranck (1975, 1981) have concluded that the majority of theta cells are interneurons and complex-spike cells are pyramidal cells. Conversely, Leung and Yim (1986) found that CA1 pyramidal cells showed intracellular oscillations of the resting membrane potential that were phase locked to extracellularly recorded theta rhythms. Nunez, Garcia-Austt, and Buno Jr. (1987) also recorded intracellularly from CA1 cells, as well as injecting these cells with Lucifer yellow for morphological identification. These authors concluded that cells showing intracellular theta oscillations and rhythmic spike activity were pyramidal cells.

BLAND'S SENSORIMOTOR MODEL

Bland (1985, 1986) has posited a sensorimotor model to account for the appearance of type 1 and 2 theta activity. Key to his model are the assumptions that there are two distinct afferent systems which are involved in the generation of type 1 and 2 theta, and that the type 2 theta system is active during voluntary movements. A considerable amount of electrophysiological and pharmacological evidence exists which substantiates these two assumptions (see Bland, 1986 for review). The type 2 theta system is posited to monitor sensory systems. Tonic activity is reflected in LIA activity, whereas phasic changes in the sensory afferents result in the production of type 2 theta and rhythmic theta cell activity. The type 1 theta system is an indicator of the level of activation in the voluntary motor system. The type 2 theta system is considered to have two roles: (1) acting as a priming input to the voluntary motor system for the initiation of motor programs; and (2) providing sensory feedback for those motor programs already initiated. In this latter case, phasic changes in the type 2 theta system result in alterations of an already activated motor program. These alterations may be reflected in changes of the speed, intensity or pattern of an ongoing motor program. A question inherent in this model is how or why does tonic activity in the sensory systems shift to phasic activity? Bland (1986) suggests that arousal of the animal and the significance it

places on a particular stimulus event may determine whether that event results in tonic (LIA) or phasic (type 2 theta) activity. Sainsbury and colleagues have done a number of studies which address the issue of arousal and the production of type 2 theta. These researchers have found in the guinea pig and rat, that stimuli which have no "species relevance" (eg. a tone) are unlikely to elicit type 2 theta during immobility. If these stimuli are paired with "species relevant" stimuli, such as sight or sound of predators (eg. snakes and ferrets), type 2 theta is produced during immobility. Further, the production of type 2 theta in response to continuous stimulus presentation has been shown to be prone to habituation. In this case type 2 theta is replaced by LIA (Sainsbury, Heynen, and Montoya, 1987; Sainsbury and Montoya, 1984). According to Bland's sensorimotor model habituation of the type 2 theta response is an example of phasic activity being replaced by tonic activity and as a result there is a decrease in the probability of the sensory processing system priming the voluntary motor system for action. It is important to note that both differences between species (Bland, 1986; Robinson, 1980), and differences within species (Sainsbury and Montoya, 1984,) in the production of type 2 theta occur. The rabbit appears to be the most reliable producer of type 2 theta and therefore the most likely species in which to test this model (Bland, 1986).

IN VITRO THETA ACTIVITY

Bland, Konopacki, Roth, and colleagues have recently studied the production of theta activity in the hippocampal slice (*in vitro*) preparation. The major findings of their research have shown: (1) the hippocampal slice is capable of generating theta activity despite being deafferented of all extrinsic inputs such as the entorhinal cortex and medial septum (MacIver, Harris, Konopacki, Roth, and Bland, 1986); (2) theta activity can be induced by the application of carbachol and antagonized by atropine sulphate, suggesting that the cholinergic receptor mediating *in vitro* theta activity is muscarinic (Konopacki, MacIver, Bland, and Roth, 1987). Other neurotransmitters, such as dopamine, serotonin, GABA, nicotine, glutamate, and norepinephrine are unable to produce or antagonize theta generated by application of carbachol (Konopacki, Bland, and Roth, 1988b); (3) like *in vivo* preparations, the CA1 region shows smaller amplitude theta activity than the dentate region, although the 180 degree phase relationship between these two generators that is found *in vivo* is no longer present in the hippocampal slice preparation (Konopacki, Bland, and Roth, 1987). Furthermore, isolated sections (trans-slices) of just the CA1 and dentate gyrus regions can independently generate theta activity following application of carbachol (Konopacki, Bland, MacIver, and Roth, 1987); (4) intracellular recordings of cells in the CA1, CA3, and dentate gyrus regions show membrane potential

oscillations and rhythmic discharge patterns that are related to extracellularly recorded theta rhythm. This provides evidence that the theta recorded in vitro is not an epiphenomenon, but has a cellular basis (Bland, Colom, Konopacki, and Roth, 1988); and (5) a third independent generator of theta rhythm was found in the CA3c region of the hippocampus proper. Isolation of this area from the CA1 and dentate gyrus regions did not affect its ability to generate carbachol induced theta rhythm (Konopacki, Bland, and Roth, 1988a).

THE MEDIAL SEPTUM AND THETA ACTIVITY

The medial septum-diagonal band area (MSDB) is considered a pivotal region in the generation of hippocampal theta activity. The MSDB sends its efferent projections to the hippocampus via the dorsal fornix, fimbria, and possibly the cingulum (Meibach and Siegal, 1977; Swanson and Cowan, 1979). Terminals from these fibers have been found in all CA fields (1-4), the dentate gyrus region, and the subiculum (Chandler and Crutcher, 1983; Meibach and Siegel, 1977; Nyakas, Luiten, Spencer, and Traber, 1987). At one time this projection was considered to be primarily cholinergic, but recent double-labeling techniques suggest that less than 50 percent of the hippocampal innervation by the septum is cholinergic (Amaral and Kurz, 1985; Baisden, Woodruff, and Hoover, 1984). A significant portion of the septo-hippocampal projections have been found to stain positively for glutamic acid decarboxylase (GAD) the enzyme mediating synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA)

(Kohler, Chan-Palay, and Wu, 1984). The MSDB receives ascending inputs from the hypothalamus and a number of brain stem nuclei (Segal and Landis, 1974; Swanson and Cowan, 1979); as well, the hipocampal formation projects back to the MSDB via the lateral septum which in turn projects to the MSDB. These efferents from the hippocampus arise from the CA1 - CA3 regions and the subicular region (Swanson and Cowan, 1979).

Lesions of the MSDB abolish hippocampal theta activity in both generator sites (Andersen, Bland, Myhrer, and Schwartzkroin, 1979; Donovick, 1968; Sainsbury and Bland, 1981), as well as cause a significant decrease in acetylcholinesterase (ACHE) activity (Mellgren and Srebro, 1973). Sainsbury and Bland (1981) suggest that MSDB projections mediating CA1 and dentate theta are at one point anatomically distinct. These researchers found that lesions of the lateral septum abolished theta activity only in the CA1 region, whereas medial septal lesions abolished theta in both generator regions. The mediation of CA1 theta by the lateral septum is likely to be polysynaptic. Studies have shown that injection of retrograde label into the CA1 region, following recordings of theta activity with HRP-filled microelectrodes, result in labelling of cells only in the medial portions of the MSDB (Monmaur and Thompson, 1983; Oka and Yoshida, 1985).

Kramis and co-workers have demonstrated that stimulation of the MSDB with frequency trains equal to the frequencies of naturally occurring theta produces hippocampal driving. Moreover, this driving of theta activity was found to be independent of ongoing behaviour.

Behaviours normally correlated with theta activity would still occur despite stimulation induced desynchrony in the hippocampus. Conversely, behaviours correlated with LIA were not disrupted by stimulation induced synchrony (Kramis and Routtenberg, 1977; Kramis and Vanderwolf, 1980). These two studies provide evidence that the medial septum provides a rhythmic input to the hippocampus and that that behaviours associated with hippocampal theta activity are not necessarily dependent upon, or the result of, theta activity.

Petsche, Stumpf, and Gogolak (1962) considered the septal area (MSDB) to be the "pacemaker" of hippocampal theta rhythm. Based on lesion and stimulation experiments, Petsche, Stumpf and collegues hypothesized that the septal area transformed the steady flow of brain stem impulses into rhythmic discharges which were passed onto the hippocampal formation and resulted in theta activity (Gogolak, Stumpf, Petsche, and Sterc, 1968; Petsche, Gogolak, and Sterc, 1968). More recent studies by Brazhnik, Vinogradova and co-workers have substantiated this assertion. Within the MSDB a number of cells show rhythmic bursting which persists despite deafferentation from both the hippocampal formation and ascending brain stem pathways. In the former case no theta is present in the hippocampal EEG. The spontaneously rhythmic bursting displayed by these cells is within the normal theta frequencies of 4-5 Hz (bursts/sec). Stimulation of afferent MSDB pathways results in an increase of bursts per second as well as a corresponding increase in hippocampal theta frequency. An interesting finding noted by these researchers was that during driving of frequencies outside those which occurred spontaneously, a number of

rhythmic septal cells would jump to higher or lower harmonics of the stimulation frequency. Others would reset to baseline spontaneous levels of bursting (Brazhnik and Vinogradova, 1986; Brazhnik, Vinogradova, and Karanov, 1985; Vinogradova, Brazhnik, Karanov, and Zhadini, 1980a,b).

Recording of MSDB cells during pharmacological manipulations has led to conflicting reports between researchers. Brazhnik and Vinogradova (1986) found that pentobarbital and atropine sulphate eliminated hippocampal theta but had relatively small effects on rhythmic MSDB neurons, which continued to burst in a rhythmic manner. Physostigmine (an ACHE inhibitor) administration was also without effect on MSDB neurons, although theta was now present in the hippocampus. Following physostigmine, MSDB neurons typically failed to respond to afferent stimulation (Brazhnik and Vinogradova, 1986). Conversely, Lamour, Dutar, and Jobert (1984) found that rhythmic MSDB neurons showed excitation following administration of cholinergic agonists (carbachol, physostigmine, and acetylcholine), and inhibition following antagonism by the muscarinic blocker, atropine sulphate. In a recent study by these researchers GABA was found to have a marked inhibitory effect on MSDB cell firing (Bassant, Jobert, Dutar and Lamour, 1988). The conflicting results by the above two groups is most likely due to the experimental methods employed. The studies by Brazhnik and Vinogradova were done with freely moving rabbits and drugs were administered intravenously (IV). Conversely, Lamour, Dutar, Jobert and associates used urethane anesthetized preparations and drugs were iontophoretically applied onto the cell. Recently,

Stewart and Fox (1989) found in the anesthetized preparation, MSDB neurons that were both resistant and sensitive to IV atropine sulphate. Recordings obtained from pairs of MSDB neurons showed that a close coupling existed between cells irrespective of whether they were atropine sensitive or resistant. These researchers are currently investigating whether the atropine-resistant neurons are involved in the generation of atropine-resistant (type 1) theta.

ENTORHINAL CORTEX AND THETA ACTIVITY

The entorhinal cortex is considered the largest source of afferent innervation to the hippocampal formation (Blackstad, 1958; Lorento de No, 1934). The entorhinal area projects to the hippocampus via three pathways: the perforant path, alvear bundle, and crossed ammonic path. The main entorhinal input, the perforant path, arises from both the lateral and medial entorhinal cortices and projects to the dendritic branches of the CA1-CA3 pyramidal cells, as well as dentate granule cells (Blackstad, 1958; Hjorth-Simonsen and Jeune, 1972). The perforant path gives rise to the trisynaptic pathway, which is frequently used in the study of long term potentiation phenomena. This synaptic loop begins with the perforant path impinging upon the distal dendrites of granular cells. The granule cells send out axons (mossy fibers) which innervate pyramided cells of the CA3 region, which in turn send axonic processes (Schaffer collaterals) to the apical dendrites of CA1 pyramidal cells. High frequency stimulation of this pathway is known to create long lasting

enhancement of synaptic transmission, which may be important in such processes as learning and memory. The crossed ammonic pathway originates in the lateral entorhinal area and like the perforant path, innervates the CA1-CA3 regions and dentate gyrus (Blackstad, 1958; Hamilton, 1976). The alvear path originates in the medial entorhinal cortex and terminates in the subiculum and on the basal dendrites of CA1 pyramidal cells (Swanson and Cowan, 1977). The entorhinal cortex receives afferent connections from the hippocampal formation as well as a number of cortical and subcortical sensory areas (Sorenson, 1985).

Vanderwolf and Leung (1983) studied the effects of total entorhinal aspiration and disconnection lesions on theta activity in the freely moving rat. Lesions were found to selectively abolish all theta activity in the CA1 region, whereas dentate theta was left intact, although it was not always present during spontaneous walking. In the intact animal, type 1 theta activity (atropine-resistant) always accompanies voluntary movements such as walking; following entorhinal lesions this correlation was absent. Further, the theta activity that did periodically occur during walking was abolished by anticholinergic agents (atropine sulphate and muscarine), suggesting that the theta present following the lesions was cholinergically mediated (type 2 theta), and that entorhinal lesions had eliminated the type 1 theta system. Vanderwolf and Leung (1983) concluded that the type 2 theta system is active during type 1 theta and related behaviours, and that entorhinal lesions selectively eliminate type 1 theta. Montoya and Sainsbury (1985) chose to study the effects of

entorhinal lesions on theta activity in the guinea pig, as this species is a reliable producer of type 2 theta during immobility. Vanderwolf and Leung (1983) were unable to examine the effects of these lesions on naturally occurring type 2 theta, as rats rarely produce theta during immobility. In agreement with Vanderwolf and Leung (1983) theta was not always present during walking following entorhinal lesions. A similar decrease in the incidence of theta activity during immobility was also found. Administration of atropine sulphate abolished all theta activity during both walking and immobility (Montoya and Sainsbury, 1985). These researchers concluded that entorhinal lesions, in addition to abolishing type 1 theta, interrupt the production of type 2 theta during immobility.

HYPOTHALAMUS AND THETA ACTIVITY

The hypothalamus is considered to be a nodal point in the ascent of synchronizing and desynchronizing brain stem influences on hippocampal formation theta activity (see Vertes, 1981 for review). Numerous studies have shown that electrical stimulation of the dorso-medial posterior hypothalamus (DMPH) results in synchronous activity (theta) in the hippocampal formation of both freely moving and anesthetized animals (Bland and Vanderwolf, 1972; Bland, Andersen, and Ganes, 1975; Colom, Ford, and Bland, 1987; Christie, 1989; Destrade, 1982; Destrade and Ott, 1982; Vanderwolf, Bland, and Whishaw, 1973). Bland and Vanderwolf (1972), in the freely moving rat, demonstrated that stimulation of the DMPH induced voluntary motor movements (eg.

running) and concomitant hippocampal theta activity. These researchers found a direct relationship between stimulation intensity, theta frequency, and running speed. This synchronizing influence is likely mediated via the medial septal region as anatomical evidence has shown that the DMPH not only innervates the hippocampal region, but also sends efferents to the septum (Riley and Moore, 1981; Ter Horst and Luiten, 1986). Wilson, Motter, and Lindsley (1976) found that DMPH stimulation evoked theta activity in the hippocampus, and firing at theta frequencies by rhythmic bursting septal cells. Lateral hypothalamic (LH) stimulation resulted in hippocampal desynchronization as well as disrupting rhythmic septal cell activity. Hippocampal desynchrony following LH stimulation has also been shown in the freely moving rat (Whishaw, Bland, and Vanderwolf, 1972). Colom, Ford, and Bland (1987) demonstrated that theta related cells in the hippocampus coded the level of activation of the DMPH. As theta frequency in the hippocampus increased as a result of DMPH stimulation, so did the discharge rates of theta-on cells. Following administration of atropine sulphate, theta-on cells no longer coded DMPH stimulation.

The hypothalamus is also known to possess circuitry involved in the modulation of locomotion. Stimulation of various sites, including the DMPH, result in hindlimb and forelimb stepping movements in the anesthetized rat (Parker and Sinnamon, 1983; Sinnamon, 1984; Sinnamon and Stopford, 1987). According to Bland (1986) these movements could possibly be accompanied by atropine-resistant (type 1) theta activity, as well as rhythmic bursting by theta-related cells. To date no

studies have been done which directly test this possibility, although Colom et al. (1987) did describe a hippocampal theta-on cell which continued to code the level of DMPH stimulation following atropine sulphate administration. Research in this area may provide definitive answers to what anatomical and pharmacological systems are involved in the generation of type 1 theta activity.

In the experiments to follow, DMPH stimulation was used to produce theta activity in the hippocampal formation. Dorso-medial posterior hypothalamic stimulation has been shown to be an effective way of producing varying frequencies of theta activity in the urethane anesthetized preparation (Christie, 1989; Colom et al, 1987).

BRAIN STEM AND THETA ACTIVITY

A number of brain stem regions are believed to have synchronizing and desynchronizing influences upon hippocampal theta activity. Vertes (1982, 1985) provides a comprehensive review of the studies demonstrating these effects. Robinson and Vanderwolf (1978) stimulated throughout the brain stem of the freely moving rat and found that all stimulation sites resulted in synchronous theta activity, except the locus coeruleus which was ineffective for both synchrony and desynchrony. Vertes (1980) replicated Robinson and Vanderwolf's (1978) results in the anesthetized rat; although contrary to these researchers, he showed that stimulation of the median raphe resulted in hippocampal desynchrony. The nucleus pontis oralis was reported as the most effective brain stem synchronizing site, where

pronounced theta of high frequency (8 Hz or greater) and amplitude could be produced. This finding has also been replicated in the cat (Macadar, Chalupa, and Lindsley, 1974), and freely moving guinea pig (Faris and Sainsbury, 1989). In response to Vertes' (1984) assertion that the nucleus pontis oralis is the critical brain stem site in the generation of hippocampal theta activity, Faris and Sainsbury (1989) did lesions of this area and recorded hippocampal field activity. No significant effects on the frequency or amplitude of hippocampal theta were observed following lesions. Other brain stem areas which have been implicated in playing a synchronizing role in hippocampal field activity are the nucleus pontis caudalis, pontine central grey region, nucleus gigantocellularis, and nucleus raphe magnus (Macadar et al, 1974; Robinson and Vanderwolf, 1978; Vertes, 1980, 1981, 1982). Vertes (1981) found that these ascending synchronizing influences followed three major pathways: (1) the medial longitudinal fasciculus; (2) the medial forebrain bundle; and (3) the central tegmental tract. These fiber bundles exert their influences on the hippocampal activity both directly and via other known hippocampal afferents such as the hypothalamus and MSDB region (for review see Vertes, 1985).

Desynchronization of hippocampal field activity appears to be solely mediated by the median raphe of the brain stem (Vertes, 1985). Although discrepancies in the literature exist, most brain stem stimulation and lesion studies suggest that this area exerts a desynchronizing influence on hippocampal theta (Macadar et al, 1974; McNaughton, Azmitta, Williams, Buchan, and Gray, 1980; Vertes, 1980, 1981). Assaf and Miller (1978) demonstrated that stimulation of the

median raphe resulted in desynchronization of hippocampal theta as well as producing asynchronous firing of rhythmically bursting septal cells. These researchers believed that the median raphe exerted its desynchronizing effects via septal pacemaker cells. Similarly, McNaughton et al (1980) found that lower stimulation intensities were needed to drive hippocampal theta from the septum following lesions of ascending median raphe pathways. Lesions of the median raphe nucleus have been shown to cause a release of theta activity in the rat. Following lesions, theta activity was often present during immobility; a behaviour typically associated with hippocampal LIA activity (Maru, Takahashi, and Iwahara, 1979). This desynchronizing influence has been shown to reach the hippocampus and theta-related nuclei via the ventromedial aspect of the medial forebrain bundle (Segal and Landis, 1974; Vertes, 1981, 1986).

The Locus Coeruleus and Theta Activity

The locus coeruleus is a compact nucleus located on the floor of the fourth ventricle in the brain stem. It is the largest group of norepinephrine containing neurons in the brain and it sends projections to virtually all areas of the cerebral hemispheres and spinal cord (see Amaral and Sinnamon, 1977 for review). Estimates suggest that it provides up to 95 percent of the norepinephrine (NE) found in the rat brain (Descarries, and Saucier, 1972; Kobayashi, Palkovits, Kopin, and Jacabowitz, 1974). Moore and co-workers have used a number of anatomical mapping techniques (eg. anterograde and retrograde labeling, fluorescent immunohistochemistry, and

autoradiography) to study the locus coeruleus (LC) innervation of the hippocampal formation. These researchers have found that the LC innervates the hippocampus proper at all CA (1-4) fields; typically at the soma and apical dendrites of the pyramidal cells. The dentate gyrus and hilar regions receive considerably heavier projections, with processes ending adjacent to the granular layer (Jones and Moore, 1977). Projections to other limbic areas such as the medial septum, hypothalamus, cingulate and entorhinal cortices have also been demonstrated (Jones, Halaris, McIlhany, and Moore, 1977). Projections to the hippocampal formation leave the LC via the dorsal and ventral bundles, and enter the MFB at the level of the thalamus. From the MFB three groups of fibers branch off to innervate the hippocampus. One group of fibers pass through the medial septum and enter the hippocampus via the fornix. A larger group of fibers, the fasciculus cinguli, continue from the MFB into the cingulate cortex and enter the subiculum and hippocampus proper. A third fiber bundle enters ventrally into the hippocampus via the ventral amygdaloid bundle (Jones and Moore 1977; Loy, Koziell, Lindsey, and Moore, 1980; Riley and Moore, 1981). Electron microscopic visualization of the innervation patterns in the hippocampus has shown that the majority (80 percent) of NE containing fibers have varicosities which do not make conventional synaptic contacts with the post synaptic membrane (Koda and Bloom, 1977; Koda, Shulman, and Bloom, 1978). This type of nonsynaptic innervation is typical of what is found at postganglionic fibers in the autonomic nervous system (Burnstock and Costa, 1975).

Electrical stimulation of the LC has proven it to be an

ineffective site for the production of hippocampal theta activity (Robinson and Vanderwolf, 1978; Robinson, Vanderwolf, and Pappas, 1977; Vertes, 1980, 1981, 1982). Findings by Macadar et al. (1974) are the exception, as they found the LC to be an effective theta inducing site in the cat. Vertes (1982) challenged this finding by noting that these researchers used stimulation intensities three to four times greater than needed to generate theta from other brain stem synchronizing sites. Vertes proposed that these researchers may have been stimulating synchronizing sites outside the LC with such high current levels. Electrolytic lesions of the LC have not been found to directly affect type 1 or type 2 theta activity (Kolb and Whishaw, 1977), although these authors did see a "release" of 5-6 Hz theta activity during spontaneous immobility and in response to sensory stimulation (eg. hand claps). Administration of atropine sulphate abolished this immobility related theta, suggesting a cholinergic basis. A similar release of theta activity accompanying immobility has been shown in rats receiving intraventricular injections of 6-hydroxydopamine (6OHDA), a neurotoxin of NE and dopamine containing fibers (Whishaw, Robinson, Schallert, De Ryck, and Ramirez, 1978). These researchers also demonstrated that atropine sulphate abolished this immobility related theta activity. Robinson, Vanderwolf, and Pappas (1977) found a release of immobility related theta activity following reserpine treatment (known to deplete monoamine stores in the brain) in both control and 6OHDA treated animals. The above studies suggest that hippocampal noradrenergic innervation from the LC may play an inhibitory role on mechanisms underlying the production

of type 2 theta activity. Disruption of this influence, either by LC lesions or NE depletions, appear to release type 2 theta activity.

Segal and Bloom (1974a, 1974b, 1976a, 1976b) studied the involvement of the LC in presumed hippocampal pyramidal cell activity. During and following stimulation of the LC the majority of cells studied showed a marked reduction in discharge rate which often persisted for several seconds. Similar inhibition of spontaneous hippocampal cell activity was shown following presentation of sensory stimuli, such as tones and flashes of light. In all instances these inhibitory responses could be antagonized by drugs interfering with NE transmission. In addition to inhibitory effects, Segal (1977, 1982) demonstrated that LC stimulation could also enhance excitatory evoked potentials produced by stimulation of known hippocampal inputs. Segal (1982) concluded that LC/NE involvement in hippocampal functioning was to decrease spontaneous noise (spike activity) and increase the efficacy of neurotransmission in excitatory pathways. The end result of this processes being a greater signal to noise ratio.

Locus Coeruleus Cell Activity

Locus coeruleus neurons have been shown to have low spontaneous discharge rates which fluctuate systematically with environmental events and changes in animal behaviour (Aston-Jones and Bloom, 1981b; Jones, Segal, Foote, and Bloom 1979). During periods of slow wave sleep these neurons fire at reduced rates (1 Hz) and during REM there is almost a complete absence of activity (Aston-Jones and Bloom, 1981a). Similarly, during what Foote, Aston-Jones, and Bloom (1980)

termed "low vigilant states", such as quiet-awakening, grooming, and eating, LC neurons exhibited low levels of spontaneous firing. The highest levels of activity were found during the presentation of sensory stimuli (eg. tones, hand claps, light flashes). Comparable firing rates have also been shown in response to more complex stimuli such as food and the presentation of a novel object in the animal's environment (Aston-Jones and Bloom, 1981b; Foote et al, 1980). Repeated presentations of these stimuli result in habituation where these neurons no longer change their rate of firing in response to the stimulus. This type of neuronal activity appears to be characteristic of the majority of cells in the LC.

NEUROTRANSMITTERS OF THE HIPPOCAMPAL FORMATION

A number of putative transmitters have been localized to the hippocampal formation and it is well beyond the scope of this discussion to review each one in detail. Storm-Mathisen and Otterson (1984), and Haas (1983) provide reviews of the transmitter candidates, their sites of origin, and terminal fields within the hippocampus.

Acetylcholine

The cholinergic innervation of the hippocampal formation arises primarily from neurons in the MSDB region (Lewis and Shute, 1967). A stratified pattern of acetylcholine esterase (ACHE; degradative enzyme of acetylcholine) and choline acetyltransferase (CHAT; enzyme necessary for the synthesis of acetylcholine) staining has been shown

above and below the pyramidal cell layer of the hippocampus proper and the granule cell layer of the dentate gyrus (Storm-Mathisen, 1970; Storm-Mathisen and Fonnum, 1969). Cholinergic fibers from the MSDB ascend to the hippocampal formation via the fimbria and dorsal fornix; transections of this fiber pathway result in a decrease of ACHE and CHAT activity (Mellgren and Srebro, 1973; Storm-Mathisen and Guldberg, 1974). Similarly, lesions of the MSDB deplete the amount of acetylcholine (ACH) that can be collected from the surface of the dorsal hippocampus, whereas stimulation of this region increases hippocampal ACH levels (Dudar, 1975). Dudar, Whishaw, and Szerb (1979) demonstrated an increase in the release of ACH from the hippocampus during sensory stimulation and running in freely moving rats. Localization of cholinergic receptors in the hippocampus have shown that muscarinic receptors predominate throughout the hippocampus proper and dentate gyrus, whereas nicotinic receptors are more prevalent in the latter region (Kuhar and Yamamura, 1975; Polz-Tejera, Schmidt, and Karten, 1975). Whether there is an intrinsic ACh system in the hippocampus is debatable, although Storm-Mathisen (1977) describes an area within the CA1 region (area 31) which retains ACHE and CHAT activity following lesions of the cholinergic MSDB input. This residual ACH activity may be the result of cholinergic inputs from brain areas other than the MSDB.

Microinfusions of muscarinic agonists into the hippocampal formation produce theta activity in both the freely moving (Malisch and Ott, 1982) and anesthetized rat (Rowntree and Bland, 1985). This theta activity is abolished by subsequent infusions of muscarinic

blocking agents. Studies using the hippocampal slice preparation have shown similar results with the use of cholinergic agonists and antagonists (Konopacki, MacIver, Bland, and Roth, 1987). At the cellular level application of ACH on neurons in the CA1 and dentate regions results in excitation (Krnjevic, Reiffenstein, and Jobert, 1981), which can be antagonized by atropine sulphate (Bland, Kostopoulos, and Phillis, 1974). Bland et al (1974) found that cells in the dentate gyrus had a faster onset of excitation following ACH iontophoresis than cells recorded in the CA1 region. Cell excitation in the dentate region was also less likely to be blocked following atropine sulphate administration. Storm-Mathisen (1977) suggests that the differences in response to ACH by cells in the CA1 and dentate regions may have been due to a larger number of nicotinic receptors in dentate versus CA1 region.

Serotonin

Histochemical studies suggest that the serotonergic activity found in the hippocampal formation is the result of neuronal fibers projecting to the hippocampus from the median raphe nucleus of the brain stem (Fuxe, 1965; Moore and Halaris, 1975). Terminals from the median raphe are found primarily in the moleculare layer of the hippocampus proper and adjacent to granule cells of the dentate gyrus (Moore and Halaris, 1975). Lesions of the median raphe or its pathways to the hippocampus result in an almost total depletion of serotonin (5HT) (Kuhar, Aghajanian, and Roth, 1972a; Storm-Mathisen and Guldberg 1974).

Serotonin is likely involved in the generation of type 1 (atropine-resistant) theta activity as administration of parachlorophenylalanine, a 5HT synthesis inhibitor, abolishes type 1 theta (Vanderwolf and Baker, 1986). Similarly, lesions of the serotonergic pathways entering the hippocampus via the entorhinal cortex also have been found to abolish type 1 theta activity (Vanderwolf and Leung, 1983). Application of 5HT onto presumed pyramidal cells results in an inhibition of spontaneous activity which can be antagonized by 5HT blocking drugs (Segal, 1975, 1976). Colino and Halliwell (1986) provide evidence suggesting that the $5HT_{1A}$ receptor subtype mediates the inhibition produced by application of 5HT.

Gamma-aminobutyric Acid

Immunohistochemical studies have shown that cells containing glutamic acid decarboxylase (GAD), the synthesizing enzyme for gamma-aminobutyric acid (GABA) are found throughout all layers and fields of the hippocampal formation (Barber and Saito, 1976; Ribak, Vaughn, and Saito, 1976). Immunohistochemical visualization of GABA containing neurons have shown similar results as those done on GAD (Storm-Mathisen, Leknes, Bore, Line Vaaland, Edminson , Haug, and Otterson, 1983). GAD containing neurons appear to have nerve terminals which form a plexus around the soma and dendrites of hippocampal pyramidal and granule cells. This suggests that these cells may be inhibitory interneurons (basket cells) (Barber and Saito, 1976). These GABA containing cells appear indigenous to the

hippocampus as deafferentation of the hippocampus from its afferent pathways result in a negligible decrease of GAD activity (Storm-Mathisen, 1972). Also a number of the GABA containing cells appear to be projection cells both within and outside the hippocampus (Ribak, Seress, Peterson, Seroogy, Fallon, and Schmued, 1986; Shinoda, Tohyama, and Shiotani, 1987). Shinoda et al (1987) using double labeling methods, found that GABA containing cells in the CA1 - CA3 regions have both ipsilateral and contralateral projections to the MSDB region. GABA containing neurons within the MSDB are also believed to synapse back upon inhibitory neurons in the hippocampal formation (Freund and Antal, 1988), creating a GABA-ergic loop between these two structures.

Iontophoretic application of GABA onto hippocampal pyramidal cells have been shown to produce both inhibitory and excitatory effects (Alger and Nicoll, 1982; Andersen, Bie, and Ganes 1982). Contradictions between studies also exist with respect to what part of the pyramidal cell body GABA acts upon to produce inhibition and excitation (see Schwartzkroin, 1986 for review). Conflicting reports are most likely the result of differences in the site of iontophoresis and the hippocampal region from which the pyramidal cells are obtained. To date no studies have been done to determine if GABA plays a role in the production of theta activity in the intact animal, although Konopacki, Bland, and Roth (1988b) have found that GABA had no effect on carbachol induced theta in the slice preparation.

Glutamate

Glutamate, an excitatory amino acid, is believed to act as a neurotransmitter in the hippocampus. Evidence suggests that glutaminergic activity in the hippocampus may be the result of intrinsic and/or extrinsic innervation. Storm-Mathisen (1981) has demonstrated that glutamate acts as a neurotransmitter in the perforant path input to the hippocampus from the entorhinal cortex. Glutaminergic activity has also been demonstrated at dentate granule cells, and CA3 - CA4 pyramidal cells. Stimulation of the perforant path input has been shown to cause a two-fold increase above basal levels of glutamate in the hippocampus (Crawford and Conner, 1973). Wheal and Miller (1980) demonstrated that application of glutamate onto dentate granule cells mimicked the excitation produced by perforant path stimulation. Further, administration of glutamate diethylester (GDEE), a post synaptic glutaminergic antagonist, blocked the excitation produced by both perforant path stimulation and glutamate application. Pyramidal cells of the hippocampus proper also exhibit excitation following glutamate application (Dudar, 1974; Schwartzkroin and Andersen, 1975). These results are consistent with the finding that glutaminergic receptors have been localized in both the hippocampus proper and dentate gyrus (Monaghan, Holets, Toy, and Cotman, 1983). Neurons in the hippocampus proper have been shown to have a glutaminergic projection to the lateral septum (Zaczek, Hedreen, and Coyle, 1979).

The majority of research into the role of glutamate in the hippocampus has focused on long term potentiation (LTP) phenomena (for

review see Raicine and Kairiss, 1987). Interestingly, recent studies suggest that the theta activity may be involved in LTP. Greenstein, Pavlides, and Winson (1987) have shown that enhanced, evoked synaptic potentials (ESP) in dentate granule cells occurred when stimulation of the perforant path (PP) had a periodicity (5 Hz) within the range of theta activity. These researchers have also demonstrated that ESP's are most likely to occur when PP stimulation is applied at the peak rather than the trough of the locally recorded theta rhythm (Pavlides, Greenstein, Grudman, and Winson, 1988). Similar LTP effects have been shown with theta frequency stimulation of the Schaffer collaterals in freely moving animals (Staubli, and Lynch, 1987).

Norepinephrine

A diffuse noradenergic innervation of the hippocampus originating the from locus coeruleus exists. Based on autoradiographic studies, the distribution of noradrenergic receptors have also been found to be diffuse. Both alpha and beta receptors, and their subtypes have been localized in the hippocampus proper and dentate gyrus regions (Crutcher and Davis, 1980; Rainbow, Parsons, and Wolfe, 1984; Young and Kuhar, 1980). Segal and Bloom (1974a,b, 1976a,b) were amongst the first researchers to study the effects of NE on hippocampal cell activity. These researchers found that spontaneously occurring and ACH induced firing of cells in the CA1-CA3 regions were depressed following application of NE. This inhibition of firing was long lasting (several seconds) and mediated by beta receptors. Based on the finding that 6OHDA treated rats

showed higher spontaneous hippocampal cell activity as compared to controls, these researchers hypothesized that the LC-NE innervation of the hippocampus was inhibitory in nature. In addition to the inhibitory responses described above, these researchers found that NE application also enhanced excitatory hippocampal cellular responses.

Since this early work by Segal and Bloom a number of studies have further investigated the role of NE in the hippocampus, although none have been done which directly test the role of NE in theta production. Rather, these studies have primarily dealt with the effects of NE on evoked potentials. The general finding has been that application of NE enhances the evoked potential (population spike) produced by trisynaptic pathway activation in the CA1 and CA3 pyramidal cell regions. This finding has been demonstrated in both in vivo and in vitro preparations (Hopkins and Johnston, 1984; Madison and Nicoll, 1986; Mueller, Hoffer, and Dunwiddie, 1981; Mueller, Palmer, Hoffer, and Dunwiddie, 1982). Mueller et al. (1982) found that at higher concentrations of NE the population spike was depressed rather than enhanced. These authors hypothesized that higher concentrations of NE no longer mimicked the effects of endogenously released NE and therefore care should be taken in interpreting results where high volumes or concentrations of exogenously applied neurotransmitters are used. The excitatory effects produced by NE application appear to be mediated by beta adrenoreceptors, as beta agonists mimic NE application and beta antagonists block these effects.

The effects of NE on evoked potentials in the dentate gyrus have

been shown to be more variable than that found in the hippocampus proper. Harley and co-workers have found in the in vitro hippocampal slice preparation that iontophoretic application of NE onto the granule cell region, as well as application of NE into the bathing medium, results in enhanced population spikes. This effect was found to be mediated by stimulation of beta receptors as beta antagonists abolished this response. (Lacaille and Harley, 1985; Neuman and Harley, 1983). Contrary to these findings, Swanson and Dahl (1985) have shown in the anesthetized preparation that iontophoresis of NE onto granule cells has no effect on perforant path evoked population spikes, although beta receptor activation enhances the population spikes and $alpha_1$ receptor activation causes inhibition. When NE was applied to the apical dendrites of the granule cells inhibition was found. Subsequent application of various receptor agonists showed that both beta and alpha receptors mediated this inhibition.

Investigations into the role of NE in the hippocampal formation has also looked at the response of individual neurons in this area. Otmakhov and Bragin (1982), using the in vitro hippocampal slice preparation, studied the effects of NE application on two populations of cells in the CA1 and CA3 regions. Based on firing repertoires, the two categories of cells studied were complex spike (pyramidal) cells and simple spike (interneurons) cells. Both types of cells showed enhanced firing rates when NE application preceded mossy fiber stimulation. Spontaneous complex cell firing was inhibited following NE administration, whereas it was facilitated in simple spike cells. The enhanced spontaneous activity exhibited by simple spike cells was

found to be dose-related and mediated by $alpha_1$ receptor activation. Recently, Pang and Rose (1987) used cell firing patterns to classify cells into complex spike and theta neurons, and investigated the effects of NE on their activity. These researchers found that NE inhibited the majority of complex spike cells while exciting theta neurons in both the CA1 and dentate gyrus. The inhibition of complex spike cells was mediated by $alpha_1$ receptors and theta cell excitation was mediated by $alpha_2$ and beta receptor activation. Complex spike cells were excited when $alpha_2$ and beta agonists were applied. It appears that when NE was applied to complex spike cells the inhibitory response mediated by $alpha_1$ receptor activation dominated over the excitation produced by $alpha_2$ and beta receptor activation.

The above findings provide evidence that NE does act upon the hippocampus at the cellular level, with the receptor subtype involved dramatically influencing the type of activity seen. A number of reasons can account for the often conflicting nature of some of these studies. Firstly, the type of animal preparation used may influence the end results. Some studies were undertaken in anesthetized animals, whereas others were done in the <u>in vitro</u> preparation. Whether the cells studied were located in the hippocampus proper or in the dentate gyrus may also lead to conflicting reports as the principal cell in these two areas differ from one another. It is also possible that these researchers were not recording from the principal cells in the area. In iontophoretic studies, whether the drug is applied to the soma or dendrites of the cell can also lead to varying results. Lastly, $alpha_2$ and $beta_2$ receptors are known to occur both

postsynaptically and presynaptically. The $alpha_2$ receptor plays an autoinhibitory role presynaptically where stimulation of this receptor reduces cell firing and release of NE. The $alpha_2$ receptor is also known to occur postsynaptically at the terminal of ACH containing neurons. Activation of the receptor in this instance results in an inhibition of ACH release (for review see Vizi, 1974a). Stimulation of presynaptic beta receptors has the opposite effect on adrenergic neurons, causing an increase in cell firing and release of NE. Therefore, the possibility exists that both presynaptic and postsynaptic events, not limited just to the NE system, may be occurring in some of the above studies.

EXPERIMENT I

The purpose of this first experiment was to determine the role NE plays in the production of type 2 theta activity. Based on prior observations made by researchers, it appears that disruption of the LC/NE system results in an increase in the production of type 2 theta in freely moving animals (Robinson et al, 1977; Kolb and Whishaw, 1977; Whishaw et al, 1978). One may conclude from these findings that, when intact, the LC/NE system plays an inhibitory role in the production of type 2 theta activity. Therefore, direct application of NE into the hippocampal formation should result in a reduction or inhibition of type 2 theta activity; the following experiments were designed to test this assumption.

The experiment was performed using the urethane anesthetized rat preparation. This allowed for control of a number of variables such as heart rate and core temperature. Although not lending itself to the analysis of animal behaviour, this type of preparation is known to produce long trains of spontaneous type 2 theta activity (Bland, 1986), which is often difficult to elicit in the freely moving rat (Sainsbury et al. 1987).

In order to bypass systemic effects which are likely to occur when intravenous or intraperitoneal administration of drugs are used, drugs were infused directly into the hippocampus at the level of the stratum moleculare of the dentate gyrus. This procedure has proven fruitful in past research concerning the cholinergic basis of type 2 theta activity (Rowntree and Bland, 1986). The procedure employed in

this study was similar to that of Rowntree and Bland (1986) with the exception that these experiments included a stimulating electrode in the DMPH. Stimulation of the DMPH has been shown to be a reliable procedure for producing varying frequencies of type 2 theta in the urethane anesthetized preparation (Christie, 1989; Colom et al, 1987).

Based on pilot research conducted prior to these experiments, only hippocampal theta activity recorded ipsilateral to the site of infusion was considered in the analysis. Effects occurring in the hippocampus contralateral to hippocampus of infusion were believed to be suspect as they appeared to occur as a result of drug spread outside the hippocampus rather than within it. Therefore, the hippocampus contralateral to the site of infusion served as a control throughout all experiments. Any changes in the contralateral hippocampus appearing to mimic that of the side receiving a microinfusion were considered to be signs that the drug was no longer confined to the hippocampus receiving microinfusion, and the animal was discarded from the analysis. Also, microinfusions of drugs were limited to a volume of 2.5 ul, as histological analysis had previously shown that a 2.5 ul infusion of a marker dye had a spread confined to the dentate gyrus and did not extend outside this area.

Power spectral analyses were performed on hippocampal field activity before and after drug infusions. This analysis has been proven to be an effective method for quantifying hippocampal field activity (Leung, 1984, 1985; Leung et al. 1982).

METHODS

Subjects and Surgical Procedure

Data for the present experiment were obtained from 10 male, Long Evans black-hooded rats bred at the University of Calgary, Psychology Department. Rats were raised in groups of three or four, in clear polycarbonate cages with bedding covering the floor of the cage to a depth of two cm. Animals were given free access to food (Rat Blox) and water, and were maintained on a 12 hour on, 12 hour off light/dark cycle. The animal room was kept at a temperature of 24 degrees celsius.

All animals were initially anesthetized with halothane, had their heads shaven, and then had tracheal and jugular catheters inserted. The rats were then maintained for the duration of experimentation with urethane (ethyl carbamate 0.8 g/ml) administered via the jugular catheter. After surgical anesthesia was achieved, the animal was placed in a stereotaxic instrument, a midline incision made and the wound margins infiltrated with local anesthetic (Lidocaine Hydrochloride). The scalp was then retracted to expose the skull and the plane between bregma and lambda was leveled to horizontal.

An uninsulated tungsten wire was placed in the cortex below the frontal bone to serve as an indifferent electrode during field activity recordings. Electrolytically sharpened, Kynar insulated, tungsten microelectrodes (0.2-0.4 Mohms) were used to record field activity from the dentate molecular layer of each hippocampus. The coordinates used were: 3.5 mm posterior to bregma and 2.5 mm lateral

to the midline suture. Each microelectrode was lowered to a depth where high amplitude (1-2 mV) theta could be recorded. Theta was found to be maximal between 2.2 and 2.8 mm ventral to the dural surface.

A bipolar (250 um), stainless steel electrode, insulated to the tip was used for stimulation of the DMPH. The electrode was lowered through a 0.5 mm hole drilled 0.5 mm lateral to the midline and 3.3 mm posterior to bregma. The stimulating electrode was lowered and affixed to the skull when low levels of stimulation (0.5 mA) reliably produced peripheral (i.e. vibrissae movements, pupil dilation, and increased respiration) and central (i.e. theta activity) effects consistent with DMPH stimulation. All electrodes were affixed to the skull with stainless steel jewelers screws and dental acrylic (Caulk Weld).

Throughout experimentation the rat's body temperature was maintained at 37 degrees celsius using a thermostatically regulated heating pad (Harvard Instruments Servosystem). Heart rate was monitored using a Grass EKG tachograph and a digital heart display designed by Tech Services at the University of Calgary.

Recording and Stimulation Apparatus

Brain activity was passed through two Grass, wide-band AC preamplifiers (model P511K) to a Grass polygraph (model 7D) with amplifiers set at one and 35 Hz for the low and high amplitude filter settings respectively. Hippocampal field activity was displayed on a Tektronix (model 5441) storage oscilloscope. Data was stored on

video cassettes using a TEAC XR-30, 7 channel FM tape recorder which had a voice channel so that notes could be made throughout the experiment. Data from the tapes were later taken for off line analysis.

A Grass multifunction stimulator (model S88) connected to a photoelectric stimulus isolation unit (model PSIU6) was used to deliver square-wave pulses (0.1 ms) at a frequency of 100 Hz. Periods when stimulation was applied were recorded onto the video tape via a synchronizing pulse from the stimulator.

Infusion Apparatus

The hippocampal infusion cannula was positioned 0.5 - 1.0 mm posterior to one of the recording electrodes and lowered to a depth equivalent to that of the electrode. Following insertion of the cannula, a 30 minute period for stabilization of the hippocampal field activity was allowed before infusions were begun. The guide tube for the cannula was constructed of 23 gauge stainless steel tubing. The infusion cannula consisted of 30 gauge stainless steel tubing, connected by PE50 intramedic tubing to a 2 CC glass syringe. When inserted in the guide tube, the cannula protruded 2.0 mm past the end of the tube. A multispeed Harvard Apparatus servo-control system (model 2990) and a Harvard Apparatus multi-speed dual infusion/withdrawal pump (model 2202) were used to generate and maintain a constant infusion rate during experimentation.

Drugs

The agent infused in this experiment was D,L norepinephrine hydrochloride (Sigma). The drug was freshly made prior to each experiment, in a 0.9 percent physiological saline vehicle. The drug was pH balanced to between 6.8 and 7.2 by adding sodium hydroxide to the solution.

Procedure

Following cannula insertion, baseline recordings of field activity, both spontaneously occurring, and in response to random presentations of 0.5 mA and 1.0 mA DMPH stimulation (20 seconds in duration) were carried out. An minimum interval of 30 seconds was given between each stimulation epoch so that no cumulative effects of stimulation would occur. Following baseline recordings, a 5 minute microinfusion of norepinephrine, at a rate of 0.5 ul/min was performed. Upon completion of the infusion, a one minute period was given to allow for the infused drug to diffuse away from the cannula. Recordings of spontaneous and stimulation induced field activity were once again done.

Upon completion of the experiment, each animal's cannula placement was marked with either pyronin red placed on the tip of the cannula, or an infusion of Evan's blue dye (3 mg/ml). Bright field and fluorescence microscopy have shown that Evan's blue dye has an infusion spread similar to that of norepinephrine and its receptor agonists and antagonists (Flicker and Geyer, 1982). This allowed for verification of cannula placement and physical spread of the drug.

Histology

At the conclusion of the experiment each rat was euthanized by an overdose of urethane, and perfused through the heart with saline followed by a 10 percent formalin solution. The brain was removed and left in a formalin solution until sectioning. Brains were frontally sectioned on a CO_2-freezing michrotome at 40 um intervals. Sections were mounted on slides and verification of all electrode and cannula sites were made relative to Paxinos and Watson's (1986) stereotaxic atlas.

Data Analysis

Upon completion of each experiment a polygraph chart record of the experiment was created by playing the FM tape back through a Grass polygraph (model 7D). As the chart record was being plotted, all notes made on the audio channel of the FM tape were written down beside corresponding sections of hippocampal field activity. The record was then used to select data for subsequent power spectral analysis. For each animal, 15 seconds of spontaneous theta activity and 0.5 mA and 1.0 mA DMPH stimulation induced theta, both before and after drug infusion were analyzed. The power spectra for these data were obtained by taking the signal from the FM tape and passing it through a Keno (type VBF8) dual acoustic variable filter (1.0 Hz high pass), and into a Bruel and Kjaer (type 2032) dual channel signal analyzer (Hanning window in place). Records of power spectra and corresponding hippocampal theta activity were plotted using a Bruel

and Kjaer (type 2319) graphics plotter. Four measures were obtained from each power spectrum: (1) peak theta frequency (Hz), which was considered to be the largest power peak in the power spectrum; (2) power (dB) of the peak theta frequency; (3) noise level (dB), which was obtained by measuring the power of the signal at five points (20,21,22,23 and 24 Hz) on the spectrum and then calculating a mean for those five points. This procedure was considered the most appropriate method for obtaining a noise value as the slope of the power spectrum levelled off past 18 Hz; and (4) signal to noise ratio, calculated by dividing the power of the peak frequency (2) by the noise (3).

To determine if microinfusions of NE had any significant effects on the four dependant measures a repeated measures analysis of variance (ANOVA) was carried out. Tests for sphericity were calculated and if found to be significant the Greenhouse-Geisser adjustment for degrees of freedom was applied. If required, multiple comparisons were done using the Tukey B procedure.

RESULTS

Histology

Histological analysis indicated that all stimulation and recording electrodes were positioned in the DMPH and stratum moleculare of the dentate gyrus, respectively. Figure 2 shows a schematic diagram of the 10 NE cannulae placements in the hippocampal formation. All placements were found to be in or near the stratum moleculare layer of the dentate gyrus. Figure 3 provides photographs of a brain receiving a 2.5 ul infusion of Evan's blue dye (3.0 mg/ml). Dye spread is shown to encompass a large portion of the dentate gyrus region.

Figure 2. Norepinephrine Microinfusion Sites

Coronal section through the dorsal hippocampus showing the 10 histologically verified microinfusion sites for norepinephrine (1.0 ug/ul). The three infusion sites in the neocortex overlying the hippocampus produced no effect on hippocampal field activity.

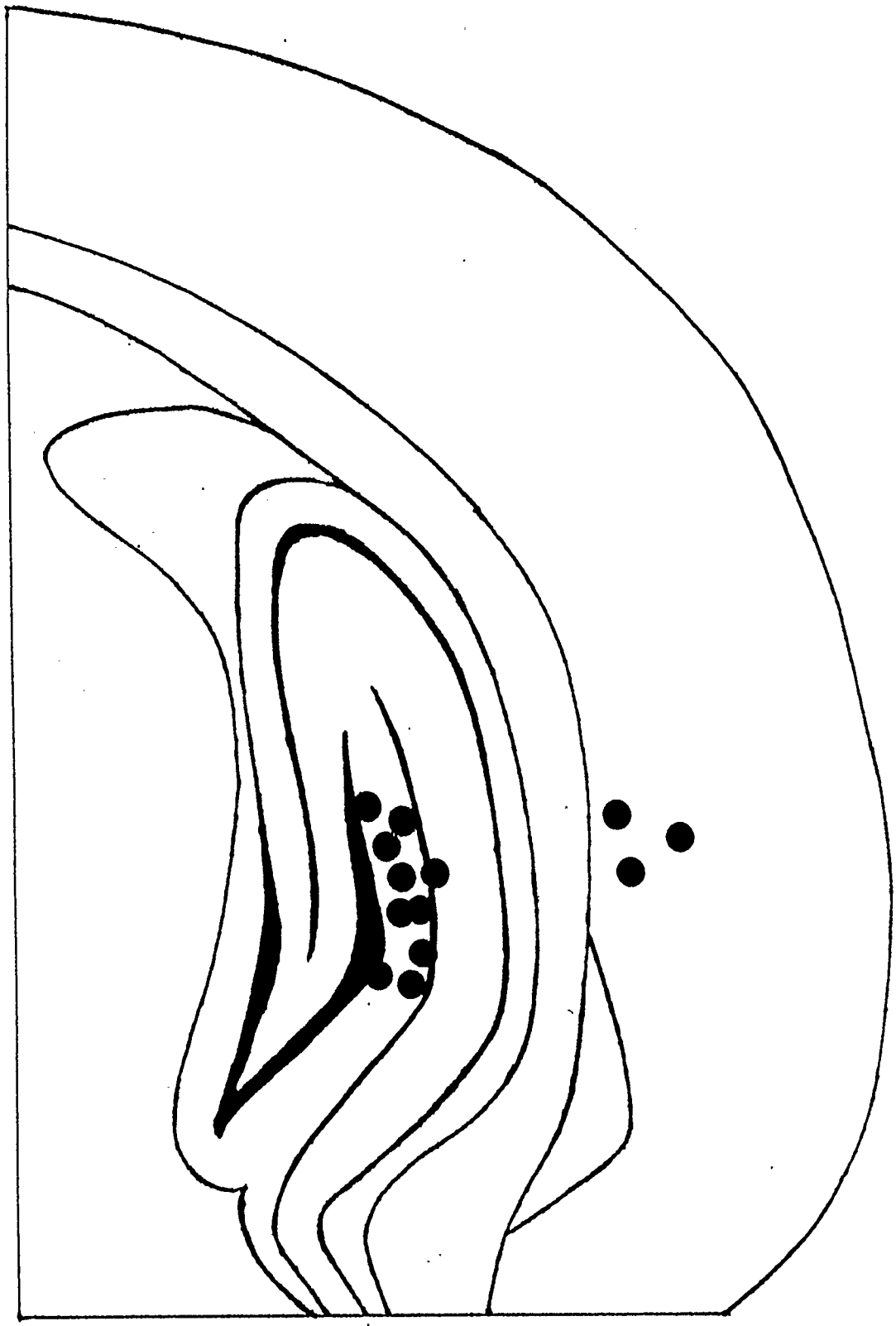
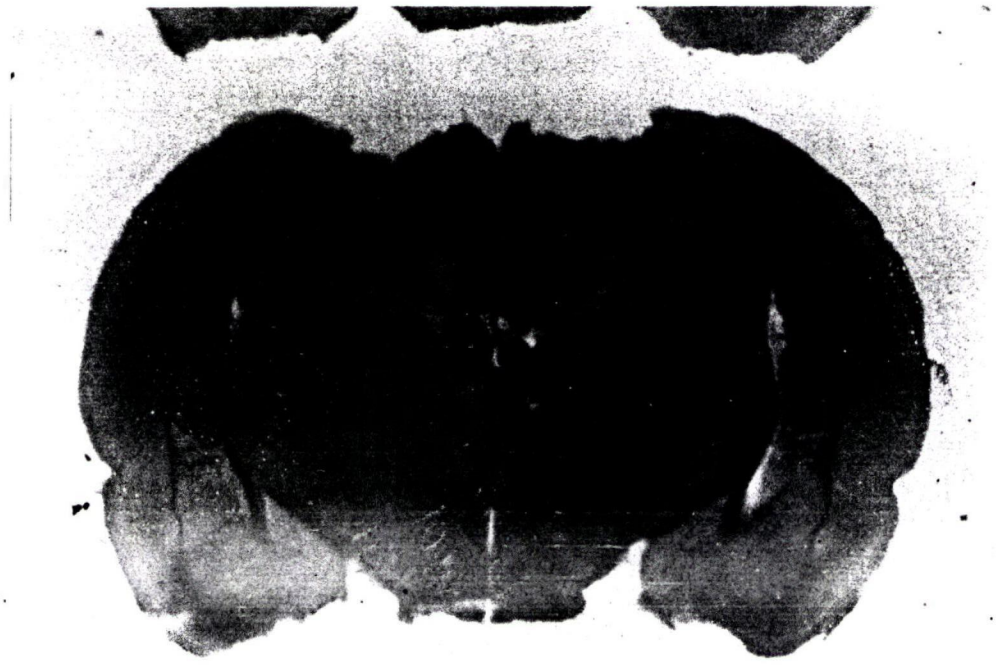
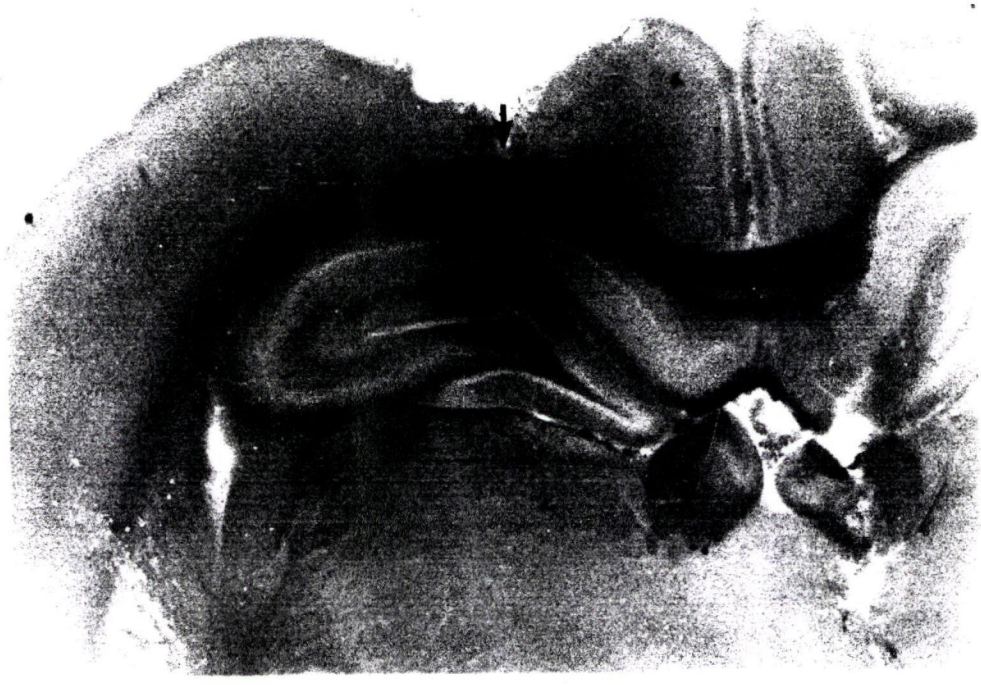


Figure 3. Dye Infusion Photographs

The top photograph shows a whole brain section through the dorsal hippocampus. The arrow in this photograph points to the hippocampus receiving a 2.5 ul infusion of Evan's blue dye (3.0 mg/ml). The lower photograph provides a higher magnification of the hippocampus receiving dye infusion. The arrow indicates the point of entry for the infusion cannula and the asterisk denotes depth where the tip of the cannula was positioned. Notice the spread of dye encompasses a large area of the dentate gyrus, both above (stratum moleculare) and below (hilus) the granule cell layer. The dye spread above the CA1 region at the point of cannula entry is due to leakage from the cannula as it was reinserted.





Microinfusions of Norepinephrine

All animals received a 2.5 ul microinfusion of NE (1.0 ug/ul). Following infusion a noticeable reduction in theta amplitude was observed in the polygraph chart records. This apparently was an effect of drug action and not due to the volume of the solution, as three animals receiving 10.0 ul control microinfusions of saline showed no depression of hippocampal theta activity. Three animals receiving 2.5 ul microinfusions of NE into the neocortex overlying the hippocampus also showed no apparent changes in hippocampal field activity. There were no instances where microinfusions of NE totally abolished stimulation induced or spontaneously occurring theta activity. The effects of NE were long lasting, as alterations in hippocampal field activity persisted for up to five hours following microinfusion. Figure 4 provides examples of hippocampal theta activity before and after microinfusions of NE.

A repeated measures ANOVA showed that there was a significant effect of NE microinfusion on noise level (F (1,9) = 21.80 $p < .01$). Prior to infusion the mean level of noise was 18.31 dB (SD = 2.02) and following microinfusion the level of noise dropped to a mean of 14.90 dB (SD = 2.76). There was also a significant main effect of NE on the power of the peak theta frequency (F (1,9) = 73.20, $p < .01$). The mean powers of the peak theta frequencies before and after microinfusion of NE were 48.21 dB (SD = 3.31) and 43.63 dB (SD = 2.26), respectively. No other main effects or interactions involving NE were found. Figure 5 provides the power spectra for a subject receiving a microinfusion of NE. Notice the drop in power of the

peak theta frequency and noise level following microinfusion, whereas theta frequency remains relatively unaltered.

Stimulation of the DMPH was found to have a significant effect on peak theta frequency (F (2,18) = 160.75, p < .01). Post-hoc analyses revealed that the frequency of spontaneously occurring theta (M = 4.75 Hz, SD = 0.37), and in response to 0.5 mA (M = 5.62 Hz, SD = 0.29), and 1.0 mA (M = 6.70 Hz, SD = 0.31) DMPH stimulation, all varied significantly from one another. Stimulation of the DMPH also had a significant effect on the powers of the peak theta frequencies (F (2,18) = 6.35, p < .01). The post-hoc analysis demonstrated that the power at the peak theta frequency during 1.0 mA (M = 46.74 dB, SD = 2.76) and 0.5 mA (M = 46.31 dB, SD = 2.29) stimulation were significantly larger than the power during spontaneous theta activity (M = 44.72 dB, SD = 3.33). No other main effects or interactions were obtained in the analysis (see Appendix A for summary tables).

Figure 4. Hippocampal Theta Activity Before and After Norepinephrine Microinfusion

Representative 8 second samples of hippocampal theta activity from a subject before (predrug) and after infusion of norepinephrine. Predrug column shows the theta occurring spontaneously (SPON) and in response to 0.5 and 1.0 mA DMPH stimulation. Column labelled Norepinephrine shows corresponding field activity following a 2.5 ul infusion of norepinephrine (1.0 ug/ul). Note the considerable drop in amplitude of the signal following norepinephrine infusion whereas frequency appears to be only slightly altered.

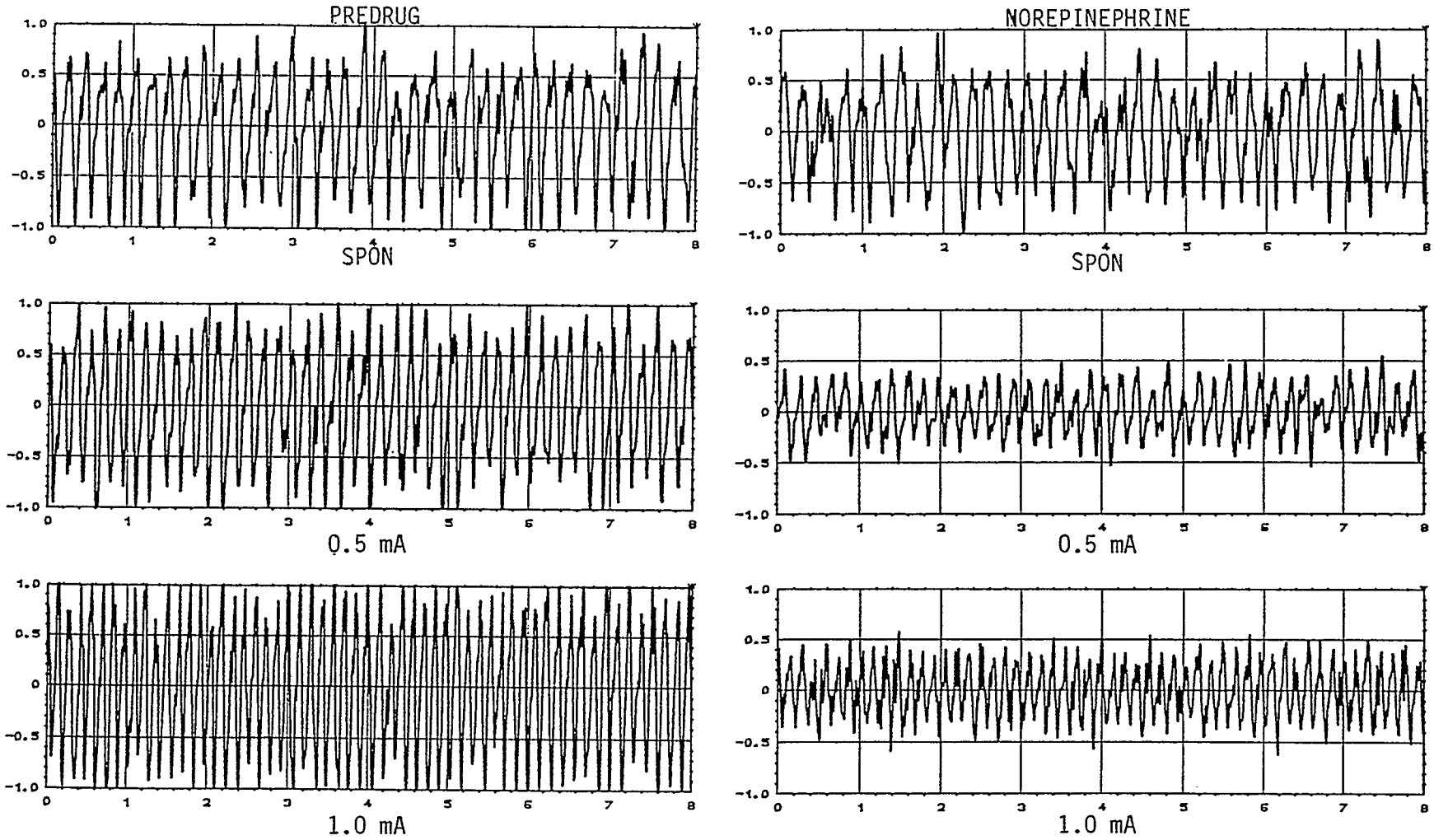
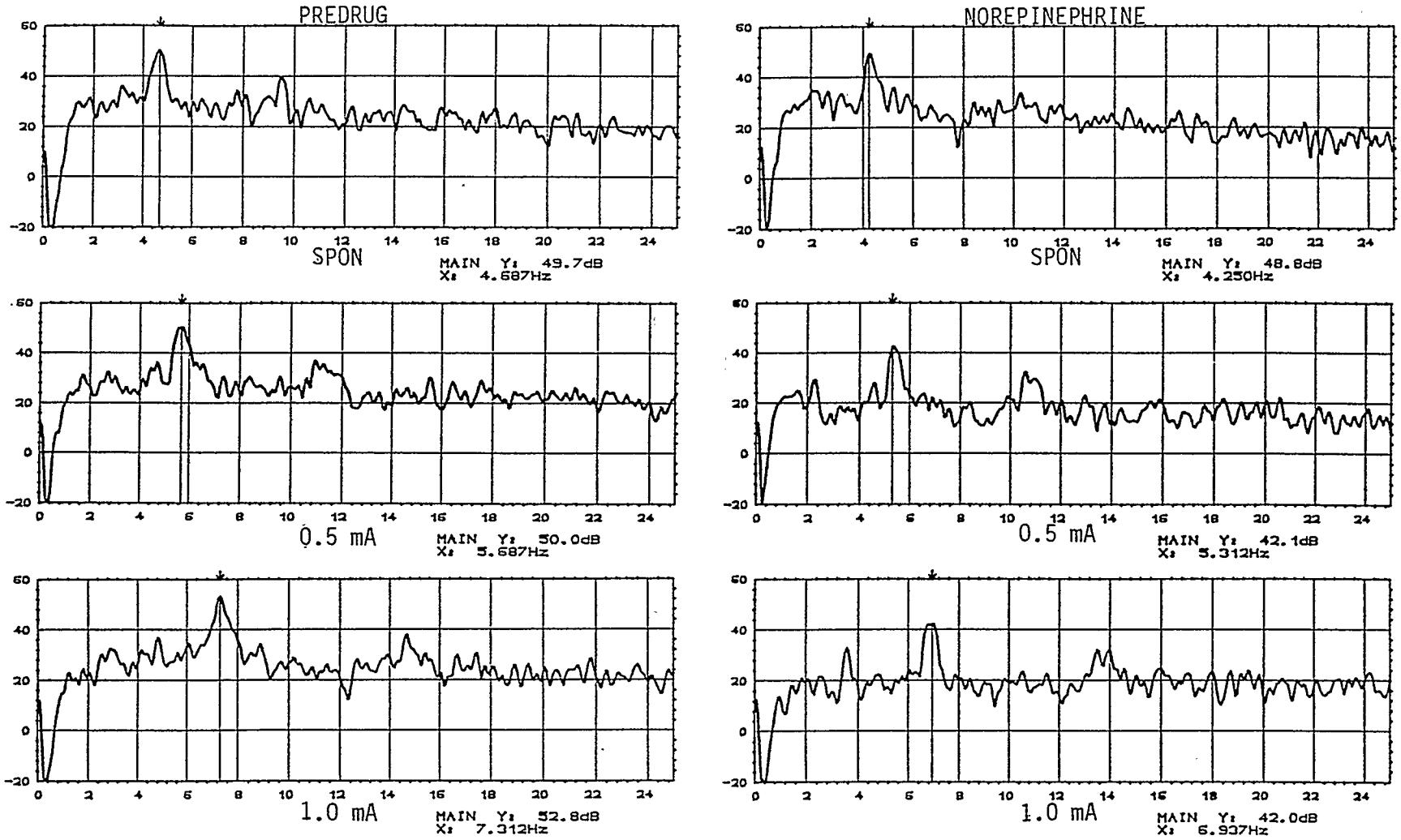

PREDRUG
NOREPINEPHRINE
SPON
SPON
0.5 mA
0.5 mA
1.0 mA
1.0 mA
1.0
0.5
0
-0.5
-1.0
0
1
2
3
4
5
6
7
8

Figure 5. Power Spectra Before and After Norepinephrine microinfusion

Corresponding power spectra for hippocampal field activity presented in figure 4. Measurements for power (dB) are located on the y axis, whereas frequency (1-25 Hz) is located on the x axis. Located at the bottom right hand corner of each spectrum are two measures: (1) main y: provides the power of the largest peak in the window; this peak is found underneath the arrow at the top of the window. (2) X: corresponds to the frequency of the main power peak. Note the increase in frequency of the main power peak as increasing intensities of DMPH stimulation are applied. Also note the drop in peak power (main y value) following infusion of norepinephrine.


PREDRUG
SPON
MAIN Y: 49.7dB
X: 4.687Hz
0.5 mA
MAIN Y: 50.0dB
X: 5.687Hz
1.0 mA
MAIN Y: 52.8dB
X: 7.312Hz
NOREPINEPHRINE
SPON
MAIN Y: 48.8dB
X: 4.250Hz
0.5 mA
MAIN Y: 42.1dB
X: 5.312Hz
1.0 mA
MAIN Y: 42.0dB
X: 6.937Hz

DISCUSSION

Histological analyses of dye spreads produced by microinfusion of 2.5 ul of Evan's blue dye demonstrated that the dye was confined to the hippocampus of infusion, concentrated primarily in the dentate gyrus region. Therefore the same volume of drug was used in the experiment to follow.

Microinfusions of NE were found to result in a decrease in theta amplitude on the polygraph chart record, as well as a decrease in power of the theta peak as ascertained by spectral analysis. Leung (1985) has demonstrated a similar decrease in theta power following atropine sulphate administration in control and eserine-treated rats. The present study also demonstrated a decrease in power of frequencies between 20-25 Hz (noise), which likely accounts for NE not having a significant effect on signal to noise ratio, as both signal and noise covaried (decreased) together. Unlike microinfusions of atropine sulphate, which have been found to totally eliminate type 2 theta (Rowntree & Bland, 1985), NE microinfusion only attenuated the amplitude of spontaneous and stimulation induced type 2 theta. Rowntree and Bland's (1985) findings are strong evidence that type 2 theta is cholinergically mediated, whereas the present results suggest that NE may play a role in modulating this cholinergic activity. Prior research supports the contention that NE modulates ACH activity. Vizi (1980b) demonstrated that chemical (6OHDA lesions) and electrical (LC lesions) destruction of the noradrenergic innervation of the cerebral cortex resulted in an increase of ACH release. Similarly, enhancement of the NE system, by electrical stimulation of

the LC, resulted in a decrease of cortical ACH release. It is possible that microinfusions of NE into the hippocampus inhibit the production of cholinergically mediated type 2 theta by decreasing the amount of ACH release.

Increasing intensities of DMPH stimulation were found to result in a significant increase in theta frequency. This finding is consistent with Christie (1989), who showed a linear increase in theta frequency with increasing intensities of DMPH stimulation. The present results also demonstrated that the power of the theta peak increased with DMPH stimulation, although no significant difference between the power during 0.5 mA and 1.0 mA DMPH stimulation was found. It would appear that a plateau in peak power is reached with 0.5 mA DMPH stimulation. Increases in stimulation intensity above this value does not appear to significantly alter the power of the theta peak, although the frequency of theta continues to increase. Along similar lines, Leung (1984) has shown that the power of theta increased with increasing frequencies of theta in the freely moving rat, although Leung did not observe an asymptote where power no longer increased with theta frequency. Not finding a drug by stimulation interaction suggests that the DMPH exerts its effects on theta power and frequency irrespective of the presence of NE. This in turn suggests that DMPH stimulation is very effective in producing hippocampal type 2 theta, and that NE microinfusion does not totally disrupt this cholinergic system.

The purpose of experiment II was to determine which adrenergic receptor(s) are mediating the inhibitory effects produced by NE. The

four subtypes of adrenoceptor, defined pharmacologically as $alpha_1$, $alpha_2$, $beta_1$, and $beta_2$, are known to be present in the hippocampal formation (for review see Storm-Mathisen and Ottersen, 1984). Therefore, specific receptor agonists will be used in hopes of mimicking the effects of NE.

EXPERIMENT II

The purpose of experiment II was to determine which adrenergic receptor(s) mediate the inhibitory effects produced by NE microinfusion as seen in experiment I. Therefore microinfusions of specific noradrenergic agonists were performed using different concentrations of the agonists to determine dose-response curves. Upon finding the receptor(s) mediating inhibition, attempts were made to block the effect with receptor antagonists. This allowed for the determination of whether effects were receptor specific or the result of non-specific drug actions.

In experiment I, it was demonstrated that DMPH stimulation has a significant effect on theta frequency and power of the peak theta frequency. This was shown to occur irrespective of whether NE was infused into the hippocampus, which suggests that the DMPH stimulation has a very powerful effect on hippocampal type 2 theta activity. In this experiment, the effects of DMPH stimulation were tested by pooling the data from all subjects prior to drug infusion, and performing a repeated measures ANOVA. This allowed for a more sensitive analysis of the effects of stimulation on the four dependent measures.

METHOD

Sujects and Surgical Procedure

Fifty-five rats were used in this experiment; all animals underwent the same surgical procedure as described in experiment I. Recording, stimulation, and infusion apparatus were the same as in experiment I.

Drugs

In order to determine the noradrenergic receptor(s) mediating the effects obtained with NE in experiment I, the following drugs were tested: isoproterenol (beta agonist); phenylephrine ($alpha_1$ agonist); detomidine ($alpha_2$ agonist); and tolazoline ($alpha_2$ antagonist). Phenylephrine, isoproterenol, and tolazoline were all obtained from Sigma Chemical Company. Detomidine was obtained from Norden Laboratories. All drugs were dissolved in a 0.9 percent physiological saline solution and pH balanced between 6.8 and 7.2 by adding sodium hydroxide to the solution. Drugs were prepared fresh prior to each experiment.

Procedure

In this experiment, three different concentrations of noradrenergic agonists were studied in order to determine if varying drug concentration had differential effects on hippocampal theta activity. The three different concentrations used were: 0.1 ug/ul, 1.0 ug/ul, and 10.0 ug/ul. Groups of five animals were tested at each

concentration for each drug used (total n = 45). One group of five animals received a 2.5 ul microinfusion of tolazoline (1.0 ug/ul), and five more animals received a 2.5 ul microinfusion of tolazoline (1.0 ug/ul) followed by a 2.5 ul microinfusion of detomidine (1.0 ug/ul) into the same site. Following completion of experimentation each animal underwent the same histological procedure used in experiment I.

RESULTS

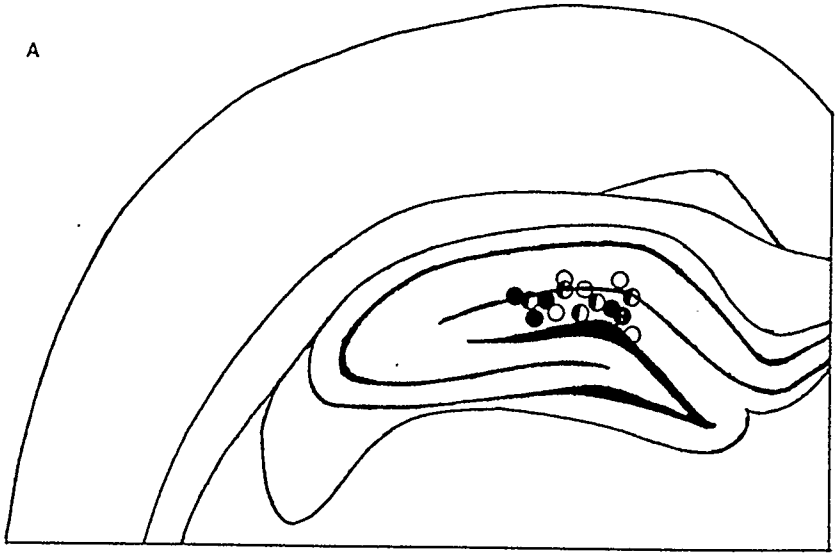
Histology

Histological analysis revealed that all recording and stimulation electrodes were positioned in the stratum moleculare layer and DMPH respectively. Histology also verified that cannulae for isoproterenol, phenylephrine, and detomidine microinfusions were situated in or bordering the stratum moleculare region of the dentate gyrus. Figure 6 provides schematic diagrams of the hippocampus and the infusion sites for all three drugs. Figure 6C shows the infusion sites for detomidine in the dentate gyrus region, and for three control infusions placed in the neocortex overlying the hippocampus. Microinfusion sites for tolazoline and tolazoline-detomidine combinations are presented in Figure 7. Cannulae placements in these instances were also found to be situated in, or proximal to the moleculare layer of the denate gyrus. Three control microinfusions of tolazoline are shown as being situated in the neocortex above the hippocampus. Neocortical control infusions for both detomidine (figure 6C) and tolazoline (figure 7) did not affect hippocampal field activity.

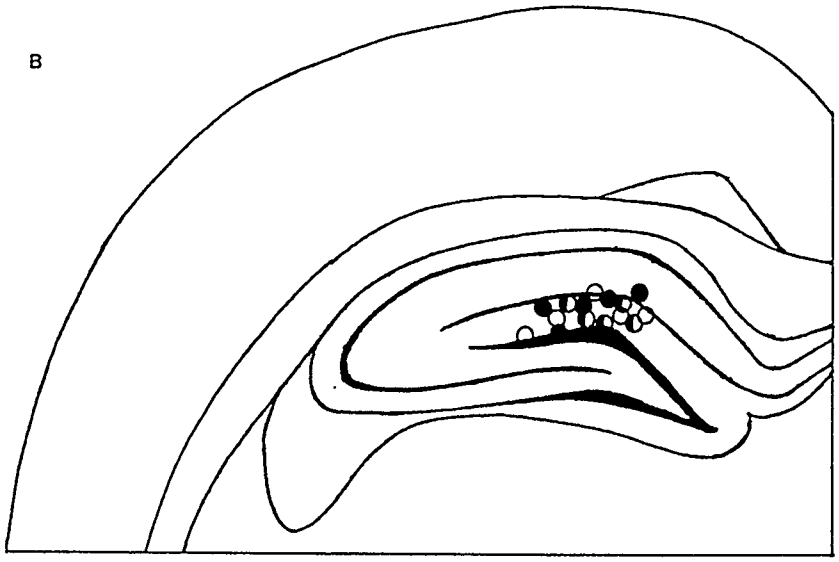
Figure 6. Infusion Sites for Specific Noradrenergic Agonists

Presented in this figure are the histologically verified infusion sites for isoproterenol (A), phenylephrine (B), and detomidine (C). Open circles denote infusion sites receiving a concentration of 0.1 ug/ul. Half-filled circles correspond to sites receiving concentrations of 1.0 ug/ul. Filled circles correspond to sites receiving infusions with a concentration of 10.0 ug/ul. Note that all three drugs had infusion sites which were in or near the stratum moleculare layer of the dentate gyrus. Infusions in this area with isoproterenol (A) and phenylephrine (B) had no effect on hippocampal field activity. All infusion sites of detomidine (C), regardless of concentration, affected hippocampal field activity. The three control infusions of detomidine (C) in the neocortex above the hippocampus had no effect.

A



B



C

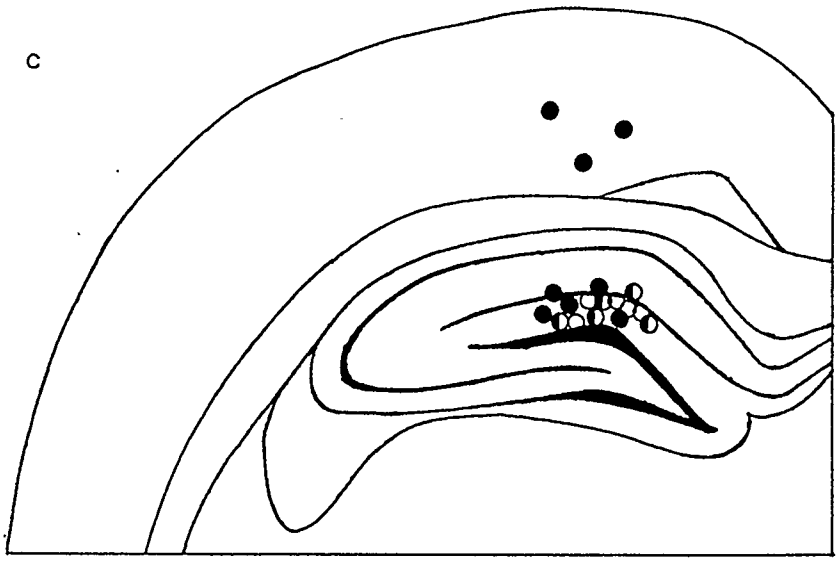
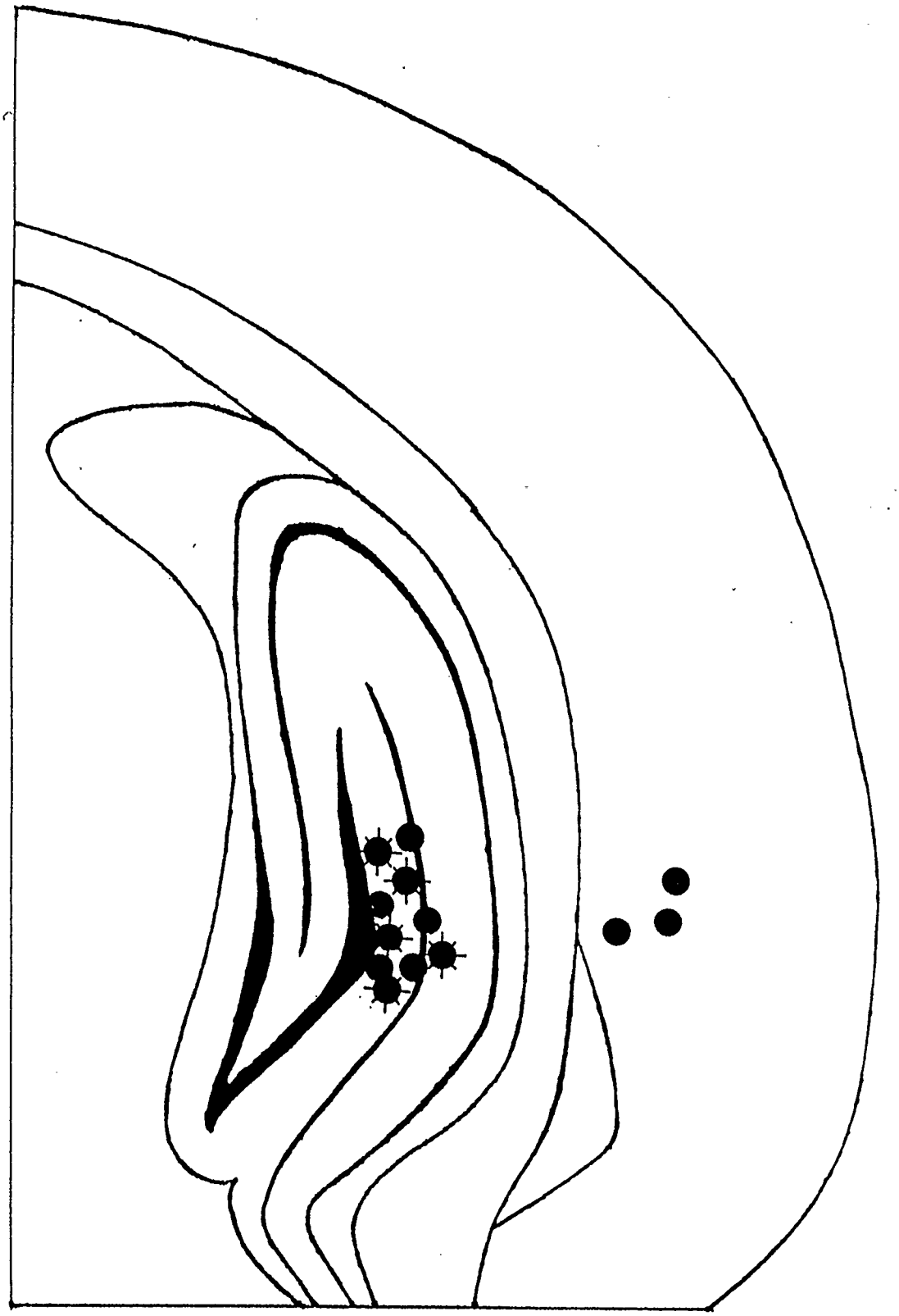


Figure 7. Infusion Sites for Tolazoline and Tolazoline - Detomidine Combinations

Histologically verified microinfusion sites for tolazoline (1.0 ug/ul) and detomidine (1.0 ug/ul). Circles correspond to sites receiving tolazoline alone. Circles with line extensions correspond to sites receiving microinfusions of tolazoline followed by detomidine. Circles in neocortex overlying hippocampus are sites where control tolazoline infusions took place, none of which affected hippocampal theta activity.



Stimulation and Theta Activity

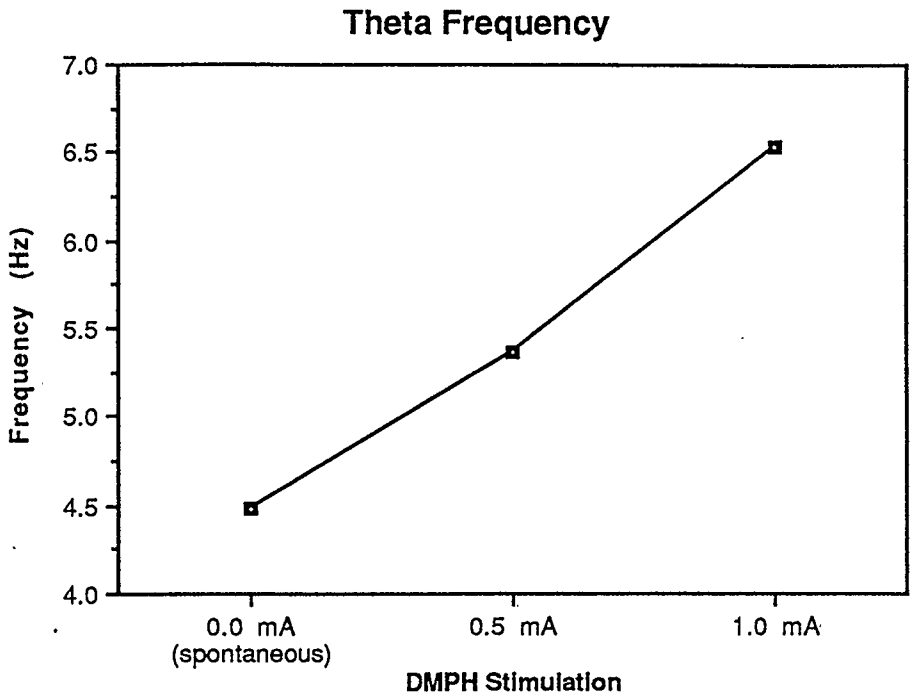
The data from all animals (n = 55) in this experiment were pooled and a repeated measures ANOVA was performed to more closely determine the effects of DMPH stimulation on the spectral characteristics of theta activity. Only data obtained prior to drug infusions were used in the analyses. Stimulation of the DMPH was found to have a significant effect on peak theta frequency (F (2,108) = 234.00, p < .01). The post-hoc analyses demonstrated that the frequency of spontaneously occurring theta (m = 4.48 Hz, SD = 0.51), and in response to 0.5 mA (m = 5.36 Hz, SD = 0.60) and 1.0 mA (m = 6.53 Hz, SD = 0.88) DMPH stimulation, all varied significantly from one another. A significant effect of DMPH stimulation on the power of the peak theta frequency was also demonstrated (F (2,108) = 52.21, p < .01). The power of the theta peak during 0.5 mA (m = 48.33 dB, SD = 2.96) and 1.0 mA (M = 48.52 dB, SD = 2.40) DMPH stimulation was found to be significantly greater than the power found during spontaneously occurring theta (M = 44.61 dB, SD = 3.18). No other significant results were found in the analyses (Appendix B contains source tables for the analysis). A graphic representaion of these results is presented in figure 8. Apparent in this figure is that theta frequency continues to increase with increasing intensities of DMPH stimulation, whereas the power of the peak theta frequency tends to level off at approximately 48.0 dB. Based on the overall analysis of the effects of DMPH stimulation on the spectral characteristics of hippocampal field activity, it can be assumed that any subsequent deviations by the groups to follow are the result of random

variability, unless an interaction is present. Therefore, only instances where stimulation is found to occur within an interaction will be presented.

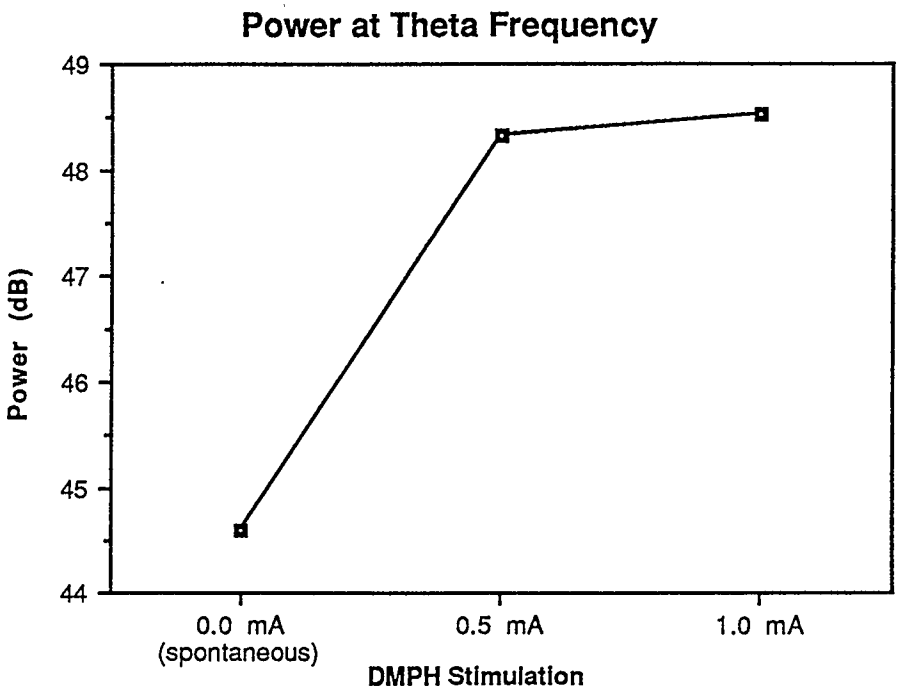
Figure 8. Frequency and Peak Frequency Power During DMPH Stimulation

Figure 8A shows the relationship between theta frequency and levels of DMPH stimulation. Notice the increase in theta frequency as higher intensities of DMPH stimulation were used. Figure 8B depicts how the power of the theta peak increases as one goes from spontaneous activity to 0.5 mA and 1.0 mA stimulation induced theta activity. Notice how the power of the theta peak appears to reach asymptote at 0.5 mA. Data for this figure were obtained by pooling results from all 55 animals participating in experiment II.

A


Theta Frequency
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Frequency (Hz)
0.0 mA
(spontaneous)
0.5 mA
1.0 mA
DMPH Stimulation

B


Power at Theta Frequency
49
48
47
46
45
44
Power (dB)
0.0 mA
(spontaneous)
0.5 mA
1.0 mA
DMPH Stimulation

Microinfusions of Noradrenergic Agonists

All microinfusions of the three agonists used in this experiment were limited to a volume of 2.5 ul. Isoproterenol (beta agonist) and phenylephrine ($alpha_1$ agonist) infusions, regardless of dose, did not result in any noticeable changes in theta activity as seen in the polygraph records. Conversely, all microinfusions of detomidine ($alpha_2$ agonist) irrespective of dose, resulted in a noticeable reduction in theta amplitude as visualized in the polygraph chart records. No instances were found where spontaneous or stimulation induced theta was totally abolished following detomidine microinfusion. Alterations in hippocampal theta activity following detomidine microinfusions persisted for several hours, and did not return to baseline values during the experimental period. Figure 9 provides eight second samples of hippocampal theta activity from a subject before and after a 2.5 ul microinfusion of detomidine (1.0 ug/ul). The reduction in signal amplitude following detomidine is quite apparent, and mimics the effects found in experiment I following infusions of NE.

Spectral analyses performed on the theta activity obtained from the isoproterenol and phenylephrine groups did not show any obvious variation. ANOVA with repeated measures statistically confirmed this observation as no significant changes in the four dependant variables of interest were obtained. Conversely, a repeated measures ANOVA performed on the detomidine group demonstrated that drug had a significant effect on the power of the peak theta frequency. (F (1,12) = 15.98, $p < .01$). The mean power of the peak theta frequency prior

to microinfusion was 48.35 dB (SD = 2.34), while following infusion it dropped to 46.60 dB (SD = 2.74). The drop in power of the peak theta frequency is reflected in the decrease in amplitude of the theta activity presented in figure 9. Detomidine microinfusions were also found to have a significant effect on noise level (F (1,12) = 16.99, $p < .01$). Prior to microinfusion the mean power of noise was 18.60 dB (SD = 2.48), while following infusion the power dropped to 17.12 dB (SD = 2.73). These effects appear to be the result of drug actions occurring within the hippocampus, as control infusions of saline into the hippocampus, and detomidine infusions into the neocortex above the hippocampus did not alter theta activity. No other main effects or interactions involving detomidine were obtained (refer to Appendix B for source tables). Figure 10 provides the power spectra for a subject receiving a 2.5 ul microinfusion of detomidine (1.0 ug/ul). Power of the peak theta frequency (main y value) and noise level show a decrease following detomidine, whereas frequency of theta remains relatively unaltered. An animal receiving a microinfusion of detomidine with a concentration of 1.0 ug/ul was chosen for this figure as the obtained values closely approximate the means obtained for the group.

Figure 9. Hippocampal Theta Activity Before and After Detomidine Microinfusion

Representative 8 second samples of hippocampal theta activity from one subject before (predrug) and after 2.5 ul microinfusion of detomidine (1.0 ug/ul). Note the obvious decrease in amplitude of field activity following detomidine, whereas frequency appears unaltered.

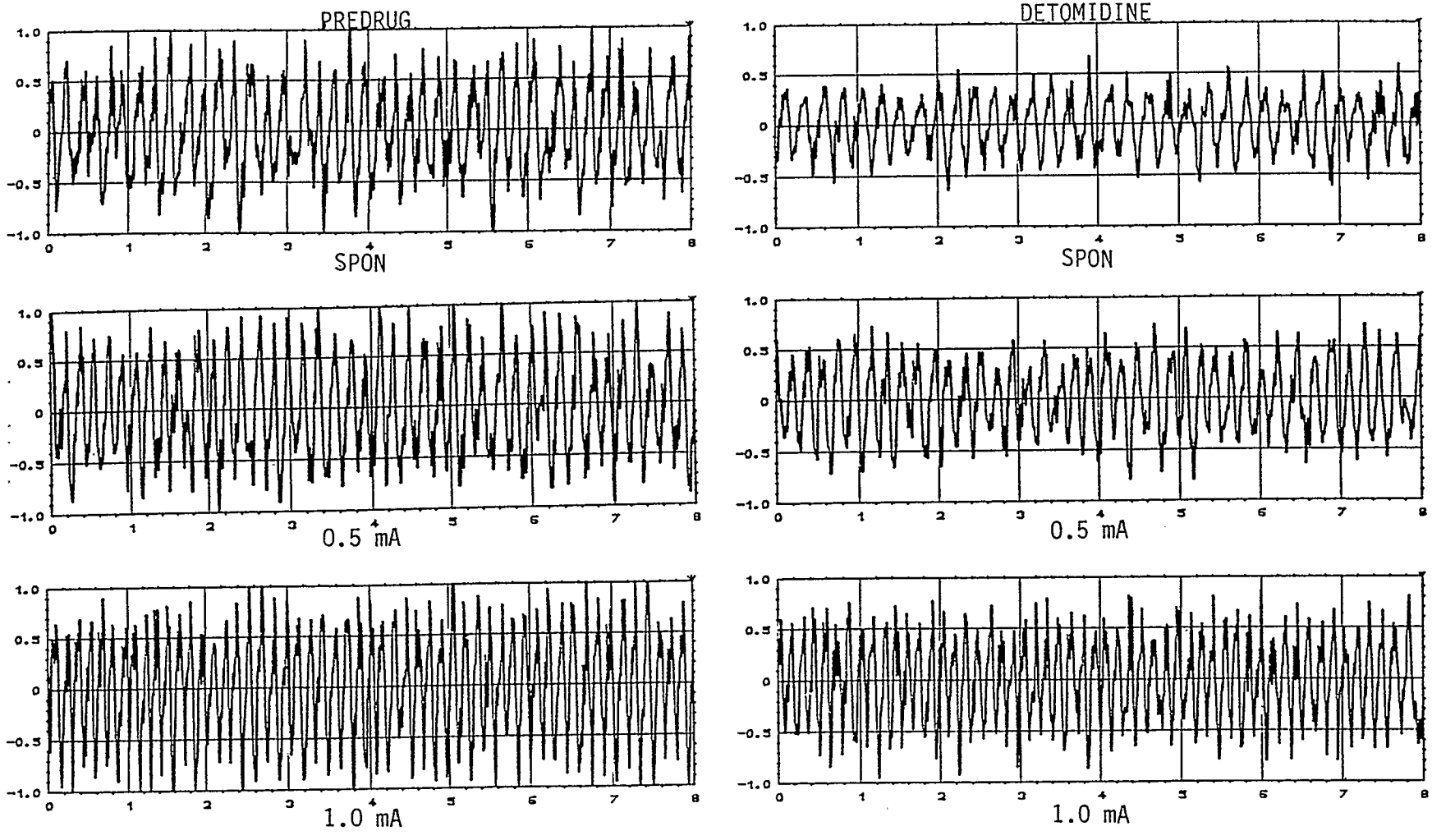
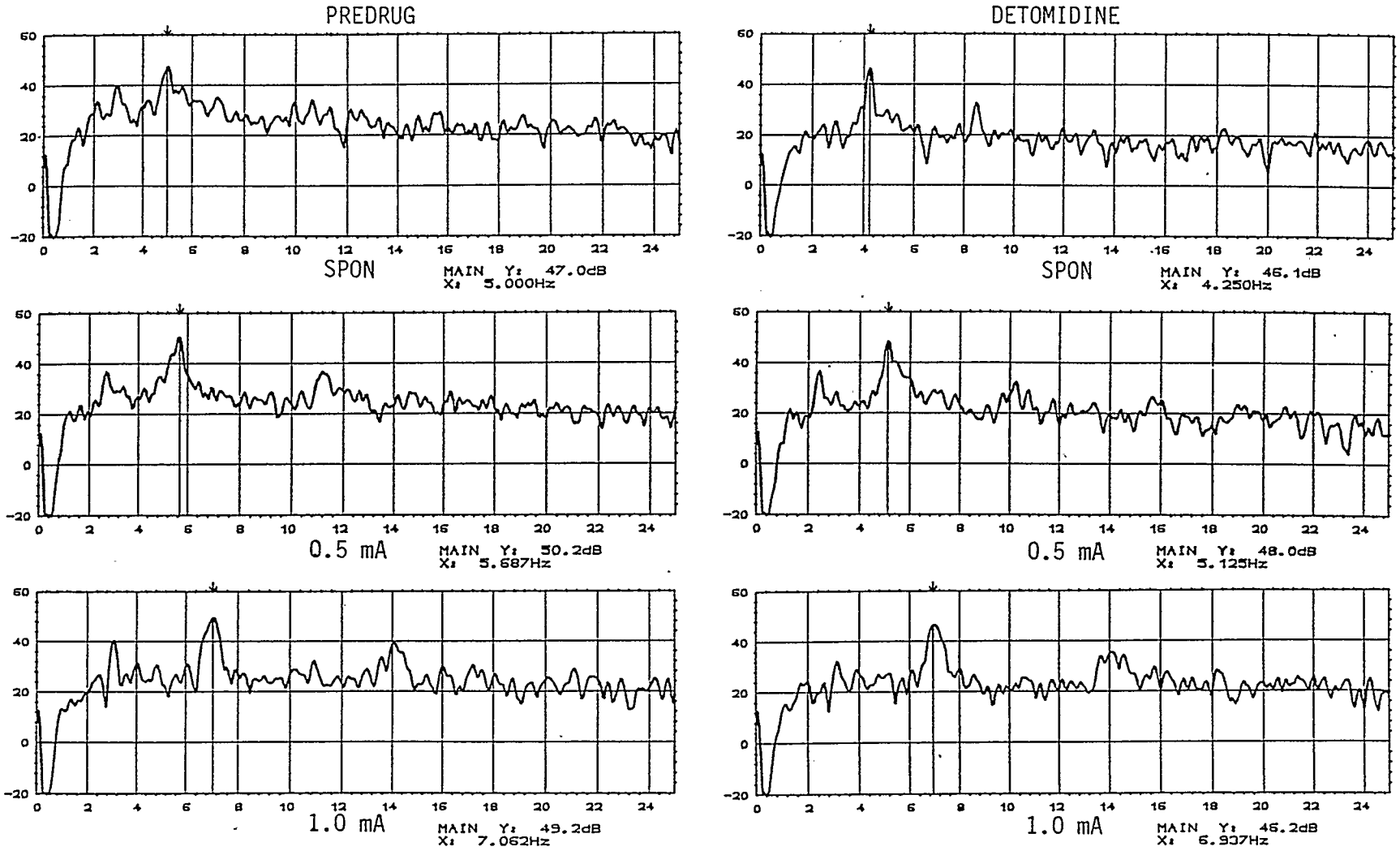

PREDRUG
DETOMIDINE
SPON
SPON
0.5 mA
0.5 mA
1.0 mA
1.0 mA
1.0
0.5
0
-0.5
-1.0
0
1
2
3
4
5
6
7
8

Figure 10. Power Spectra Before and After Detomidine Microinfusion

Power spectra corresponding to hippocampal theta activity presented in figure 8. Notice that following microinfusion of 2.5 ul of detomidine (1.0 ug/ul) that the power of the theta peak (main y) decreases. Also notice how the power in the spectra between 20-25 Hz appears to decrease after detomidine. Noise values were obtained from this area.


PREDRUG
DETOMIDINE
SPON
MAIN Y: 47.0dB
X: 5.000Hz
SPON
MAIN Y: 46.1dB
X: 4.250Hz
0.5 mA
MAIN Y: 50.2dB
X: 5.687Hz
0.5 mA
MAIN Y: 48.0dB
X: 5.125Hz
1.0 mA
MAIN Y: 49.2dB
X: 7.062Hz
1.0 mA
MAIN Y: 46.2dB
X: 6.937Hz

Microinfusions of Tolazoline and Tolazoline-Detomidine Combinations

Tolazoline (1.0 ug/ul) microinfusions were performed on five animals to determine if $alpha_2$ receptor blockade had any effects on hippocampal theta activity. Observations of polygraph chart records failed to find any noticeable change in the amplitude or frequency of the hippocampal signal. Subsequent spectral and statistical analyses substantiated these observations, as no significant effects of the drug were obtained. Figure 11 provides examples of hippocampal theta activity before and after a 2.5 ug/ul microinfusion of tolazoline (1.0 ug/ul) into the hippocampus of one animal. The similarities between theta activity occurring before and after microinfusion is reflected in the corresponding power spectra presented in figure 12.

Although not quantified, an observation made during these experiments merits comment at this point. Three of the five animals receiving microinfusions of tolazoline exhibited LIA immediately before and immediately after DMPH stimulation during predrug recordings. This is a common occurrence for animals deeply anesthetized with urethane. Following microinfusions of tolazoline, LIA activity was still present prior to DMPH stimulation; however after stimulation LIA no longer occurred, but was replaced by theta activity which persisted throughout the remainder of the experiment. Stimulation of the DMPH still proved effective in altering the frequency of theta activity, but the occurrance of LIA

Figure 11. Hippocampal Theta Activity Before and After Tolazoline Microinfusion

Representative 8 second samples of hippocampal theta activity before and after a 2.5 ul infusion of the $alpha_2$ blocker, tolazoline (1.0 ug/ul). Note that spontaneous hippocampal theta activity, and theta activity produced by 0.5 mA and 1.0 mA DMPH stimulation appears unaltered following tolazoline microinfusions.

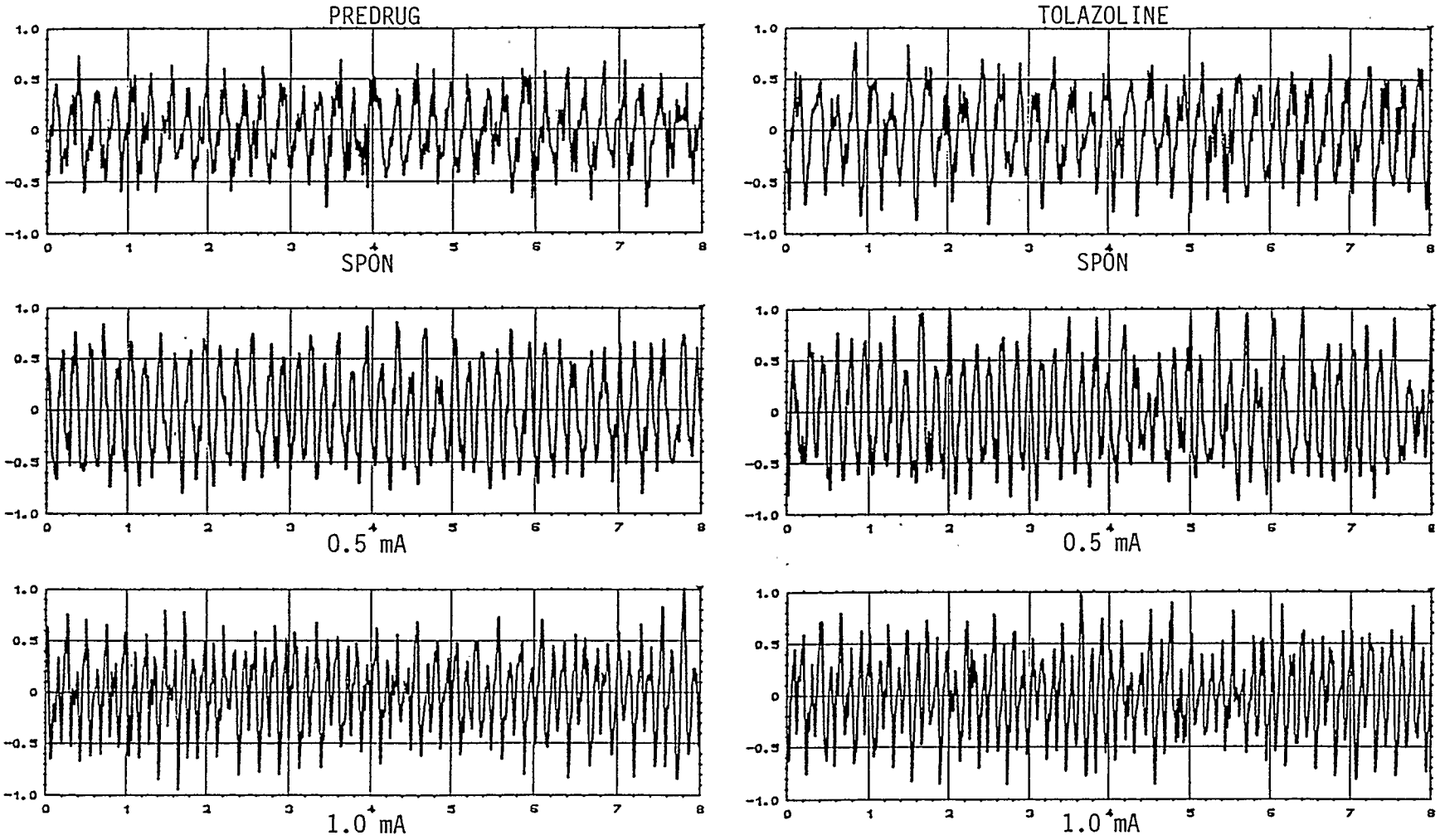
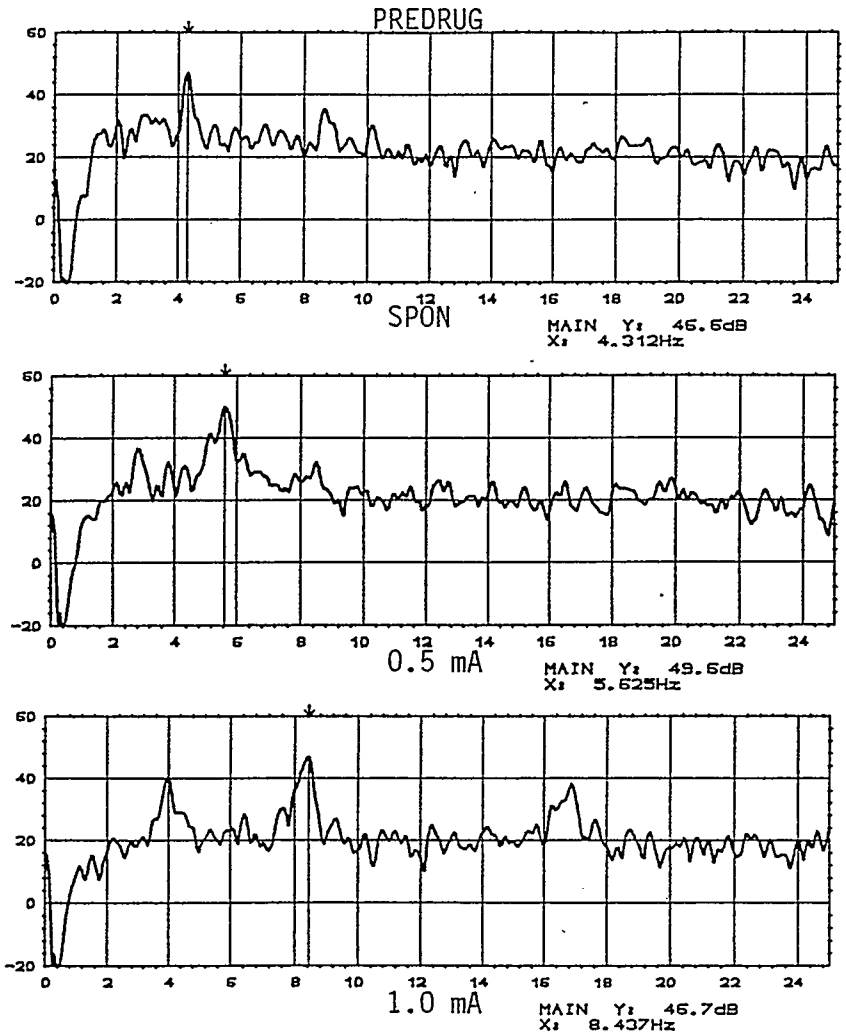
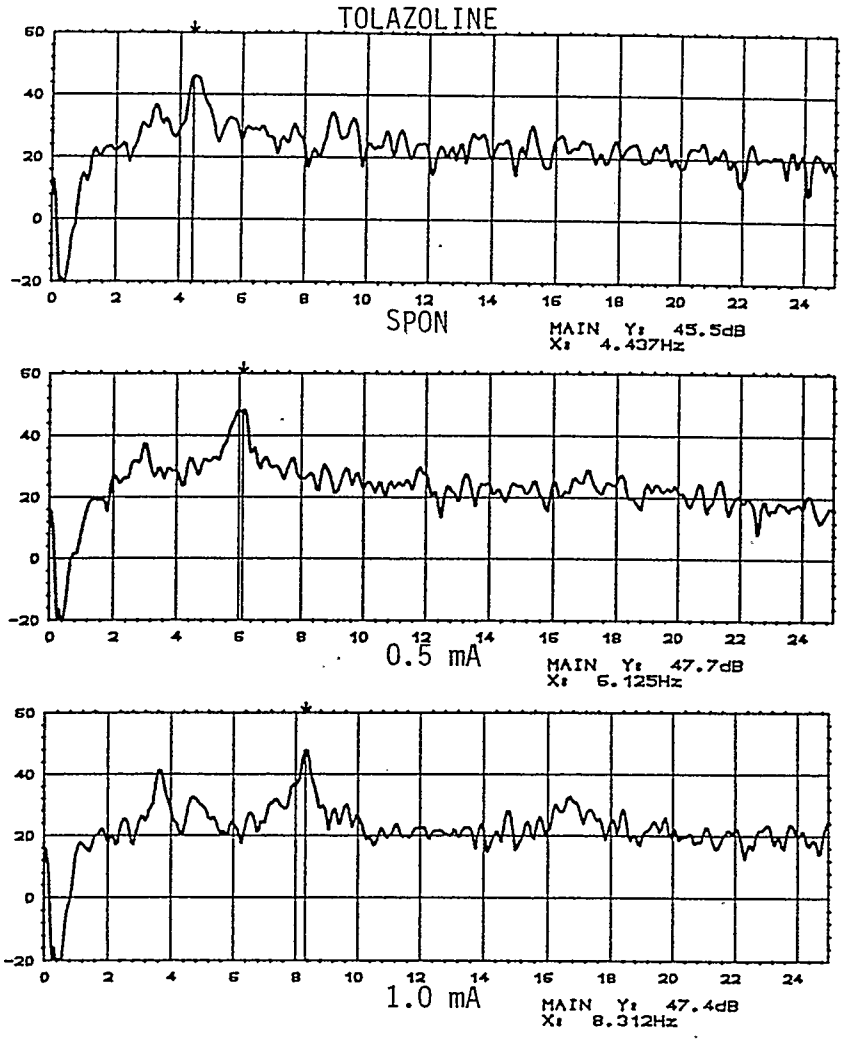

PREDRUG
TOLAZOLINE
SPON
SPON
0.5 mA
0.5 mA
1.0 mA
1.0 mA
1.0
0.5
0
-0.5
-1.0
0
1
2
3
4
5
6
7
8

Figure 12. Power Spectra Before and After Tolazoline Microinfusion

Power spectra corresponding to the samples of hippocampal field activity presented in figure 11. Notice that the power spectra before and after microinfusions of tolazoline (1.0 ug/ul) appear similar. Also notice the appearance of a large power peak in the 2-4 Hz range in a number of the spectra; this was a common occurrence across animals.


PREDRUG
SPON
MAIN Y: 46.6dB
X: 4.312Hz
0.5 mA
MAIN Y: 49.6dB
X: 5.625Hz
1.0 mA
MAIN Y: 46.7dB
X: 8.437Hz


TOLAZOLINE
SPON
MAIN Y: 45.5dB
X: 4.437Hz
0.5 mA
MAIN Y: 47.7dB
X: 6.125Hz
1.0 mA
MAIN Y: 47.4dB
X: 8.312Hz

before and after stimulation was no longer present. Figure 13 provides eight second samples of hippocampal field activity prior, during, and after 0.5 mA DMPH stimulation for one subject. Notice that following tolazoline microinfusion theta activity is present after DMPH stimulation whereas LIA is present in the predrug state.

Statistical analyses of hippocampal theta activity for the group receiving 2.5 ul microinfusions of tolazoline (1.0 ug/ul) followed by a 2.5 ul infusion of detomidine (1.0 ug/ul) proved none significant. Figures 14 and 15 present the hippocampal theta activity and spectral analyses for an animal receiving the above manipulations. As seen in figure 14, the field activity following tolazoline infusion does not appear to change after subsequent infusion of detomidine. Power spectra for the corresponding theta activity corroborate this observation.

Figure 13. Hippocampal Field Activity in Response to 0.5 mA Stimulation Before and After Tolazoline

Eight second samples of hippocampal field activity immediately before (A), during (B), and immediately after (C) 0.5 mA DMPH stimulation. Notice that before tolazoline microinfusions (predrug) LIA precedes and follows DMPH stimulation. After microinfusion of tolazoline (1.0 ug/ul) DMPH stimulation is followed by theta activity. Theta activity persisted for several seconds following stimulation in this instance. Three of the five animals receiving tolazoline microinfusions exhibited this effect. Calibration: 1.0 sec., 500 mv.

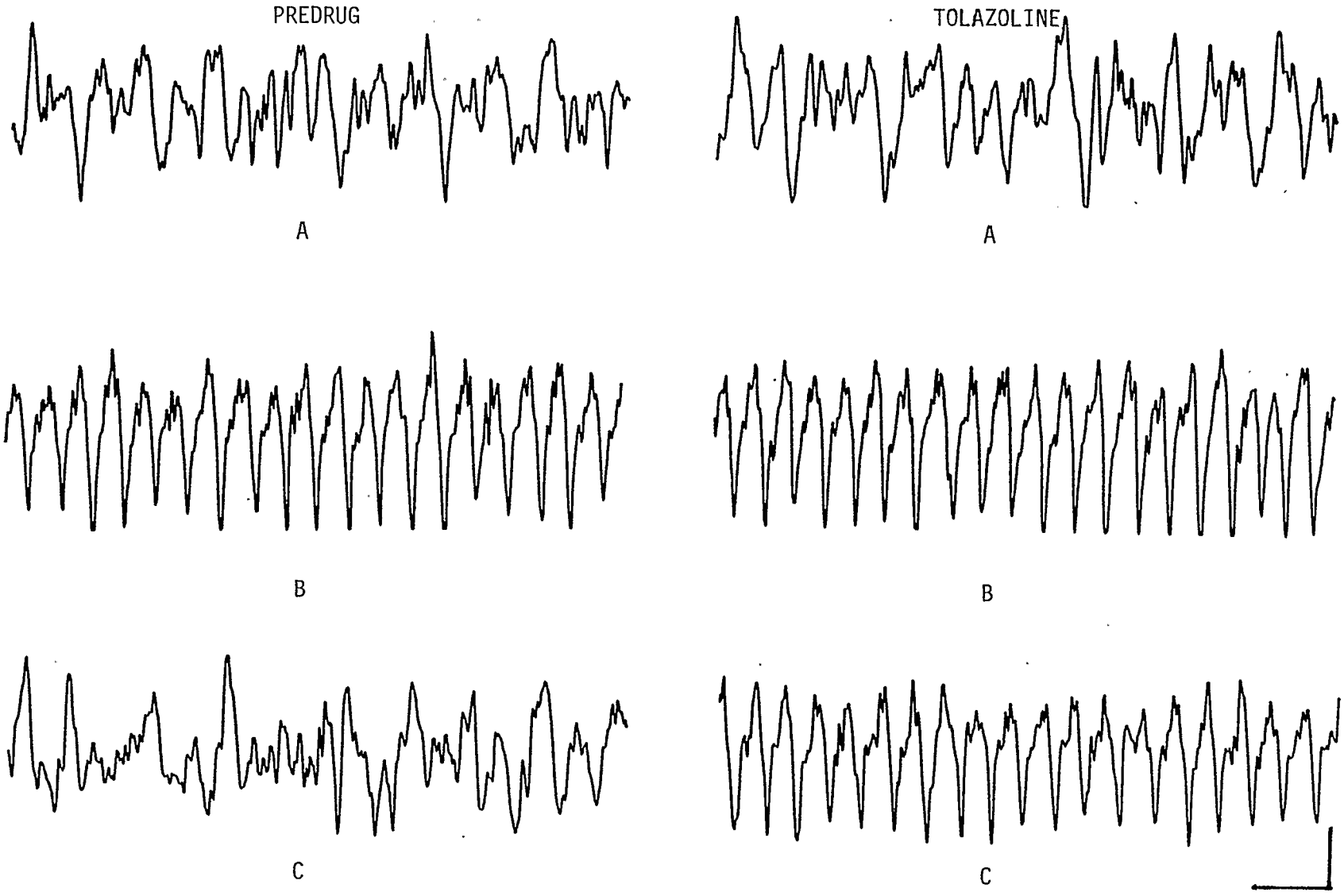

PREDRUG
TOLAZOLINE
A
A
B
B
C
C

Figure 14. Hippocampal Theta Activity For Microinfusions of Tolazoline Followed by Detomidine

Eight second samples of hippocampal theta activity obtained from one subject following tolazoline (1.0 ug/ul), and subsequent microinfusion of detomidine (1.0 ug/ul) into the same site. Notice that the theta activity following tolazoline alone and following a combination of tolazoline-detomidine are similar in both instances. Tolazoline microinfusion blocked the inhibitory effects of detomidine microinfusion.

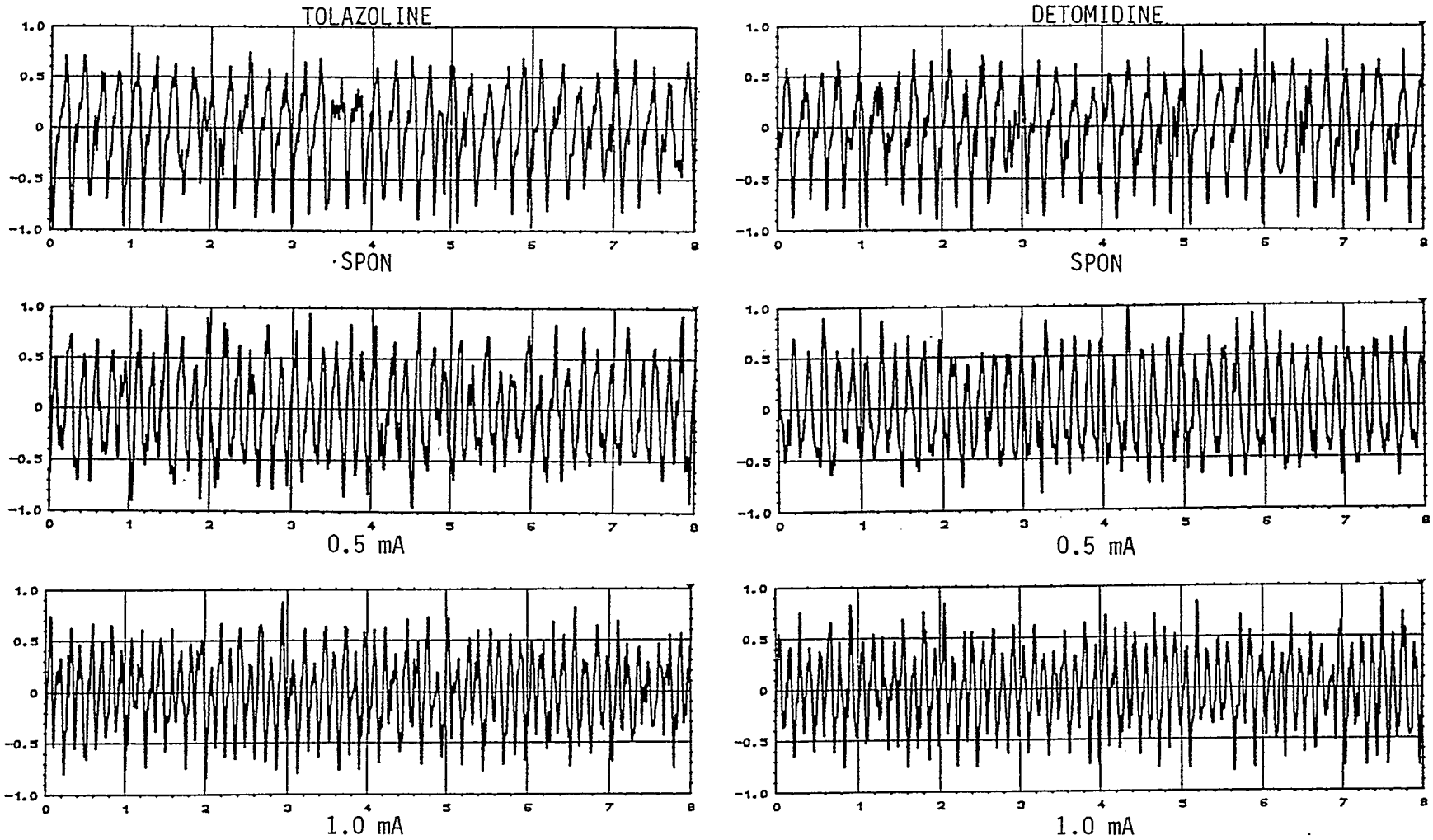
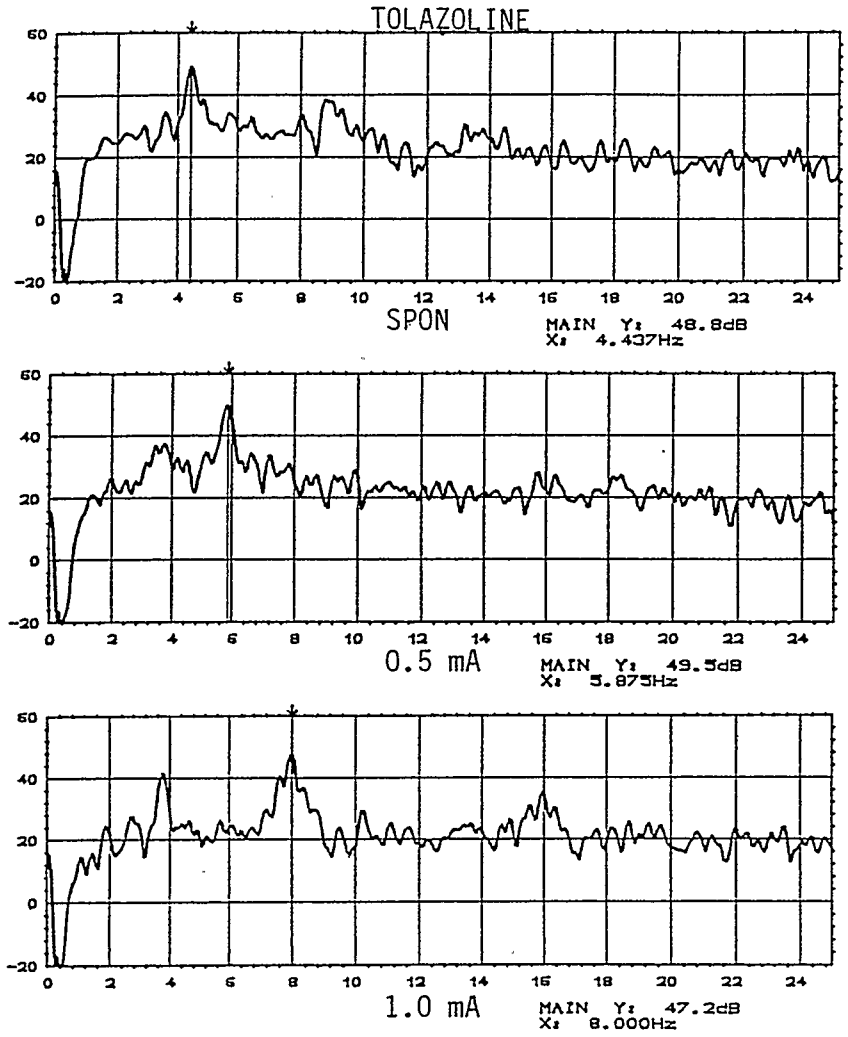
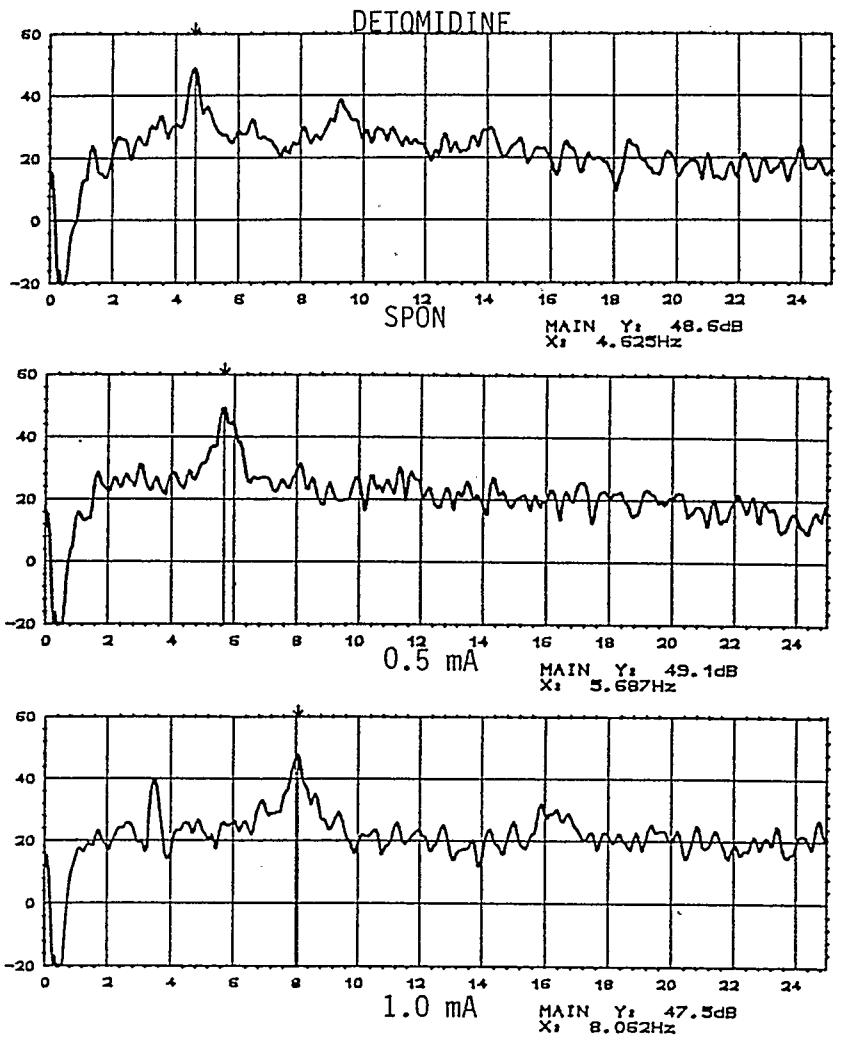

TOLAZOLINE
DETOMIDINE
SPON
SPON
0.5 mA
0.5 mA
1.0 mA
1.0 mA
1.0
0.5
0
-0.5
-1.0
0
1
2
3
4
5
6
7
8

Figure 15. Power Spectra for Tolazoline Followed by Detomidine Microinfusion

Power spectra corresponding to hippocampal field activity presented in figure 14. This figure shows the power spectra following tolazoline (1.0 ug/ul) microinfusion, and then following detomidine (1.0 ug/ul) microinfusion into the same site. Notice that the power spectra in both instances appear relatively equal, a finding confirmed by the main y and x values shown.


TOLAZOLINE
SPON
MAIN Y: 48.8dB
X: 4.437Hz
0.5 mA
MAIN Y: 49.5dB
X: 5.875Hz
1.0 mA
MAIN Y: 47.2dB
X: 8.000Hz


DETOMIDINE
SPON
MAIN Y: 48.6dB
X: 4.625Hz
0.5 mA
MAIN Y: 49.1dB
X: 5.687Hz
1.0 mA
MAIN Y: 47.5dB
X: 8.062Hz

DISCUSSION

As in experiment I, histological analyses proved that cannulae were confined to the dentate gyrus region. This, and the finding that control infusions into the neocortex above the hippocampus did not affect field activity, are evidence that drug effects were localized to the dentate region.

The analyses, testing for the effects of stimulation on hippocampal spectral characteristics, replicated the findings of experiment I. Dorso-medial posterior hypothalamus stimulation resulted in a significant increase in theta frequency; as higher intensities of stimulation were applied, theta frequency was found to increase. DMPH stimulation also resulted in an increase in power of the theta peak, although no significant difference in theta peak power was found when comparing 0.5 mA to 1.0 mA stimulation levels. There appears to be an upper limit in the power of the theta peak where, following attainment, increasing intensities of DMPH stimulation does not result in a substantial increase. Christie (1989) demonstrated that increasing intensities of DMPH stimulation resulted in marked increases of theta frequency, whereas amplitude (power) of stimulation induced theta did not vary in any noticeable fashion. The results of the present study are in agreement with Christie's (1989) findings. It appears as though the MSDB codes increasing activation of the DMPH with linear increases of frequency, whereas amplitude, once reaching its maximum, no longer varies with stimulation intensity. This frequency modulated response is then passed onto the hippocampus where increased theta frequencies are recorded in response to greater DMPH

activation of the MSDB. A more thorough examination of how DMPH stimulation intensity is related to the spectral characteristics of hippocampal field activity should be performed, varying intensity in much finer increments than done in the present experiments.

Once again, as in experiment I, no interactions involving stimulation were found, which suggests that the above effects of stimulation were still present despite pharmacological manipulations within the hippocampus. Finally, the analyses performed on stimulation did not show a significant main effect of stimulation on signal to noise ratio.

Microinfusions of the beta agonist isoproterenol, and the $alpha_1$ agonist phenylephrine, were found to have no effect on hippocampal theta activity. As experimentation was taking place, no changes in theta activity were observed on the polygraph chart records. Subsequent spectral and statistical analyses substantiated these observations. The lack of any noticeable effect by these agonists was somewhat unexpected given the number of studies which have found that administration of these drugs, especially in the case of isoproterenol, result in marked changes in hippocampal cell activity. The work by Segal and Bloom (see Segal 1980 for review) typically found that isoproterenol iontophoresed onto hippocampal cells resulted in a similar inhibition of spontaneous cell activity as seen during NE iontophoresis and LC stimulation. Moreover, all inhibitory responses produced by the above manipulations were blocked by the administration of beta receptor antagonists. Beta receptor agonists have also been found to excite hippocampal cells. Most pertinent to

this discussion are the findings by Pang and Rose (1987). Upon differentiating between complex spike and theta cells, these researchers found that theta neurons exhibited excitation following iontophoresis of NE, which was mediated by beta and $alpha_2$ receptors. It is possible that these cellular responses are occurring in the present study but are not affecting hippocampal theta activity in any observable manner. Combined field and single unit recordings during manipulations similar to those used in the present study may prove fruitful in determining how changes at the cellular level are reflected in hippocampal field activity.

Microinfusions of the $alpha_2$ agonist detomidine, resulted in an attenuation in power of the hippocampal theta peak, as well as noise level, whereas the frequency and signal to noise ratio remained unaffected. These results correspond well with the effects found for NE in experiment I. Moreover the inhibitory effects of detomidine appear to be receptor specific as infusion of the $alpha_2$ antagonist, tolazoline, blocked these effects. The attenuation of theta produced by detomidine occurred irrespective of dose which suggests that smaller concentrations of drug are needed to observe dose-response curves. Binding studies have shown detomidine to be a very specific and potent $alpha_2$ adrenoceptor agonist, having weak affinity for $alpha_1$ and beta receptors and able to produce a marked displacement of other $alpha_2$ ligands (Virtanen, Savola, Saano, and Nyman, 1988).

The fact that NE and detomidine both caused an inhibiton of type 2 theta activity rules out the possibility that this effect was mediated by presynaptic $alpha_2$ receptors. Stimulation of the

presynaptic $alpha_2$ receptor would have caused a decrease in the amount of NE available to bind postsynaptically; therefore infusions of NE should not have caused inhibition, since NE would also diffuse to postsynaptic sites and offset the loss of endogenously released NE. Further support for an inhibitory $alpha_2$ postsynaptic site is evident from the results of the tolazoline group. Tolazoline microinfusion would have likely resulted in an increase in the release of NE, due to presynaptic $alpha_2$ receptor blockade. Despite the probable pooling of NE in the synapse, hippocampal theta activity remained unaffected. This is likely due to the ability of tolazoline to bind postsynaptically and block any inhibitory effects endogenously released NE might have produced. Postsynaptic $alpha_2$ receptors have been shown to be present on cells intrinsic to the hippocampus, as well as on the terminal fields of neurons innervating the hippocampus (Madison and Nicoll, 1986; Segal, 1981; Vizi, 1983). The $alpha_2$ receptors located postsynaptically have been found to be unaffected by 6OHDA lesions which result in destruction of presynaptic $alpha_2$ receptors (U'Prichard, Bechtel, Rouot, and Snyder, 1979). The role of $alpha_2$ receptors located on the presynaptic endings of non-adrenergic neurons appears to be inhibitory in nature. Maura, Gemigenoni, and Raiteri (1982) demonstrated that 5HT fibers within the hippocampus have nerve terminals that are endowed with $alpha_2$ adrenoceptors, which modulate the release of 5HT. Stimulation of these receptors by $alpha_2$ agonistic drugs inhibits the release of 5HT. Relevant to the effects found in the present study is the finding that postsynaptic $alpha_2$ receptors are localized on the

synaptic terminals of cholinergic fibers innervating both the neocortex and hippocampus, stimulation of which results in a decrease of ACH release (see Vizi, 1983 for review). It is likely that the inhibitory effects produced by NE and detomidine were the result of postsynaptic activation of $alpha_2$ receptors, located on the synaptic endings of cholinergic fibers within the hippocampus. This may help to explain the results obtained for those animals receiving tolazoline microinfusions. During baseline recordings, LIA followed very shortly after DMPH stimulation. Following the first epoch of DMPH stimulation after tolazoline microinfusion, LIA no longer occurred, but was replaced by theta activity which persisted throughout the remainder of experimentation. Similarly, Vizi (1980) found that administration of an $alpha_2$ antagonist resulted in long lasting enhancement of stimulation induced ACH release from neocortex, whereas resting release of ACH remained unaltered. A similar process may be occurring in the present study, whereby stimulation induced release of ACH by DMPH stimulation is no longer modulated by endogenous NE due to $alpha_2$ receptor blockade at the ACH terminal.

GENERAL DISCUSSION

The results of the present experiments demonstrated that microinfusion of NE, likely acting via a postsynaptic $alpha_2$ receptor, has an inhibitory influence on the production of hippocampal type 2 theta activity. This inhibitory influence was reflected in a drop in theta peak power and noise level, whereas frequency was unaffected. It is apparent from the present and previous studies (see Bland, 1986 for review), that the the primary drive for type 2 theta is cholinergic, and that NE modulates this activity.

The hypothesis that NE modulates the principal neurotransmission between structures is not new. Numerous studies have demonstrated that NE can both facilitate and inhibit the principal neurotransmission already taking place within a defined system (Harley, 1989; Vizi, 1981, 1983). The anatomical makeup of the NE/LC system would appear to serve this purpose. The LC shows widespread innervation throughout the brain, making few classical synaptic contacts. Rather, LC fibers possess varicosities which allows for the liberation of NE into the extracellular medium anywhere along its axonic length. This in turn allows for the effects of NE to be far reaching, although relatively slow in onset. The actions of NE are typically found to be long and tonic, lasting several seconds, whereas other neurotransmitters such as ACH and glutamate, show relatively short, phasic actions which last on the order of milliseconds. Such long lasting activity exhibited by NE allows for an overall pacing of activity within a given system.

Within the hippocampal formation NE can be seen as modulating the interactions between this structure and areas such as the medial septum, entorhinal cortex, and various brain stem nuclei. Having not found any other adrenoceptor responses in the current study appeared puzzling given the evidence for the contrary (refer to introduction). It may be that NE exerts its modulatory role on the cholinergic activities between the MSDB/DMPH and hippocampus via $alpha_2$ receptors, whereas the glutaminergically mediated interaction between the entorhinal cortex and hippocampus may be modulated by NE in the form of beta receptor activities. Harley (1989) provides a review of the modulatory actions of NE in the development of long term potentation and population spike activity in the hippocampus, as induced by entorhinal/perforant path stimulation. In virtually all studies cited, the glutaminergic activity mediating these phenomena were found to be enhanced by beta adrenoceptor activity, whereas alpha receptor activity was of no consequence. It is possible that the LC/NE system interacts with the various hippocampal inputs, such as the median raphe (serotonin), entorhinal cortex (glutamate), and MSDB (acetylcholine), via different receptor subtypes. This may provide a partial explanation for why no other receptor involvement was exhibited in the present study.

Furthermore, testing the effects of NE microinfusion in the freely moving animal would allow for a more precise evaluation of the modulatory role of NE on theta activity, as atropine-resistant, (i.e. noncholinergic) type 1 theta is produced during this state. Flicker and Geyer (1982) have found marked behavioural changes in rats

receiving hippocampal microinfusions of NE, which were mediated by beta receptor activation.

Interestingly, comparisons can be made between activity in the hippocampus and that which occurs in the LC. Neurons within the LC have been shown to increase activity during arousal states, and in response to the presentation of sensory stimuli (Aston-Jones and Bloom, 1981b; Foote et al, 1980). Similar behavioural and sensory events have been correlated with the production of type 2 theta activity (Bland, 1986: Sainsbury, 1985). How these two structures interact during such events is currently unknown but warrants further study. The lesion studies done by Kolb and Whishaw (1977), Robinson et al., (1977), and Whishaw et al., (1978), were correct in concluding that the LC/NE system did not play a principal role in the production of theta activity. Careful observations made by these researchers, of the release of type 2 theta activity occurring following lesions, provided much of the impetus for the present work.

The contention that the inhibitory effects of detomidine are a result of inhibition of ACH release via activation of $alpha_2$ receptors is speculative. This is partially based upon personal observations; during similar microinfusion experiments low concentrations (< 5.0 ug/ul) of atropine sulphate resulted in an inhibition of type 2 theta. Amplitude (power) of the theta activity was considerably reduced, whereas spontaneous production and frequency of theta appeared unaltered. Partial disruption of the cholinergic system mediating type 2 theta, either by presynaptic inhibition (detomidine) or partial postsynaptic blockade (atropine sulphate), appear to

result in similar reductions in hippocampal theta activity. Further investigations will prove whether these observations are of significance.

Undoubtedly the interaction between the NE and ACH systems is very complex, requiring considerably more study. The hippocampal formation's well-defined electrophysiological activity and the behavioral correlates of this activity, provides a prime testing ground for further research on how these two systems interact with one another.

References

Alger, B. E., and Nicoll, R. A. (1982). Pharmacological evidence for two kinds of GABA receptors on rat hippocampal pyramidal cells studied in vitro. Journal of Physiology. 328, 125-141.

Alonso, A., and Garcia-Austt, E. (1987a). Neuronal sources of theta rhythm in the entorhinal cortex of the rat I. Laminar distribution of theta field potentials. Experimental Brain Research. 67, 493-501.

Alonso, A., and Garcia-Austt, E. (1987b). Neuronal sources of theta rhythm in the entorhinal cortex of the rat II. Phase relations between unit discharge and theta field potentials. Experimental Brain Research. 67, 502-509.

Amaral, D. G., and Kurz, J. (1985). An analysis of the origins of the cholinergic and non-cholinergic septal projections to the hippocampal formation of the rat. The Journal of Comparative Neurology. 240, 37-59.

Amaral, D. G., and Sinnamon, H. M. (1977). The locus coeruleus: Neurobiology of a central noradrenergic nucleus. Progress in Neurobiology. 99, 147-196.

Andersen, P., Bie, B., and Ganes, T. (1982). Distribution of GABA sensitive areas on hippocampal pyramidal cells. Experimental Brain Research. 45, 357-363.

Andersen, P., Bland, B. H., Myhrer, T., and Schwartzkroin, P. A. (1979). Septohippocampal pathway necessary for dentate theta production. Brain Research. 165, 13-22.

Andersen, P., Eccles, J. C., and Loyning, P. (1964). Pathway of postsynaptic inhibition in the hippocampus. Journal of Neurophysiology. 27, 608-618.

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

Aston-Jones, G., and Bloom, F. E. (1981a). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. Journal of Neuroscience. 1, 876-886.

Aston-Jones, G., and Bloom, F. E. (1981b). Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. Journal of Neuroscience. 1, 887-900.

Baisden, R. H., Woodruff, M. L., and Hoover, D. B. (1984). Cholinergic and non-cholinergic septohippocampal projections: A double-label horseradish peroxidase-acetylcholinesterase study in the rabbit. Brain Research. 290, 146-151.

Barber, R., and Saito, K. (1976). Light microscopic visualization of GAD and GABA-T in immunocytochemical preparations of rodent CNS. In E. Roberts, T. W. Chase, and D. B. Tower (Eds.), GABA in Nervous System Function, Kroc Foundation Series, Volume 5. (pp. 113-132). Raven Press: New York.

Bassant, M. H., Jobert, A., Dutar, P. and Lamour, Y. (1988). Effect of psychotropic drugs on identified septohippocampal neurons. Neuroscience. 27, 911-920.

Bayer, S. A. (1985). Hippocampal region. In G. Paxinos (Ed.) The Rat Nervous System Volume 1: Forebrain and Midbrain. New York: Academic Press.

Blackstad, T. W. (1956). Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. The Journal of Comparative Neurology. 105, 417-537.

Blackstad, T. W., (1958). On the termination of some afferents to the hippocampus and fascia dentata: An experimental study in the rat. Acta Anatomica. 35, 202-214.

Bland, B. H. (1985). Behavioral correlates and afferent control of hippocampal theta cells discharges. In G. Buszaki and C. H. Vanderwolf (Eds.), Electrical activity of the archicortex. (pp. 125-142). Budapest: Akademiai Kiado.

Bland, B. H. (1986). The physiology and pharmacology of hippocampal formation theta rhythms. Progress in Neurobiology. 26, 1-54.

Bland, B. H., Andersen, P., and Ganes, T. (1975). Two generators of hippocampal theta activity in rabbits. Brain Research. 94, 199-218.

Bland, B. H., and Colom, L. V. (1988). Responses of phasic and tonic hippocampal theta-on cells to cholinergics: differential effects of muscarinic and nicotinic activation. Brain Research. 440, 167-171.

Bland, B. H., Colom, L. V., and Ford, R. D. (In Press). Responses of septal theta-on and theta-off cells to activation of the dorsomedial-posterior hypothalamic region. Brain Research.

Bland, B. H., Colom, L. V., Konopacki, J., and Roth, S. H. (1988). Intracellular records of carbachol-induced theta rhythm in hippocampal slices. Brain Research. 447, 364-368.

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

Bland, B. H., Sainsbury, R. S., and Creery, B. (1979). Anatomical correlates of rhythmical slow activity (theta) in the hippocampal \formation of the cat. Brain Research. 161, 199-209.

Bland, B. H., Sainsbury, R. S., Seto, M., Sinclair, B. R., and Whishaw, I. Q. (1981). The use of sodium pentobarbital for the study of immobility-related (type 2) hippocampal theta. Physiology and Behavior. 27, 363-368.

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

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

Bland, B. H., and Vanderwolf, C. H. (1972). Diencephalic and hippocampal mechanisms of motor activity in the rat: Effects of posterior hypothalamic stimulation on behavior and hippocampal slow wave activity. Brain Research. 43, 67-88.

Bland, B. H., and Whishaw, I. Q. (1976). Generators and topography of hippocampal theta (RSA) in the acute and freely moving rabbit. Brain Research. 118, 259-280.

Bland, S. K., and Bland, B. H. (1986). Medial septal modulation of hippocampal theta cell discharges. Brain Research. 375, 102-116.

Brazhnik, E. S., and Vinogradova, O. S. (1986). Control of the neuronal rhythmic bursts in the septal pacemaker of theta-rhythm: Effects of anaesthetic and anticholinergic drugs. Brain Research. 380, 94-106.

Brazhnik, E. S., Vinogradova, O. S., and Karanov, A. M. (1985). Frequency modulation of neuronal theta-bursts in rabbit's septum of low-frequency repetitive stimulation of the afferent pathways. Neuroscience. 14, 501-508.

Burnstock, G., and Costa, M. (1975). Adrenergic Neurons, their Organization, Function and Development in the Peripheral Nervous System. London: Chapman and Hall.

Buszaki, G. (1986). Generation of hippocampal EEG patterns. In R. L. Isaacson and K. H. Pribram (Eds.) The Hippocampus. New York: Plenum Press

Chandler, J. P., and Crutcher, K. A. (1983). The septohippocampal projection in the rat: an electron microscopic horseradish peroxidase study. Neuroscience. 10, 685-696.

Christie, B. R. (1989). An Investigation of a Hypothalamic-Septal-Hippocampal pathway in the Urethan Anesthetized Rat. M.Sc. Thesis, University of Calgary, Calgary.

Colino, A., and Halliwell, J. V. (1986). 8-OH-DPAT is a strong antagonist of 5-HT action in rat hippocampus. European Journal of Pharmacology. 130, 151-152.

Colom, L. V., and Bland, B. H. (1987). State-dependent spike train dynamics of hippocampal neurons: Evidence for theta-on and theta-off cells. Brain Research. 422, 277-286.

Colom, L. V., Christie, B. R., and Bland, B. H. (1988). Cingulate cell discharge patterns related to hippocampal EEG and their modulation by muscarinic and nicotinic agents. Brain Research. 460, 329-338.

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

Crawford, I. L., and Connor, J. D. (1973). Localization and release of glutamic acid in relation to hippocampal mossy fibre pathway. Nature. 224, 442-443.

Creery, B. L., and Bland, B. H. (1980). Ontogeny of fascia dentata electrical activity and motor behavior in the dutch belted rabbit (orcytolagus cunniculus). Experimental Neurology. 67, 554-572.

Crutcher, K. A., and Davis, J. N. (1980). Sympathetic noradrenergic sprouting in response to central cholinergic denervation. Trends in Neuroscience. 4, 70-72.

Destrade, C. (1982) Two types of diencephalically driven RSA (theta) as a means of studying memory formation in mice. Brain Research. 234, 486-493.

Destrade, C., and Ott, T. (1982). Is a retrosplenial (cingulate) pathway involved in the mediation of high frequency hippocampal rhythmical slow activity (theta)? Brain Research. 252, 29-37.

Descarries, L. and Saucier, G. (1972). Disappearance of the locus coeruleus in the rat after intraventicular 6-hydroxidopamine. Brain Research. 100, 310-316.

Donovik, P. J. (1968). Effects of localized septal lesions on hippocampal EEG activity and behavior in rats. Journal of Comparative Physiological Psychology. 66, 569-578.

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

Dudar, J. D., Whishaw, I. Q., and Szerb, J. C. (1979). Release of acetylcholine from the hippocampus of freely moving rats during sensory stimulation and running. Neuropharmacology. 18, 673-678.

Faris, P. D., and Sainsbury, R. S. (Submitted). The role of the pontis oralis in the generation of RSA activity in the hippocampus of the guinea pig.

Feenstra, B. W. A., and Holshiemer, J. (1979). Dipole-like neuronal sources of theta rhythm in dorsal hippocampus dentate gyrus and cingulate cortex of the urethane-anaesthetized rat. Electroencephalography and Clinical Neurophysiology. 47, 532-538.

Flicker, C., and Geyer, M. A. (1982). Behavior during hippocampal microinfusions. I. Norepinephrine and diversive exploration. Brain Research Reviews. 4, 79-103.

Foote, S. L., Aston-Jones, G., and Bloom, F. E. (1980). Impulse activity of locus coeruleus neurons in awake rates and monkeys is a function of sensory stimulation and arousal. Proceedings of the National Academy of Science. 77, 3033-3037.

Ford, R. D., Colom, L. V., and Bland, B. H. (1989). The classification of medial septum-diagonal Band cells as theta-on or theta-off in relation to hippocampal EEG states. Brain Research. 493, 269-282.

Fox, S. E., and Ranck, J. B. (1975). Localization and anatomical identification of theta and complex spike cells in dorsal hippocampal formation of rats. Experimental Neurology. 49, 229-313.

Fox, S. E., and Ranck, J. B. (1981). Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. Experimental Brain Research. 41, 399-410.

Freund, T. F., and Antal, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature. 336, 170-173.

Fuxe, K. (1965). Evidence for the existence of monoamine containing neurons in the central nervous system. IV. The distribution of monoamine terminals in the central nervous system. Acta Physiology Scandinavia. 64, Supple. 247, 39-85.

Gogolak, G., Petsche, H., Stere, J., and Stumpf, C. (1967). Septum cell activity in the rabbit under reticular stimulation. Brain Research. 5, 508-510.

Gogolak, G., Stumpf, H., Petsche, H., and Sterc, J. (1968). The firing pattern of septal neurons and the form of the hippocampal theta wave. Brain Research. 7, 201-207.

Green, J. S., and Arduini, A. (1954). Hippocampal electrical activity in arousal. Journal of Neurophysiology. 17, 533-557.

Greenstein, Y. J., Pavlides, C., and Winson, J. (1987). Long-term potentiation in the dentate gyrus is preferentially induced at theta rhythm periodicity. Brain Research. 438, 331-334.

Haas, H. L. (1983). Amine transmitter actions in the hippocampus. In W. Siefert (Ed.) Molecular, Cellular, and Behavioral Neurobiology of the Hippocampus. New York: Academic Press.

Hamilton, L. W. (1976). Basic limbic system anatomy of the rat. New York: Plenum Press.

Harley, C. W. (1989). A role for norepinephrine in arousal, emotion and learning?: Limbic modulation by norepinephrine and the Kety hypothesis. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 11, 419-458.

Hjorth-Simonsen, A., and Jeune, B. (1972). Origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. The Journal of Comparative Neurology. 144(2), 215-232.

Holshiemer, J. (1982). Generation of theta activity (RSA) in the cingulate cortex of the rat. Experimental Brain Research. 47, 309-312.

Hopkins, W. F., and Johnston, D. (1984). Frequency-dependent noradrenergic modulation of long-term potentiation in the hippocampus. Science. 226, 350-351.

Jones, B. E., Halaris, A. E., McIlhany, M., and Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. I. Axonal transport in central noradrenaline neurons. Brain Research. 127, 1-22.

Jones, B. E., and Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. Brain Research. 127, 23-53.

Jones, G., Segal, M., Foote, S. L., and Bloom, F. E. (1979). Locus coeruleus neurons in freely moving rats exhibit pronounced alterations of discharge rate during sensory stimulation and stages of the sleep-wake cycle. In E. Usdin, I. J. Kopin, and J. Barchas (Eds.) Catecholamines: Basic and Clinical Frontiers. (pp. 643-645) New York: Pergamon.

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

Kobayashi, R., Palkovits, Jacobowitz, D., and M., Kopin, I. J. (1975). Biochemical mapping of the noradrenergic projection from the locus coeruleus. Neurology. 25, 223-233

Koda, L. Y., Schulman, J. A., and Bloom, F. E. (1978). Ultrastructural identification of noradrenergic terminals in the rat hippocampus: unilateral destruction of the locus coeruleus with 6-hydroxydopamine. Brain Research. 145, 190-195.

Kohler, C., Chan-Palay, V., and Wu, J. Y. (1984). Septal neurons containing glutamic acid decarboxylase immunoreactivity project to the hippocampal region in the rat brain. Anatomical Embryology. 169, 41-44.

Kolb, B., and Whishaw, I. Q. (1977). Effects of brain lesions and atropine on hippocampal and neocortical EEG in the rat. Experimental Neurology. 56, 1-22.

Konopacki, J., Bland, B. H., MacIver, M.., and Roth, S.H. (1987). Cholinergic theta rhythm in transected hippocampal slices: Independant CA_1 and dentate generators. Brain Research. 436, 217-222.

Konopacki, J., Bland, B. H., and Roth, S. H. (1987). Phase shifting of CA1 and dentate EEG theta rhythms in hippocampal formation slices. Brain Research. 417, 399-402.

Konopacki, J., Bland, B. H., and Roth, S. H. (1988a). Carbachol-induced EEG 'theta' in hippocampal formation slices: Evidence for a third generator of theta in CA3c area. Brain Research. 451, 33-42.

Konopacki, J., Bland, B. H., and Roth, S. H. (1988b). Evidence that activation of In Vitro hippocampal theta rhythm involved only muscarinic receptors. Brain Research. 455, 110-114.

Konopacki, J., MacIver, M. B., Bland, B. H., and Roth, S. H. (1987). Carbachol-induced EEG 'theta' activity in hippocampal brain slices. Brain Research. 405, 196-199.

Kramis, R., C., and Routtenberg, A. (1969). Rewarding brain stimulation, hippocampal activity, and footstomping in the gerbil. Physiology and Behavior. 4, 7-11.

Kramis, R. C., and Routtenberg, A. (1977). Dissociation of hippocampal EEG from its behavioral correlates by septal and hippocampal electrical stimulation. Brain Research. 125, 37-49.

Kramis, R., and Vanderwolf, C. H. (1980). Frequency specific RSA like hippocampal patterns elicited by septal, hypothalamic and brainstem stimulation. Brain Research. 192, 383-398.

Kramis, R., Vanderwolf, C. H., and Bland, B. H. (1975). Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethylether, urethane and pentobarbital. Experimental Neurology. 49, 58-85.

Krnjevic, K., Reiffenstein, R. J., and Ropert, N. (1981). Disinhibitory action of acetylcholine in the rat's hippocampus: extracellular observations. Neuroscience. 6, 1465-1474.

Kuhar, M. J., Aghajanian, G. K., and Roth, R. H. (1972). Synthesis of catecholamines in the locus coeruleus from 3H-tyrosine in vivo. Biochemical Pharmacology. 21, 2280-2282.

Kuhar, M. J., and Yamamura, H. I. (1975). Light autoradiographic localization of cholinergic muscarinic receptors in rat brain by specific binding of a potent antagonist. Nature. 253, 560-561.

Lacaille, J. C., and Harley, C. W. (1985). The action of norepinephrine in the dentate gyrus: Beta-mediated facilitation of evoked potentials in vitro. Brain Research. 358, 210-220.

Lamour, Y. P., Dutar, O., and Jobert, A. (1984). Septo-hippocampal and other medial septum-diagonal band neurons: electrophysiological and pharmacological properties. Brain Research. 309, 227-239.

Le Moal,M., and Cardo, B. (1975). Rhythmic slow wave activity recorded in the ventral mesencephalic tegmentum in the rat. Electroencephalography and Clinical Neurophysiology. 38, 139-147.

Leung, L. S. (1984). Theta rhythm during REM sleep and waking: correlations between power, phase and frequency. Electroencephalography and Clinical Neurophysiology. 58, 553-564.

Leung, L. S. (1985). Spectral analysis of hippocampal EEG in the freely moving rat: effects of centrally acting drugs and relations to evoked potentials. Electroencephalography and Clinical Neurophysiology. 60, 65-77.

Leung, L. S., and Borst, J. G. G. (1987). Electrical activity of the cingulate cortex. I. Generating mechanisms and relations to behaviour. Brain Research. 407, 68-80.

Leung, L. S., Lopes da Silva, F. H., and Wadman, W. J. (1982). Spectral characteristics of the hippocampal EEG in the freely moving rat. Electroencephalography and Clinical Neurophysiology. 54, 203-291.

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

Lewis, P. R., and Shute, C. C. D. (1967). The cholinergic limbic system: projection to the hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system and the subfornical organ and supraoptic crest. Brain. 90, 521-537.

Lewis, P. R., Shute, C. C. D., and Silver, A. (1967). Confirmation from choline acetylase analyses of a massive cholinergic innervation of the rat hippocampus. London Journal of Physiology. 191, 215-224.

Lorente de No, R. (1934). Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. Journal fur Psychologie und Neurologie. 46, 113-177.

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

Macadar, A. W., Chalupa, L. M., and Lindsley, D. B. (1974). Differentiation of brain stem loci which affect hippocampal and neocortical electrical activity. Experimental Neurology. 43, 499-514.

MacIver, M. B., Harris, D. P., Konopacki, J., Roth, S. H., and Bland, B. H. (1986). Carbachol-induced rhythmical slow wave activity recorded from dentate granule neurons in vitro. Proceedings of the Western Pharmacological Society. 29, 159-161.

Madison, D. V., and Nicoll, R. A. (1986). Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurones, in vitro. Journal of Physiology. 372, 221-244.

Malisch, R. and Ott, T. (1982). Rhythmical slow wave electroencephalographic activity elicited by hippocampal injection of muscarinic agents in the rat. Neuroscience Letters. 28, 113-118.

Maru, E., Takahashi, L. K., and Iwahara, S. (1979). Effects of median raphe nucleus lesions on hippocampal EEG in the freely moving rat. Brain Research. 163, 223-234.

Maura, G., Gemignani, A., and Raiteri, M. (1982). Noradrenaline inhibits central serotonin release through alpha$_2$-adrenoceptors located on serotonergic nerve terminals. Naunyn-Schmeideberg's Archives of Pharmacology. 320, 272-274.

McNaughton, N., Azmitia, E. C., Williams, J. H., Buchan, A., and Gray, J.A. (1980). Septal elicitation of hippocampal theta rhythm after localized de-afferentation of serotoninergic fibers. Brain Research. 200, 259-269.

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

Mellgren, S. I., and Srebro, B. (1973). Changes in acetylcholinesterase and distribution of degenerating fibers in the hippocampal region after septal lesions in the rat. Brain Research. 52, 19-36.

Meuller, A. L., Hoffer, B. J., and Dunwiddie, T. V. (1981). Noradrenergic responses in rat hippocampus: Evidence for mediation by alpha and beta receptors in the in vitro slice. Brain Research. 214, 113-126.

Meuller, A. L., Palmer, M. R., Hoffer, B. J., and Dunwiddie, T. V. (1982). Hippocampal noradrenergic responses in vitro and in vivo, characterization of alpha and beta components. Naunyn-Schmiedeberg's Archives. 318, 249-266.

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

Monaghan, D. T., Holets, V. R., Toy, D. W., and Cotman, C. W. (1983). Anatomical distributions of four pharmacologically distinct 3H-L-glutamate binding sites. Nature. 306, 176-179.

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

Montoya, C. P., and Sainsbury, R. S. (1985). The effects of entorhinal cortex lesions on type 1 and type 2 theta. Physiology and Behavior. 35, 121-126.

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

Morris, R. G. M., Black, A. H., and O'Keefe, J. (1976). Hippocampal EEG during a ballistic movement. Neuroscience Letters. 3, 102.

Neuman, R. S., and Harley, C. W. (1983). Long-lasting potentiationof the dentate gyrus population spike by norepinephrine. Brain Research. 273, 162-165.

Nunez, A., Garcia-Austt, E., and Buno Hr., W. (1987). Intracellular theta-rhythm generation in identified hippocampal pyramids. Brain Research. 416, 289-300.

Nyakas, C., Luiten, P. G. M., Spencer, D. G., and Traber, J. (1987). Detailed projection patterns of septal and diagonal band efferents to the hippocampus in the rat with emphasis on innervation of CA1 and dentate gyrus. Brain Research Bulletin. 18, 533-545.

Oka, A., and Yoshida, K. (1985). Septohippocampal connections to field CA1 of the rat identified with field potential analysis and retrograde labeling by horseradish peroxidase. Neuroscience Letters. 58, 19-24.

Otmakhov, N. A., and Bragin, A. G. (1982). Effects of norepinephrine and serotonin upon spontaneous activity and responses to mossy fiber stimulation of CA3 neurons in hippocampal slices. Brain Research. 253, 173-183.

Pang, K. and Rose, G. M. (1987). Differential effects of norepinephrine on hippocampal complex-spike and theta neurons. Brain Research. 425, 146-158

Papanicolaou, J., Summers, R. J., Vajda, F. J. E., and Louis, W. J. (1982). The relationship between alpha$_2$-adrenoreceptor selectivity and anticonvulsant effect in a series of clonidine-like drugs. Brain Research. 241, 393-397.

Parker, S. M., and Sinnamon, H. M. (1983). Forward locomotion elicited by electrical stimulation in the diencephalon and mesencephalon of the awake rat. Physiology and Behavior. 31, 581-587.

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

Paxinos, G., and Watson, C. (1986). The Rat Brain in Stereotaxic Atlas. New York: Academic Press.

Petsche, H., and Stumpf, C. (1960). Topographic and toposcopic study of the origin and spread of regular synchronized arousal pattern in the rabbit. Electroencephalography. 12, 589-600.

Petsche, H., Stumpf, C., and Gogolak, G. (1962). The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. I. The control of hippocampus arousal activity by septum cells. Electroencephalography and Clinical Neurophysiology. 14, 202-211.

Polz-Tejera, G., Schmidt, J., and Karten H. (1975). Autoradiographic localization of alpha-bungarotoxin-binding sites in the central nervous system. Nature. 258, 349-351.

Racine, R. J., and Kairiss, E. W. (1987). Long-term potentiation phenomena: The search for the mechanisms underlying memory storage processes. Neuroplasticity, Learning, and Memory. 173-197.

Rainbow, T. C., Parsons, B., and Wolfe, B. B. (1984). Quantitative autoradiography of $beta_1$ and $beta_2$ adrenergic receptors in rat brain. Proceedings of the National Academy of Science. 81, 1585-1589.

Ranck, J. B. (1973). Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. Part I. Behavioral correlates and firing repertoires. Experimental Neurology. 41, 461-555.

Ribak, C. E., Seress, L. Peterson, G. M., Seroogy, K. B., Fallon, J., H., and Schmued, L. C. (1986). A GAGAergic inhibitory component within the hippocampal commissural pathway. Journal of Neuroscience. 6, 3492-3498.

Riley, J. N., and Moore, R. Y. (1981). Diencephalic and brainstem afferents to the hippocampal formation of the rat. Brain Research Bulletin. 6, 437-444.

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

Robinson, T. E., and Green, D. J. (1980). Effects of hemicholinium 3 and choline on hippocampal electrical activity during immobility and movement. Electroencephalography and Clinical Neurophysiology. 50, 314-323.

Robinson, T. E., and Vanderwolf, C. H. (1978). Electrical stimulation of the brainstem in freely moving rats. II. Effects on hippocampal and neocortical electrical activity and relations to behavior. Experimental Neurology. 61, 485-515.

Robinson, T. E., Vanderwolf, C. H., and Pappas, B. A. (1977). Are the dorsal noradrenergic bundle projections from the locus coeruleus important for neocortical or hippocampal activation? Brain Research. 138, 75-98.

Robinson, T. E., and Whishaw, I. Q. (1974). Effects of posterior hypothalamic lesions on voluntary behaviour and hippocampal electroencephalograms in the rat. Journal of Comparative and Physiological Psychology. 86, 768-786.

Roth, S. H., Konopacki, J. and Bland, B. H. (1987). Pharmacological demonstration of two EEG 'theta'generators in hippocampal slices. Proceedings Western Pharmacological Society. 30, 37-40.

Rowntree, C. I., and Bland, B. H. (1986). An analysis of cholinoceptive neurons in the hippocampal formation by direct microinfusion. Brain Research. 362, 98-113.

Sainsbury, R. S. (1970). Hippocampal activity during natural behaviors in the guinea pig. Physiology and Behavior. 5, 317-324.

Sainsbury, R. S. (1985). Type 2 theta in the guinea pig and the cat. In Buszaki, G., and Vanderwolf, C. H. (Eds.) Electrical Activity of the Archicortex (pp. 11-22). Budapest: Akademiai Kiado.

Sainsbury, R. S., and Bland, B. H. (1981). Effects of selective septal lesions on theta production in the CA1 and dentate gyrus of the hippocampus. Physiology and Behavior. 26, 1097-1011.

Sainsbury, R. S., Harris, J. L., and Rowland, G. L. (1987). Sensitization and hippocampal type 2 theta in the rat. Physiology and Behavior. 41, 489-493.

Sainsbury, R. S., Heynen, A. J., and Montoya, C. P. (1987). Behavioral correlates of hippocampal type 2 theta in the rat. Physiology and Behavior. 39, 513-519.

Sainsbury, R. S., and Montoya, C. P. (1984). The relationship between type 2 theta and behavior. Physiology and Behavior. 33, 621-626.

Schwartzkroin, P. A. (1986). Regulation of excitability in hippocampal neurons. in R. L. Isaacson and K. H. Pribham (Eds.) The Hippocampus, Volume 3. New York: Plenum Press.

Schwartkroin, P. A., and Andersen, P. (1975). Glutamic acid sensitivity of dendrites in hippocampal slices in vitro. In G. Kreutzberg (Ed)Properties of Dendrites. Advanceds in Neurology. New York: Raven Press.

Segal, M. (1975). Physiological and pharmacological evidence for a serotonergic projection to the hippocampus. Brain Research. 94, 115-131.

Segal, M. (1976). 5-HT antagonists in rat hippocampus. Brain Research. 103, 161-166.

Segal, M. (1977). Effects of brainstem priming stimulation on hippocampal responses to interhemispheric stimulation in the awake rat. Experimental Brain Research. 28, 529-541.

Segal, M. (1980a). The action of serotonin in the rat hippocampus. Journal of Physiology. 303, 423-439.

Segal, M. (1980b). The noradrenergic innervation of the hippocampus. In J. A. Hobson and M. A. B. Brazier (Eds.) The Reticular Formation Revisited. New York: Raven Press.

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

Segal, M., and Bloom, F. E. (1974a). The action of norepinephrine in the rat hippocampus. I. Iontophoretic studies. Brain Research. 72, 79-97.

Segal, M., and Bloom, F. E. (1974b). The action of norepinephrine in the rat hippocampus II. Activation of the input system. Brain Research. 72, 99-114.

Segal, M., and Bloom, F. E. (1976a). The action of norepinephrine in the rat hippocampus. III. Hippocampal cellular responses to locus coeruleus stimulation in the awake rat. Brain Research. 107, 499-507.

Segal, M., and Bloom, F. E. (1976b). The action of norepinephrine in the rat hippocampus. IV. The effects of locus coeruleus stimulation on evoked hippocampal unit activity. Brain Research. 107, 513-525.

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

Shinoda, K., Tohyama, M., and Shiotani, Y. (1987). Hippocampal gamma-aminobutyric acid (GABA)-containing neuron system in the rat: a study using a double-labeling method that combines retrograde tracing and immunohistochemistry. Brain Research. 409, 181-186.

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

Sinnamon, H. M. (1984). Forelimb and hindlimb stepping by the anesthetized rat elicited by electrical stimulation of the diencephalon and mesencephalon. Physiology and Behavior. 33, 201-208.

Sinnamon, H. M., and Stopford, C. K. (1987). Locomotion elicited by lateral hypothalamus stimulation in the anesthetized rat does not require the dorsal midbrain. Brain Research. 402, 78-86.

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

Stewart, M. and Fox, S. E. (1989). Two populations of rhythmically bursting neurons in rat medial septum are revealed by atropine. Journal of Neurophysiology. 61, 982-994.

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

Storm-Mathisen, J. (1972). Glutamate decarboxylase in the rat hippocampal region after lesions of the afferent fiber system. Evidence that the enzyme is localized in intrinsic neurones. Brain Research. 40, 215-235.

Storm-Mathisen, J. (1977). Localization of transmitter candidates in the brain: the hippocampal formation as a model. Progress in Neurobiology. 8, 118-181.

Storm-Mathisen, J. (1981). Autoradiographic and microchemical localization of high affinity glutamate uptake. In P. J. Roberts, J. Storm-Mathisen, and G. A. R. Johnston (Eds.) Glutamate: Transmitter in the Central Nervous System. Chicester: Wiley.

Storm-Mathisen, J., and Fonnum, F. (1969). Neurotransmitter synthesis in excitatory and inhibitory synapses of rat hippocampus. In R. Paoletti, R. Fumagalli, and C. Galli (Eds.), Second International Meeting of the International Society for Neurochemistry. (p. 382). Tamburini: Milano.

Storm-Mathisen, J., and Guldberg, J. C. (1974). 5-Hydroxytryptamine and noradrenaline in the hippocampal region: Effect of transection of afferent pathways on endogenous levels, high affinity uptake and some transmitter-related enzymes. Journal of Neurochemistry. 22, 793-803.

Storm-Mathisen, J., Leknes, A. K., Bore, A. T., Line Vaaland, J., Edminson, P. Haug, F. M. S. and Otterson, O. P. (1983). First visualization of glutmate and GABA in neurones by immunocytochemistry. Nature. 301, 517-520.

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

Storm-Mathisen, J., and Ottersen, O. P. (1984). Neurotransmitters in the hippocampal formation. In F. Reinoso-Suarez and C. Ajome-Marsan (Eds.) Cortical Integration: Basic, Archicortical, and Cortical Association levels of Neural Integration. New York: Raven Press.

Stumpf, C., Petsche, H., and Gogolak, G. (1962). The significance of the rabbit's septum as a relay station between the midbrain and hippocampus. II. The differential influence of drugs upon both the septal cell firing pattern and the hippocampal theta activity. Electroencephalography and Clinical Neurophysiology. 14, 212-219.

Swanson, L. W., and Cowan, W. M. (1977). An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. Journal of Comparative Neurology. 172, 49-84.

Swanson, L. W., and Cowan, W. M. (1979). Connections of the septal region in the rat. The Journal of Comparative Neurology. 186, 621-656.

Swanson, J., and Dahl, D. (1985). Action of norepinephrine in the dentate gyrus. II Iontophoretic studies. Experimental Brain Research. 59, 497-506.

Ter Horst, G. J., and Luiten, P. G. M. (1987). Phaseolus vulgaris leuco-agglutinin tracing of intrahypothalamic connections of the lateral, ventromedial, dorsomedial, and paraventricular hypothalamic nuclei in the rat. Brain Research Bulletin. 18, 191-203.

U'Prichard, D. C., Betchel, W. D., Rouot, B. M., and Snyder, S. H. (1979). Multiple apparent alpha-noradrenergic receptor binding sites in rat brain: effect of 6-hydroxydopamine. Molecular Pharmacology. 16, 47-60.

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

Vanderwolf, C. H. (1971). Limbic-diencephalic mechanisms of voluntary movement. Psychology Reviews. 78, 83-113.

Vanderwolf, C. H. (1975). Neocortical and hippocampal activation in relation to behavior: Effects of atropine, eserine phenothiazines and amphetamine. Journal of Comparative and Physiological Psychology. 88, 300-323.

Vanderwolf, C. H., and Baker, G. B. (1986). Evidence that serotonin mediates non-cholinergic neocortical low voltage fast activity, non-cholinergic hippocampal rhythmical slow activity and cognitive abilities. Brain Research. 374, 342-356.

Vanderwolf, C. H., Bland, B. H., and Whishaw, I. Q. (1973). Diencephalic, hippocampal and neocortical mechanisms in voluntary movement. In Maser, J. D. (Ed.) Efferent Organization and the Integration of Behavior. (pp. 229-262). New York: Academic.

Vanderwolf, C. H., Kramis, R., Gillespie, L. A., and Bland, B. H. (1975). Hippocampal rhythmic slow activity and neocortical low voltage fast activity: Relations to behavior. In R. L. Isaacson and K. H. Pribram (Eds.) The Hippocampus: Volume 2. New York: Plenum Press.

Vanderwolf, C. H., and Leung, L. W. S. (1983). Hippocampal rhythmical slow activity: A brief history and the effects of entorhinal lesions and phencyclidine. In W. Siefert (Ed.) Molecular, Cellular, and Behavioral Neurobiology of the Hippocampus. New York: Academic Press.

Vertes, R. P. (1980). Brainstem activation of the hippocampus: A role for the magnocellular reticular formation and the MLF. Electroencephalography and Clinical Neurophysiology. 50, 48-55.

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

Vertes, R. P. (1982). Brainstem generation of the hippocampal EEG. Progress in Neurobiology. 19, 159-186.

Vertes, R. P. (1985). Brainstem-septohippocampal circuits controlling the hippocampal EEG. In G. Buzsaki and C. H. Vanderwolf (Eds.), Electrical activity of the archicortex (pp. 33-45). Budapest: Akademiai Kiado.

Vertes, R. P. (1986). Brainstem modulation of the hippocampus: Anatomy, physiology, and significance. In R. L. Isaacson K. H. Pribram (Ed.) The Hippocampus: Volume 4. Plenum Publishing corporation. 41-75.

Vinogradova, O. S., Brazhnik, E. S., Karanov, A. M., and Zhadina, S. D. (1980). Neuronal activity in the septum following various types of deafferentation. Brain Research. 187, 153-168.

Vinogradova, O. S., Brazhnik, F. S., Karanov, A. M., and Zhadina, S. D. (1980). Analysis of neuronal activity in rabbit's septum with various conditions of deafferentation. Brain Research. 187, 354-368.

Virtanen, R., Savola, J. M., Saano, V., and Nyman, L. (1988). Characterization of the selectivity, specificity and potency of detomidine as an alpha$_2$-adrenoreceptor agonist. European Journal of Pharmacology. 150, 9-14.

Vizi, E. S. (1968). The inhibitory action of noradrenaline and adrenaline on release of acetylcholine from guinea-pig ileum longitudinal strips. Archives of Experimental and Pathological Pharmacology. 259, 199-200.

Vizi, E. S. (1974). Interaction between adrenergic and cholinergic systems: presynaptic inhibitory effect of noradrenaline on acetylcholine release. Journal of Neural Transmission. 11, 61-78.

Vizi, E. S. (1980). Modulation of cortical release of acetylcholine by noradrenaline released from nerves arising from the rat locus coeruleus. Neuroscience. 5, 2139-2144.

Vizi, E. S. (1981). Non-synaptic modulation of chemical neurotransmission. In L. Stjarne, P. Hedqvist, H. Lagercrantz, and A. Wennmalm (Eds.), Neutrotransmission: 75 years. (pp. 235-248) London: Academic Press.

Vizi, E. S. (1983). Non-synaptic interneuronal communication: physiological and pharmacological implications. In N. N. Osbourne (Ed.) Dale's Principle and Communication Between Neurons. Oxford: Pergamon Press.

Vizi, E. S., and Pasztor, E. (1981). Release of acetylcholine from isolated human cortical slices: inhibitory effect of norepinephrine and phenytoin. Experimental Neurology. 73, 144-153.

Vizi, E. S., Ronai, A. Z., Harsing, L. G. Jr., and Knoll, J. (1977). Presynaptic modulation by norepinephrine and dopamine of acetylcholine release in the peripheral and central nervous system. In D. J. Jenden (Ed.), Cholinergic Mechanisms and Psychopharmacology. (pp. 587-603). New York: Plenum Press.

Vizi, E. S., Harsing, L. G. Jr., and Zsilla, G. (1981). Evidence of the modulatory role of serotonin in acetylcholine release from striatal interneurons. Brain Research. 212, 89-99.

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

Whishaw, I. Q, (1972). Hippocampal electroencephalographic activity in the Mongolian gerbil during natural behaviours and wheel running in the rat during wheel running and conditioned immobility. Canadian Journal of Psychology. 26, 219-239.

Whishaw, I. Q, (1982). A simple behavioural paradigm for the study type I hippocampal rhythmical slow activity (RSA) frequency shifts. Physiology and Behaviour. 29, 751-753.

Whishaw, I. Q., Bland, B. H., and Vanderwolf, C. H. (1972). Hippocampal activity, behavior, self-stimulation, and heart rate during electrical stimulation of the lateral hypothalamus. Journal of Comparative and Physiological Psychology. 79, 115-127.

Whishaw, I. Q., Robinson, T. E., Schallert, T., Deryck, M. and Ramirez, V. D. (1978). Electrical activity of the hippocampus and neocortex in rats depleted of brain dopamine and norepinephrine: Relations to behavior and effects of atropine. Experimental Neurology. 62, 748-767.

Whishaw, I. Q., and Vanderwolf, C. H. (1973). Hippocampal EEG and behaviour; changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. Behavioural Biology. 8(4), 461-484.

Wilson, C. L., Motter, B. C., and Lindsley, D. B. (1976). Influences of hypothalamic stimulation upon septal and hippocampal electrical activity in the cat. Brain Research. 102, 55-68.

Winson, J. (1974). Patterns of hippocampal theta rhythm in the freely moving rat. Electroencephalography and Clinical Neurophysiology. 36, 291-301.

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

Winson, J. (1976b). Hippocampal theta rhythm. II. Depth profiles in the freely moving rabbit. Brain Research. 103, 71-79.

Young, W. S., and Kuhar, M. J. (1980). Noradrenergic alpha-1 and alpha-2 receptors: Light microscopic autoradiographic localization. Proceedings National Academy of Science U.S.A. 77, 1696-1700.

Zackek, R., Hedreen, J. C., and Coyle, J. T. (1979). Evidence for a hippocampal-setpal glutaminergic pathway in the rat. Experimental Neurology. 65, 145-156.

APPENDIX A

Source Tables for Experiment I

Effect of DMPH Stimulation on Peak Theta Frequency

Source	SS	df	MS	F	ω^2
Drug	0.37	1	0.37	9.25	
Error	0.34	9	0.04		
Stim	51.64	2	25.82	160.75*	0.95
Error	2.89	18	0.16		
Drug x Stim	0.08	2	0.04	0.81	
Error	0.87	18	0.05		

* $p<.01$

	SPON	0.5mA	1.0mA
SPON			
0.5mA	*		
1.0mA	*	*	

Effect of NE on Noise Level

Source	SS	df	MS	F	ω^2
Drug	174.35	1	174.35	21.80*	0.71
Error	71.99	9	8.00		
Stim	47.64	2	23.82	5.57	
Error	76.92	18	4.27		
Drug x Stim	3.25	2	1.62	0.87	
Error	33.47	18	1.86		

*$p<.01$

Effect of NE on Power at Peak Theta Frequency

Source	SS	df	MS	F	^{2}
Drug	315.10	1	315.10	73.20*	0.89
Error	38.74	9	4.30		
Stim	45.13	2	22.56	6.35*	0.41
Error	63.98	18	3.55		
Drug x Stim	1.10	2	0.55	0.22	
Error	45.85	18	2.55		

*p<.01

	SPON	0.5mA	1.0mA
SPON			
0.5mA	*		
1.0mA	*		

APPENDIX B

Source Tables for Experiment II

Effect of Stimulation on Theta Frequency

Source	SS	df	MS	F	^{2}
Stim	116.57	2	58.28	234.00*	0.81
Error	26.83	108	0.25		

*p<.01

(Greenhouse-Geisser adjustment made for analysis.)

	SPON	0.5mA	1.0mA
SPON			
0.5mA	*		
1.0mA	*	*	

Effect of Stimulation on Peak Theta Power

Source	SS	df	MS	F	^{2}
Stim	535.08	2	267.54	52.21*	0.50
Error	553.44	108	5.12		

*p<.01

(Greenhouse-Geisser adjustment made for analysis.)

	SPON	0.5mA	1.0mA
SPON			
0.5mA	*		
1.0mA	*		

Effects of Detomidine on Peak Frequency Strength

Source	SS	df	MS	F	^{2}
Dose	84.79	2	42.40	4.91	
Error	103.57	12	8.63		
Drug	68.43	1	68.43	15.98*	0.57
Drug x Dose	20.88	2	10.44	2.44	
Error	51.38	12	4.28		
Stim	183.93	2	91.97	12.07*	0.50
Dose x Stim	15.75	4	3.94	0.52	
Error	182.86	24	7.62		
Drug x Stim	19.37	1.20	9.68	2.30	
Dose x Drug x Stim	9.16	2.4	2.29	0.54	
Error	101.21	14.40	4.22		

*$p<.01$

Effect of Detomidine on Noise Level

Source	SS	df	MS	F	^{2}
Dose	60.73	2	30.36	1.21	
Error	300.60	12	25.05		
Drug	49.80	1	49.80	16.99*	0.59
Drug x Dose	3.45	2	1.73	0.59	
Error	35.17	12	2.93		
Stim	37.55	2	18.78	3.83	
Dose x Stim	5.68	4	1.42	0.29	
Error	117.52	24	4.90		
Drug x Stim	6.92	2	3.46	1.69	
Dose x Drug x Stim	7.41	4	1.85	0.90	
Error	49.23	24	2.05		

*$p<.01$