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Theta Cells in the CA1 Layer of the Hippocampal Formation: Relations to Slow Wave Activity and Motor Behavior in the Freely Moving Rabbit

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Brian R. Sinclair

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Theta Cells in the CA1 Layer of the Hippocampal Formation: Relations to Slow Wave Activity and Motor Behavior in the Freely Moving Rabbit" submitted by Brian R. Sinclair in partial fulfillment of the requirements for the degree of Master of Science.

Supervisor, B.H.Bland, Psychology

R.S.Sainsbury, Psychology

J.A.Adam, Psychology

Q.J.Pittman, Pharmacology and Therapeutics

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ABSTRACT

The firing repertoires of theta cells in the CA1 layer of the hippocampal formation of the freely moving rabbit were analysed during three behavioral conditions: 1. Voluntary motor patterns, termed Type 1 theta behaviors, 2. Automatic motor patterns, termed Type 2 LIA behaviors, 3. Alert immobility with presentation of sensory stimuli, termed Type 2 theta behavior. The firing repertoires of theta cells in the CA1 layer of the hippocampus were also analysed in relation to the simultaneously recorded slow wave activity from the same electrode and a reference electrode located in stratum moleculare of the dentate. Computer analysis of the relation of theta cell discharges to the phase of slow wave theta activity was carried out using a cross-correlation technique. Of the total cell groups that were isolated, 19 were histologically located in the CA1 layer. All theta cells had a duration of .3 to .4 msec across the negative component of the spike (narrow band) and fired in a single spike pattern. All theta groups increased their firing (approximately doubled) at the onset of Type 1 movement and continued to fire rhythmically for the duration of movement. During Type 2 LIA behaviors theta cell firing was arhythmic. During alert immobility and presentation of sensory stimuli that

elicited theta, theta cell groups again fired in a rhythmic pattern for the duration of the theta slow wave activity (there were fewer rhythmic discharges per theta wave, compared to the Type 1 theta condition). The difference in discharges between the two theta conditions was shown to be significant even when the frequencies of the slow wave theta were identical. All theta cells discharged on the negative phase of their local slow wave with CA1 layer theta cells discharging just prior to peak negativity. Theta cell discharges were arhythmic during the LIA condition. The cells could be silent for up to 1 second and discharges often occurred in bursts. Eserine administration elicited Type 2 theta, while atropine SO_4 abolished Type 2 theta and the associated unit rhythmicity. Atropine SO_4 also produced decreased discharge rates of units associated with Type 1 behavior.

The results of this study provide further support for the views that:

1. There are separate systems to the hippocampal formation providing the afferent drive for the two kinds of theta,
2. The hippocampal formation is involved in the organization of voluntary motor behavior.

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Dedication

I dedicate this thesis to my parents, David and Evelyn Sinclair. The support and encouragement that they have always shown has allowed my academic career to proceed as it has, and for this I lovingly thank them.

Table of Contents

List of Tables

List of Figures

Introduction

The purpose of this thesis was to evaluate the relations of theta cells in the CA1 layer of the hippocampal formation to slow wave activity and motor behavior. To meet this objective, the relationships of CA1 layer cells to slow wave activity and motor behavior were studied in the freely moving rabbit. This chapter will provide a background of the relevant literature on various aspects of the hippocampal formation, concluding with the rationale and the specific objectives of the thesis.

ANATOMY

The hippocampal formation is part of the telencephalon forming a relatively long, horn-shaped body along the curvature of the lateral ventricles. It can be divided into a dorsal portion lying just behind the septum, a posterior portion where it begins to curve ventrally and laterally, and a ventral portion located in the temporal part of the brain. The hippocampal region can be divided into the hippocampus proper (Ammons Horn); the fascia dentata (dentate gyrus); and the subiculum. The hippocampus itself is divided into two U-shaped sections, with the pyramidal cells of the hippocampus proper interlocking with the layer of dentate granule cells.

The hippocampus proper (Cornu Ammonis) has been further subdivided by Ramon y Cajal (1911) into the superior, intermediate, and inferior regions. Lorento de No (1934) further defined four zones: The dorsal fields of CA1 and CA2, and the lower fields of CA3 and CA4. The CA1 contains a single layer of small pyramidal cells, the CA3 a layer of large pyramidal cells, while the CA2 represents a transition area between the CA1 and CA3. The CA4 area designates the scattered cells inside the hilus of the fascia dentata. The CA4 cells, although not layered neatly like the pyramidal cells of the other fields, are included in the hippocampus

proper because of their pyramid-like characteristics, their mossy fiber reception, and their axonal output to the fimbria.

A number of architectonic layers have been identified to describe the internal structure of the hippocampus (Raisman, Cowan & Powell, 1965).

1. Alveus - containing the axons of pyramidal cells going towards the fimbria or subiculum.

2. Stratum Oriens - containing the proximal parts of pyramidal axons going towards the alveus, the start of Schaffer collaterals, the basal dendrites of pyramidal cells, as well as afferent fibers from the fimbria.

3. Stratum Pyramidale - dominated by the cell bodies of the pyramids.

4. Stratum Radiatum - containing main shafts of primary (apical) dendrites of the pyramids.

5. Stratum Lacunosum - containing primarily horizontal fibers derived in part from the Schaffer collaterals.

6. Stratum Moleculare - containing a wide variety of cell types which receive terminal dendritic plexus of pyramids as well as the myelinated fibers of the perforant path from entorhinal cortex. An additional layer is recognized in the CA3 region, the Stratum Lucidum, which is formed by the efferent (mossy) fibers from the dentate granule cells.

The fascia dentata has a simple laminated structure of three layers:

1. Stratum Granulosum, an 80 μ thick layer containing the densely-packed cell bodies of the granule cells.

2. Stratum Moleculare,continuous with both stratum moleculare and radiatum of the hippocampus proper. This 400 μ layer is formed by the interwining apical dendrites of the granule cells.

3. Stratum Polymorphe - layer in the hilus of the dentate gyrus which is not clearly separated from the CA4 subfield; contains the initial segments of the granule cell axons as they come together to form the mossy fiber bundle.

Generally, the basic pattern within the hippocampus is similar, being an ordered sheet of large neurons with a densely packed somal layer whose dendrites all travel in the same direction. Traversing at right angles to the dendrites, inputs to many sectors occur en passage within restricted dendritic regions. An important distinction between the morphology of the pyramidal cells and the granule cells is that the former has both apical and basal dendrites while the latter has only apical dendrites.

Another important cell of the hippocampus is the interneuron called the basket cell of Cajal. The basket cell

bodies are located around the cell layer with axons ascending through the layer to the dendrites of the pyramidal or granule cells. The axons giveoff a number of descending collaterals which terminate around the cell bodies in a basket-like plexus. A critical difference between the pyramidal cells and these basket cells is that the basket cells do not send their axons out of the area (that is, they are not projection cells).

The entorhinal region has been shown by a number of investigators to be the major source of afferent input into the hippocampus and dentate fascia (Ramon y Cajal 1901, Zuckerkandl 1900, Lorento de No 1933, 1934; Blackstad 1958). Axons from the cells of the entorhinal area form the perforant path, which bifurcates following the hippocampal fissure with each bifurcation laying parallel to the surfaces of the dentate fascia. The perforant path axons form numerous en passage synatpic contacts with the dendritic spines of the granule cells.

This pathway has been shown (Hjorth-Simonsen 1972) to be composed of at least two distinct fiber systems:

1. A medial perforant path coming from the medial part of the entorhinal area and terminating in the middle of the dentate molecular layer and in the deep half of the stratum lacunosum - moleculare of the hippocampal subfield CA3.

2. A lateral perforant path from the lateral part of the entorhinal area to a superficial zone in the dentate moleclar layer and to the superficial part of the stratum lacunosum - moleculare of CA3.

It is known that dentate granule cells have axons which terminate in the hippocampus. These mossy fibers have been carefully mapped by Lorento de No (1934) and Blackstad et al (1970), with the granule cells of both the infra and supra pyramidal aspects of the dentate fascia giving off such fibers. Mossy fibers travel through the hilus of the dentate toward the pyramidal cells of CA3 where giant synapses are formed with the dendritic spines of the proximal region of the apical dendrites.

The axons of the giant CA3 pyramids divide, with one branch entering the fimbria and going to the septum, while the other branch remains within the hippocampus. Within the hippocampus, the CA3 cells send a course myelinated collateral back up towards the CA1 region. This Schaffer collateral system runs in the stratum radiatum (pyramidal apical dendrites) of CA1, making synapses en passage (Lorento de No 1934, Hjorth-Simonsen 1973).

The CA1 pyramidal cells send their axons out of the hippocampus via the alveus, where they continue back to the subiculum (Hjorth-Simonsen 1973) and then to the hypothalamus (Raisman 1966).

Extensive connections exist between the two hippocampi, crossing via the ventral and dorsal commissures. The ventral path consists of fibers running in the fimbria which turn back at the rostral end of the hippocampus to run in the fimbria on the opposite side of the midline. The dorsal path consists of a thin layer of fibers which run beneath the splenium of the corpus callosum at the posterior arch of the hippocampus where it begins to turn downwards.

All of the fields of the hippocampal formation have been demonstrated to have commissural connections, but not all are of homotopic origin (Gottlieb & Cowan 1973). They have also pointed out that each field of the hippocampus which contributes to the commissural projection also gives rise to an ipsilateral association pathway which follows the same intrahippocampal course and terminates within the same region. For example, the CA1 and fascia dentate receives input from the CA3 on both sides.

The hippocampus receives extrinsic afferents from a number of other structures, including primarily the entorhinal cortex, the medial septal area, and several brain stem sites. Chronister and White (1975) have noted that because of the position of the hippocampal formation, several sources of external input are possible. The pathways must enter one of three routes:

1. Travel toward the lateral border of the hippocampal formation either superficially or in the angular bundle so as to gain access to the entorhinal region.

2. Course supracallosally (ie. cingulum and stria lancisii) to enter the hippocampal formation through extremely dorsal regions.

3. Enter the hippocampal formation through the fornix-fimbrial system.

Part of the information that is passed to the hippocampal formation by way of the perforant path is taken from the information entering the entorhinal cortex. The entorhinal cortex is in a position to receive multi-synaptic cortico-cortical inputs of visual (Cowey & Gross 1970, Gross, Bender & Rocha-Miranda 1969), auditory (Gross, Schiller, Wells & Gerstein 1967), and somesthetic (Jones & Powell 1970) modalities coming from association areas of the frontal, parietal temporal and occipital lobes. The perforant path fibers from medial entorhinal area form en passage syanpses with granule cell dendrites (Blackstad 1958, Nafstad 1967, Raisman, Cowan and Powell 1965).

A major input to the hippocampus arises in the septal area. The septohippocampal fibers travel in both the dorsal fornix and the body of the fornix to enter the alveus. Septal afferents to the hippocampus appear to arise from the

medial septal nucleus and diagonal band nucleus following through the stria of Lancisii to the dorsal fornix, alveus, and fimbria (Daitz & Powell 1954, Lewis & Shute 1967). Termination appears primarily in the hilus of the dentate fascia and the region of CA2/CA3, with target layers being the stratum oriens and stratum radiatum. Although it is assumed by many authors that the septal projection is to the pyramidal cells and granule cells, the heavy concentration of terminals in the stratum oriens and in the zone just above the cell bodies has suggested to Mosko et al (1973) that the target cells may be the basket cell interneurons.

It has generally been assumed that the main hippocampal efferents (CA1 and CA3) would travel along the alveus and fimbria to be distributed by the pre- and post-commissural fornix system to several areas of the forebrain and midbrain. This concept must be revised due to the finding (Meiback & Siegal 1977, Swansen & Sowan 1977) that there are two efferent projection areas for the axons of CA1 and CA3. One is the lateral septal area, the other is the subicular complex.

PHYSIOLOGY

With the discovery by Jung and Kornmuller (1938) of hippocampal rhythmical activity (theta rhythm) came an interest in the slow wave relationships to behavior.

Hippocampal theta rhythm is one of the largest amplitude, most regular EEG phenomenon occurring in the mammalian brain. The hippocampal slow wave rhythm is interesting to study because of its large amplitude, its relatively stable frequency, and its striking behavioral correlates.

The Jung and Kornmuller study first reported rhythmical slow wave activity in a rabbit, with the wave seen following sensory stimulation. Green and Arduini (1954) suggested the slow wave was associated with arousal. The synchronized waves, in the frequency range of 4 - 7 Hz, were called theta waves. Later studies showed variations in frequency from 2 - 12 Hz in some species.

Stumpf (1965) identified three separate waveforms within the dorsal hippocampal formation. They are:

1. RSA - rhythmical slow activity or theta. A series of roughly sinusoidal waves varying in frequency from 4 - 12 Hz.

2. LIA - large amplitude irregular activity. Contains a slow component, has occasional large spikes, and lacks rhythmicity.

3. SIA - small amplitude irregular activity. Brief 1 - 2 second trains, and appears as a sudden reduction in amplitude.

Bland and Whishaw (1976) and Vanderwolf (1969) have noted a fourth kind of activity. It is a high frequency

15 - 50 Hz irregular wave recorded from the hilus of the dentate gyrus of the rat.

The amplitude of theta varies from 0 - 50 μv to 3 mv depending on the placement of the electrode (Robinson 1980). LIA, because of its irregular form is difficult to accurately estimate an amplitude for. All three major types of activity can occur at a single electrode placement with the pattern depending on the behavior of the animal.

The studies of J.D. Green (Green & Arduini 1954, Green et al 1961, Green et al 1960, Green & Petsche 1961, Petsche & Stumpf 1960) demonstrated that the hippocampal pyramidal layer, in particular the CA1 region, was a major area for the generation of theta activity. Several studies involving laminar profiles of amplitude and phase of theta rhythm have shown that there are two anatomically separate generators in the hippocampus of rabbits (Bland, Andersen, Ganes 1975, Winson 1976). Other studies looking at the topography of hippocampal theta rhythm have confirmed the two-generator concept in rats (Bland & Whishaw 1976) and cats (Bland et al 1979). These generators, whose locations are the stratum oriens of the CA1 region and the stratum moleculare of the dentate gyrus, are approximately 180° out of phase. Signals recorded in the stratum oriens are not isomorphic with the signals recorded in the dentate.

Although the signals from the two zones are phase-locked and approximately 180° out-of-phase, they have different shapes and show independent amplitude modulation.

The distribution of theta phase and amplitude has been measured by moving a recording electrode from the surface to the depth of the hippocampal formation, or by moving the electrode from one section of the hippocampus to another. In a depth profile, small amplitude activity is recorded in the neocortex with amplitude increasing with depth to a maximum of about 1 mV in stratum oriens (Bland, Andersen & Ganes 1975, Bland & Whishaw 1976, Winson 1974, 1976a, 1976b). Theta declines through the pyramidal layer then disappears about 50 - 150 u below the cell layer. In this null zone, which separates the CA1 theta generator from the dentate theta generator, theta is replaced by irregular activity. It is this null zone, in stratum radiatum, that marks the point of phase reversal. Below the null zone, theta reappears but is now 180° out-of-phase with the CA1 theta activity (Green, Maxwell, Schindler, & Stumpf 1960, Petsche & Stumpf 1960). Theta amplitude now increases with depth until it reaches a 2 mV maximum in the stratum moleculare of the ectal blade of the dentate gyrus. Theta activity is replaced with fast 15 - 50 Hz activity in the hilus region of the dentate, but reappears as a third maxima

in the endal blade. Theta of decreasing amplitude can be recorded ventrally through the thalamus and hypothalamus (Bland, Andersen, Ganes 1975, Bland & Whishaw 1976, Whishaw, Bland, Robinson & Vanderwolf 1976, Whishaw, Bland & Bayer 1978, Winson 1974, 1976a, 1976b).

The relationship between theta amplitude and phase at different points across the surface of the hippocampal formation has also been studied. Recent findings of Bland et al (1975) found no phase shift at all between widely spread homotypic points of the hippocampal surface.

It has been suggested that the two sites are a single generator separated by an iso-electric point, but this idea has been strongly argued (Bland, Andersen, Ganes 1975, Bland & Whishaw 1976). CA1 theta is abolished by cooling or surgical interference without disrupting the dentate theta (Bland, Andersen, Ganes 1975), making the notion of a common generator unlikely. Whishaw, Bland and Bayer (1978) attempted to selectively destroy the dentate generator by x-irradiating neonatal rats. However, RSA survived in both stratum oriens and in the upper blade of the dentate, although the density of granule cells was reduced by about 85%. In the lower blade, where granule cells were eliminated, no RSA amplitude peak was found.

Developmental work on rats (Leblanc and Bland 1979) and rabbits (Creery and Bland 1980) supports the hypothesis that there are two main generators of theta activity, the CA1 pyramidal region and the dentate region. Leblanc and Bland (1979) found that despite the difference in time course of morphogenesis, the prenatally formed CA1 pyramidal region and the postnatally formed dentate granule region developed in parallel with respect to the appearance of both types of theta activity at about 10 days. Theta frequency and amplitude developed to adult values during the next two weeks, but the activity in the CA1 remained smaller than in the dentate region.

Work on hippocampal unit discharges demonstrated that the CA1 pyramids were theta generators, several studies reporting rhythmic firing of CA1 pyramids (Green & Arduini 1954, Green & Machne 1955, Green Maxwell, Schindler & Stumpf 1960, Green Maxwell & Petsche 1961, Petsche & Stumpf 1960, Noda, Manohar & Adey 1969, Von Euler & Green 1960). Studies have shown a coupling of the extracellular discharges of CA1 pyramidal cells and dentate granule cells to hippocampal theta in animals anaesthetized with urethane (Bland, Andersen, Ganes & Sveen 1980, Vinogradova 1975). Bland et al (1980) have demonstrated that virtually all CA1 pyramidal cells and dentate granule cells were phase-locked to the negative

portion of the theta waves recorded from the corresponding region. When slow wave activity was irregular, with a low amplitude, there were usually few spontaneously active CA1 or granule cells. When the slow wave activity became regular and of higher amplitude, the activity of the units also increased. It has been reported that the degree of coupling varies, with dentate granule cells having higher modulation indices than the CA1 pyramids (Berry, Rinaldi, Thompson & Verzeano 1978, Bland et al 1980). Bland et al (1980) also noted that CA3 cells and basket cells had a lesser tendency to rhythmicity, with the former having the weakest coupling.

Theta Mechanisms in the Hippocampus - Inhibitory Circuits

A series of electrophysiological studies of inhibitory circuits within the hippocampus (Kandel & Spencer 1961a, 1961b, Spencer & Kandel 1961a, 1961b, 1961c, Kandel et al 1961) showed that inhibitory potentials in pyramidal cells were mediated by a direct axonal collateral of these cells with a possible interposed interneuron. Andersen and co-workers (Andersen & Eccles 1962, Andersen et al 1964a, 1964b) report that widespread inhibition is mediated via axon collaterals onto basket cells, which project to pyramidal cells. Thus, it seems possible that such defined inhibitory systems may be involved in the intra-hippocampal theta mechanism.

Intracellur Data

Fujita and Sato (1964) recordedintracellularly from rabbit CA1 cells and found that fluctuations in the voltage of the pyramidal cells were related to extracellular rhythmical slow activity. Specifically, in 61/71 cells, an intracellular theta rhythm was in phase with the gross extracellular theta. Theta positivity indicates cellular hyperpolarization and negativity depolarization, with population spikes observed during depolarization. The authors suggest both EPSP and IPSP involvement. According to Artemenko (1972), extracellular theta recorded from the pyramidal cell area has two components, with the negative phase consisting primarily of EPSPs of the basal and proximal apical dendrites of the basket cells and the positive phase are to subsequent hyperpolarization of the pyramidal cell body and dendrites.

EXTRACELLULAR DATA

A number of studies have found hippocampal units which fire in synchrony with hippocampal theta waves (Green et al 1960, Von Euler & Green 1960a, 1960b, Macador et al 1970, O'Keefe and Dostrovsky 1971, Ranck 1973, Feder & Ranck 1973, O'Keefe 1976). Ranck has identified two populations of hippocampal neurons on the basis of firing patterns and behavioral correlates (Feder & Ranck 1973, Ranck 1973).

The two populations are:

1. Theta cells
2. Complex-spike cells

Both cell types have single action potentials, but complex-cells produce occasional clusters of high frequency discharges referred to as complex spikes. The theta units increase their firing rate whenever theta rhythm appears and usually fire in bursts, with each burst locked to a particular phase of the theta cycle. The complex-spike cells seem to have no simple relation to theta. Fox and Ranck (1975) have mapped the units and found the theta cells largely in CA1 layer stratum oriens, CA3 stratum lucidum/radiatum and moleculare, and within the hilus of the dentate, whereas the complex-spike cells were largely in the layers of projection cell somas, the stratum pyramidale and dentate granule layer.

In view of such evidence, Fox and Ranck (1975) have suggested that theta cells are the basket cell interneurons of Cajal. In conflict with these data, Bland, Andersen, Ganes and Sveen (1980) have shown that while pyramidal and granule discharges are clearly coupled to theta, the basket cell discharge is not closely coupled.

EXTERNAL CIRCUITS INVOLVED IN THETA

Early studies on theta (Green & Arduini 1954) noted that theta production depended on the integrity of a neuronal circuit originating in the mesencephalic reticular formation, extending through the hypothalamus to the septum, and thence via the fornix to the hippocampus.

Ranck has studied the neurons of the medial and lateral septal nuclei to discover the septum's role in theta (Feder & Ranck 1973, Ranck 1973, Ranck 1975). In the medial septum there was a class of cells which fired continuously during the theta rhythm, suggesting a pacemaker role which drives the hippocampus during theta-related behavior. Numerous other studies have also regarded the septal inflow to the hippocampus as a driving force for theta (Gogolak, Stumpf, Petsche & Steve 1968, Green & Arduini 1954, Macadar, Roig, Monte & Budelli 1970, Morales, Roig, Monte, Macadar & Budelli 1971, Petsche, Stumpf & Gogolak 1962, Stumpf 1965). Recently it has been shown that the septohippocampal pathway is required for the production of dentate theta (Andersen Bland, Myhrer & Schwartzkroin 1979).

The effects of selective lesions of lateral, medial, and entire septum on CA1/dentate theta production has recently been studied (Sainsbury & Bland, 1981). They found:

1. Lateral septal lesions disrupted CA1 theta but not dentate theta.

2. Medial septal lesions affected CA1 and dentate theta.

3. Entire septal lesions abolished CA1 and dentate theta.

Such findings suggest that the septohippocampal pathways mediating theta inputs to the two generators are at least at one point anatomically distinct.

PHARMACOLOGY

The hippocampus with its simple lamellar structure and defined connections is an ideal system for the study of neurotransmitters and the associated receptor sites. The basic technique has involved the microiontophoretic application of drugs onto the desired tissue via extracellular pipettes - followed with recordings of both intra and extracellular activity. Storm-Mathisen (1977) notes that strong evidence exists for the neurotransmitter role of five putative transmitters. They are:

1. Gamma-amino butyric acid (GABA)
2. Acetylcholine (ACh)
3. Noradrenaline (NA)
4. Serotonin (5-HT)
5. Histamine (HA)

Evidence suggests that the neutral amino acid, GABA, may play an important inhibitory transmitter role in the mammalian central nervous system. Storm-Mathisen and Fonnum (1972) state that nearly all GABA within the hippocampus is present within intrinsic or local neurons. They found GABA unaffected by deafferentation through lesions of the perforant path from the entorhinal cortex and/or the intrinsic mossy fibers from dentate granule cells. Fimbrial lesions reduced GABA activity only 16% whereas acetyltransferase activity was reduced 85-90% in all areas. Other studies involving hippocampal afferent pathway lesions have confirmed that GABA uptake and the GABA enzyme GAD (glutamic acid decarboxylase) are not significatly influenced by such intervention (Nadler, Cotman & Lynch 1974, Storm-Mathisen 1974, Storm-Mathisen & Guldberg 1974, Storm-Mathisen 1975).

Iontophoretic data suggest GABA to be an inhibitory transmitter (Curtis, Felix & McLennan 1970) concentrated in the molecular layer and in the cellular layers containing basket cell synaptic terminal (Storm-Mathisen & Fonnum 1971, Storm-Mathisen 1972). Storm-Mathisen and Fonnum (1971) have noted that pyramidal and granule cell layers showed uniformly high levels of GAD (and hence GABA) activity which was compatible with the axosomatic inhibitory terminals concentrated in these areas. GABA injected towards pyramidal

somal regions has been seen to inhibit the spontaneous and induced activity of these pyramidal cells (Stefanis 1964, Curtis, Duggan, Felix, Johnston, and McLennan 1971). The biochemical evidence thus far mentioned seems to support the neurophysiological studies showing that interneurons in stratum oriens mediate the inhibition of pyramidal cells (Andersen, Eccles & Loyning 1964).

The presence of acetylcholine, along with its synthetic and degradive enzymes, has been known for many years. An important note, however, is that unlike the four other putative transmitters discussed, ACh is an excitatory agent. Histochemical studies in the rat (Shute & Lewis 1961) and the cat (Krnjevic & Silver 1965) provide evidence for a system of acetylcholinesterase - containing nerve fibers extending into the hippocampus from the medial septal nucleus via the fimbria. Neuroanatomical studies (Raisman 1966) and neurophysiological studies (Andersen, Bruland & Kaada 1961) also support the existence of such a pathway.

Acetylcholinesterase containing nerve fibers have been identified in the cat, which have a laminar distribution sparing pyramidal and granular cell body regions. The distribution of ACh appears highest in the bands of tissue adjacent to the cell body layers (Storm-Mathisen & Blackstad 1964, Fonnum 1970, Mosko, Lynch & Cotman 1973).

Straughan (1975) believes acetylcholinesterase staining in the hippocampus occurs within cholinergic nerves. Levels of acetylcholinesterase and choline transferase decline substantially with medial septal nucleus lesions. Straughan thus suggests hippocampal cholinergic elements input from the septal nucleus.

Early work postulated the existence of such a cholinergic septohippocampal pathway (Green & Arduini 1954, Stumpf 1965). It was observed that fimbrial transections decreased activity of both the choline acetyltransferase and the acetylcholinesterase (Lewis & Shute 1967, Lewis, Shute & Silver 1967). Work with anesthetized rabbits showed that stimulation of the septal surface caused a two and one-half fold increase in acetycholine (ACh) efflux from the hippocampus (Smith 1972, 1974). This stimulated efflux of Ach was abolished by acute lesioning of the septohippocampal pathway.

It was also noted (Dudar 1977) that removal of the septum abolished the effect of atropine on resting ACh release and on release evoked by MRF (mesencephalic reticular formation) stimulation from both the hippocampus and the cortex.

Application of ACh and ACh agonists microiontophoretically to various hippocampal regions in anesthetized

cats has been studied by a number of researchers (Stefanis 1964, Herz & Nacimiento 1965, Biscoe & Straughan 1966).

Units excited by ACh would be put into two distinct groups:

1. Hippocampal cortex
2. Dentate gyrus

It has been noted that CA1 pyramidal cells and dentate granule cells differ in their sensitivity to applied ACh (Bland, Kostopoulos & Phillis 1974). They found a higher proportion of granule cells responding, with the granule cells excitation being of faster onset than the CA1 pyramidal cells.

Histochemical studies (Fuxe 1965, Blackstad, Fuxe & Hokfelt 1967) suggest that 5-Ht and NE in the hippocampus is contained within nerve fibers originating in the brain stem. The 5-HT fibers arise from cells of the raphe nucleus of the midbrain while the NE fibers arise from cells of the locus coeruleus of the pons. In both systems, fibers ascend in the medial forebrain bundle and largely through the dorsal route-fimbria, superior fornix, cingulum - to reach the hippocampus. Histochemical work on 5-HT distribution has shown that terminals are found mainly in the stratum lacunosum - moleculare of the

hippocampal gyrus, and the subiculum (Fuxe & Jonsson 1974). Fuxe (1965) has described the distribution of NE terminal as follows:

1. High density in the stratum radiatum of the dorsal hippocampus CA3 area and the ventral hippocampus.

2. Moderately dense in the stratum lacunosum but not the stratum radiatum of the CA1 and CA2 areas of the dorsal dippocampus.

3. High density just below the granular layer in the dentate gyrus.

In the granular zone of the dentate and in stratum radiatum, most of the terminals form axodendritic junctions.

For the most part, NE and 5-HT in the hippocampus appear to be derived from afferents into the area rather than from intrinsic systems. Storm-Mathisen and Guldberg (1974), after unilateral lesions of the dorsal afferents, found 5-HT to be reduced 85% and NE reduced 70% in the hippocampus on the lesioned side. Lesions in the medial forebrain bundle produce a 70% loss of 5-HT and NE in the hippocampus (Heller, Seiden & Moore 1966, Moore & Heller 1967).

A number of studies have explored the effects of 5-HT and NE on the hippocampus. In anesthetized cats, 5-HT and NE have been shown to be weak depressants of neuronal firing (Stefanis 1964, Herz & Nacimiento 1965, Biscoe & Straughan 1966, Salmoiraghi & Stefanis 1967). In anesthetized

rats, 5-HT and NE were shown to be essentially depressant in action on pyramidal cells (Segal and Bloom 1974a). It was seen that 5-HT depression had a shorter latency of onset and did not persist after application ceased whereas the NE depression both developed and recovered slowly.

It has been shown that electrolytic lesions of the medial forebrain bundle induce a fall in histidine decarboxylase activity (the specific synthetic enzyme of brain histamine) in the ipsilateral cerebral cortex and hippocampus of the guinea pig; these results suggest the presence of an ascending histaminergic pathway (Haas, Wolf, Palacios, Garbarg, Barbin & Schwartz 1978).

Neurophysiological studies have made it clear that there are a number of excitatory nerve terminals in the hippocampus which are intrinsic and not dependent on the afferent input via the fimbria (Andersen, Blackstad & Lomo 1966, Andersen & Lomo 1966). The neurons included are:

1. Collaterals of pyramidal axons exciting inhibitory basket cells.

2. Schaffer collaterals from CA3 pyramidal axons running to CA1 neurons.

3. Commissural fibers derived from pyramidal cells of the contralateral hippocampus.

4. Perforant fibers from the entorhinal cortex to the dentate granule cells.

5. Mossy fibers to CA3 cells.

It is possible that the excitatory transmitter present at these intrinsic excitatory terminals is the acidic amino acid glutamate. Crawford and Connor (1973) found concentrations of glutamic acid in the apical dendritic layer of CA3 (where mossy fibers terminate), but this may reflect the role of the acid as a GABA precursor. Bland, Kostopoulos and Phillis (1974) provide additional support for glutamate as an excitatory agent. In their work glutamate was demonstrated to be effective in producing excitation during the time period of the blockade of ACh excitation.

HIPPOCAMPAL FUNCTION

The role of the hippocampus with respect to psychological processes and behavior has been a complex subject to investigate. A variety of functions have been assigned to the hippocampus, including emotional, motivational, arousal, attentional, perceptual, decisional, learning, and memory processes, as well as the control of voluntary and automatic motor behavior. Investigation of hippocampal function has taken four main paths:

1. Human Studies
2. Lesion Studies
3. EEG Studies
4. Unit Recording Studies

Human Studies

Almost any type of sensory stimuli cause instantaneous activation of different parts of the hippocampus, and the hippocampus in turn distributes many outgoing signals to parts of the limbic system, including the hypothalamus. On the basis of such input, it has often been stated that the primary role of the hippocampal function is to provide a means through which different incoming sensory signals can elicit appropriate limbic reactions. Papez (1937) discussed the limbic system circuit (hippocampus, amygdala, septum, cingulate gyrus, and entorhinal cortex) in terms of control of emotion. Kluver and Bucy (1938) with temporal lobe damage, found hypersexuality, tameness and psychic blindness to result. These effects are known as the Kluver-Bucy Syndrome and have been linked to amygdala control. In comparison, situations of hippocampal damage (removal) were not seen to produce any emotional deficits.

It is possible that the hippocampus plays a role in associating the affective characteristics of different sensory signals and then transmits the correlated information to

various limbic centers to at least partially control the material that a person will learn. When the hippocampus is removed bilaterally, a person develops anterograde amnesia, thus it is difficult or almost impossible for him to store new memories. On the basis of this, it has been postulated that the hippocampus is directly involved with some facet of memory storage or retrieval (Penfield & Milner 1958, Milner & Penfield 1955, Scoville & Milner 1957).

One problem with these findings, however, is that they involved massive temporal lobe damage. Thus, in such patients, the hippocampal complex was not removed alone, but with other structures such as the uncus or amygdala. It should be noted that animal lesion studies have been unable to reproduce the memory deficits, but Milner (1970) suggests possible species differences may be involved.

Lesion Studies - Animals

A very large number of studies have been conducted in order to clarify the effects of hippocampal lesions on behavior, but it is difficult to determine how much of an increase in an understanding of hippocampal function has occurred.

The hippocampus was long believed to be largely involved in the process of olfaction. Secondary olfactory

tracts pass from the nuclei of both the medial olfactory area and the lateral olfactory area into the hippocampus. However, ablation of the hippocampus failed to produce any impairment in olfaction (Allen 1940, Brodal 1947, Swann 1934). Recent work into olfactory input to the hippocampus concluded that the pathway from the pre-pyriform cortex via lateral entorhinal cortex to hippocampal neurons may enable olfactory inputs to effectively excite hippocampal neurons (Habets, Lopes da Silva & Mollevanger 1980). So, although the hippocampus may not directly mediate olfaction, it seems that the olfactory information may be important for some aspect of hippocampal function.

In order to encompass much of the information from lesion work, a number of researchers proposed theories of unitary function of the hippocampus. The hippocampus function was seen as sensory gating to direct attentional processes (Douglas 1967, 1972, 1975 Douglas and Pribram 1966). Douglas saw the unconventional behavior of hippocampal-damaged animals as being a result of response preservation or inability to withold a prepotent response. The response inhibition deficit observed is either a deficit in inhibition of connections between stimuli-response or a deficit in inhibition of attention to and/or reception of the stimulus which normally triggers the response.

Kimble viewed the hippocampus as an organ for the control of behavior through inhibitory processes, an equivalent to Pavlov's internal inhibition (Kimble 1968, Kimble & Kimble 1970, Silveira & Kimble 1968). Thus, the lesion is seen to impair the normal curbing or braking of behavior that the development of internal inhibition normally initiates. Altman, Brunner and Bayer (1973), in reviewing the behavioral similarities between hippocampectamized adults and normal juvenile rats have proposed a response-inhibition model. When an animal is in an aroused state, the hippocampus acts to brake (supress) the arousal. The hippocampectomized animals, when aroused areless observant than control lesion counterparts - they fail to slow down and examine potential cues.

Others have seen the loss of response inhibition as a symptom of a more fundamental information processing or attention deficit (Jackson & Strong 1969, Winocur & Bindra 1976, Winocur & Brekenridge 1973, Winocur & Mills 1970). In such a theory, the hippocampus is seen as an isolator and processor of stimulus cues necessary for proper response organization. Support comes from the finding that hippocampal damage impairs the ability to adjust to environmental changes, with a difficulty in organizing response sequences.

Nadel, O'Keefe and Black (1975) have criticized the inhibition theories as oversimplifying the data and ignoring variables such as nature of the task and inter-trial intervals. They propose a spatial information processing theory in which the hippocampus is seen as part of a neural spatial mapping system. O'Keefe and Nadel (1978) note that animals with damage to the hippocampal system do not explore new environments or novel objects, show profound deficits in the reversal of spatial discriminations, and are generally less able than normals at learning complex mazes. They conclude that the hippocampus constructs and stores cognitive maps or representations which capture the spatial layout of an animal's environment.

EEG Studies

Since the discovery by Jung and Kornmuller (1938) of hippocampal rhythmical activity (theta) there has been an interest in determining its significance. Hippocampal theta rhythm is one of the largest amplitude, most regular EEG phenomenon in the mammalian brain, but discovering its function has become a most challenging and perplexing problem. The appearance of this slow wave has been linked to possible functions performed by this structure in mediating arousal (Green & Aruini 1954), attention or orienting responses to

environmental stimuli (Bennett 1969, Grastyan et al 1959), information processing and memory consolidation (Adey et al 1960), and voluntary movement (Dalton & Black 1968, Vanderwolf 1969).

The rabbit hippocampal EEG displays largely theta activity in response to attention-attracting sensory stimulii. This arousal response was first noted by Jung and Kornmuller (1938) and further documented by Green and Arduini (1954). Theta rhythm was seen following somatic auditory, visual and olfactory stimulation. Green and Arduini (1954) proposed that the hippocampus participates in a specific way in the electrical activity of the brain when the animals are stimulated in any manner likely to alert or arouse them. The hippocampal response, interpreted as an arousal reaction, was proposed to be mediated by connections passing from the ascending activating system in the brain stem forward to the septum, with the dorsal fornix completing the relay to the hippocampus. Kramis et al (1975) have argued against the arousal theory, claiming that a rabbit which is highly alert need not show theta in its hippocampal EEG activity.

Bennett and his colleagues (Bennett 1969, 1970, 1971, 1975, Bennett and Gottfried 1970, Bennett, Herbert, and Moss 1973) have linked hippocampal theta rhythm to responses requiring attention or investigation. In a

lever-pressing task there was a high incidence of theta with the cue (Bennett et al 1973), while SIA predominated without the cue (Bennett & Gottfried 1970). It was concluded that theta was a correlate of attention to environmental cues, while SIA related to attention of proprioceptive cues. This view was contrary to earlier evidence finding theta persisting throughout extensive training on various tasks (Adey, Dunlop & Hendrix 1960, Brenner 1964, Elazar & Adey 1967, Holmes & Adey 1960, Lopes da Silva & Kamp 1969, Pickenhain & Klingberg 1967, Vanderwolf & Heron 1964, Whishaw 1972, Yoshii Shimokochi, Miyamoto & Ito 1966). An inherent problem in many studies is relating theta only to a concept of behavior (ie. arousal or attention) which is difficult to define and hard to quantify.

Others found theta associated with orienting, disappearing as the animals advanced through later trials of a learning task. It was concluded that the theta rhythm indicated hippocampal inactivation and excitation of the orienting reflex, while the functioning hippocampus displayed a desynchronized pattern with an inhibition of the orienting reflex (Grastyan, Karmos, Vereczkey, & Kellenyi 1966, Grastyan, Lissak, Madarasz & Dornhoffer 1959, Lissak & Grastyan 1960).

Adey and his associates (Adey 1961, Adey, Walter & Hendrix 1961, Adey, Kado & Didio 1962, Adey 1966) concurred

with Grastyan that theta appearance may signal hippocampal mediation of attention, however, they argued that theta may additionally reflect functions performed by the hippocampus in information processing and memory consolidation during learning and performance. They reported changes in the phase relations as learning progressed and suggested that theta was a correlate of memory trace deposition and possibly was required for later recall.

To avoid problems inherent in correlating occurrence of hippocampal electrical activity with inferred conditions (ie. arousal, attention, etc.), Vanderwolf and associates stressed more rigid and exacting behavioral correlations with hippocampal EEG (Vanderwolf 1967, 1969, 1975, Bland and Vanderwolf 1972a, 1972b, Sainsbury 1970, Whishaw, Bland & Vanderwolf 1972, Whishaw & Vanderwolf 1971, 1973, Whishaw 1972, Vanderwolf, Kramis, Gillespie & Bland 1975). Basing their assertions of studies on the rat, using noninferential descriptions of the animals' behaviors, Vanderwolf (1969, 1975) reported the following correlations between hippocampal EEG and behavior:

RSA (Type 1 Theta) - walking, running straight ahead or backing up, turning, rearing, jumping, climbing, struggling when held, swimming, head movements, postural changes, manipulation and digging.

LIA - behavioral immobility, licking, chewing, chattering of the teeth, salivation, piloerection, urination, defecation, pelvic thrusting and ejaculation, face washing, licking and biting, vocalization.

SIA - drowsy rat startled leaps to feet but does not run; rat jumps out of avoidance box and almost goes over side (requiring sudden halt of alternately jumping (RSA) and clinging to the wheel (SIA).

These results were generalized as follows:

RSA - correlated with voluntary movements

LIA - associated with immobility or "automatic" responses

SIA - occurred in 1 - 2 second trains apparently associated with the sudden cessation of on-going movement.

Vanderwolf (1969) found amplitude of RSA related to the gross amount of concurrent motor activity whereas frequency increases are associated with the initiation of movement.

There are exceptions to the close association between theta and voluntary movement that was described for the rat. Under certain conditions and in certain species locomotion is not accompanied by slow wave activity. During slow wave sleep, an animal produces LIA, but during REM sleep this LIA is replaced by theta rhythm (Vanderwolf 1969). The

presence of theta (analogous to Type 1) during paradoxical sleep was a sign that behavior-producing neural mechanisms were activated, but their normal appearance was blocked at the level of the spinal cord (Jouvet 1967). However, in spite of spinal inhibition, excitatory motor impulses descending from the brain do allow visible twitches and small movements of the limbs.

A second kind of theta (Type 2) can be seen in immobile rabbits spontaneously or upon presentation of a sensory stimulus (Jung & Kornmuller 1938, Green & Arduini 1954, Kramis, Vanderwolf & Bland 1975). Others have confirmed the presence of Type 2 immobility theta in rabbits and cats (Grastyan et al 1966, Parmeggiani 1967, Brown 1968, Bennett 1969, 1970). Immobility theta cannot be recorded in rats under similar conditions, which may be a species difference related to the animals' mode of responding in the environment. Rabbits, which produce immobility theta, tend to remain still while visually exploring the environment. On the other hand, rats do not produce immobility theta and react to their environment by active exploration.

Subsequent inquiry into the existence of immobility theta, involving behavioral observation and pharmacological manipulations, confirmed the suggestion that there are two types of theta activity (Vanderwolf, Kramis, Gillespie &

Bland 1975, Whishaw 1972). If a waking rabbit is given atropine SO_4 (anticholinergic drug) all traces of Type 2 theta during immobility or face washing are abolished but the Type 1 theta associated with movement persists (Kramis et al 1975, Vanderwolf, Kramis & Robinson 1978, Whishaw 1972). Such findings have led to the suggestion of two distinct reticulohippocampal pathways, each capable of producing RSA (Vanderwolf & Robinson 1981, Bland, Seto & Sinclair 1981). One pathway, resistant to atropine, but sensitive to anesthetics, is active in waking rats and rabbits only in relation to the occurrence of Type 1 behavior, while a second pathway, sensitive to atropine but resistant to volatile anesthetics, can be active during waking immobility, especially in rabbits. The existence of two pathways has been confirmed in a study of hippocampal rhythms evoked by rhythmical bursts of electrical stimulation of the medial septum (Kramis et al 1975). Further evidence of two systems comes from ontogenic studies of hippocampal electrical activity (LeBlanc & Bland 1979, Creery & Bland 1980).

Creery and Bland (1980) found that in the rabbit, motor abilities developed in parallel with dentate EEG activity. Low-amplitude irregular activity accompanied the uncoordinated motor behaviors of rabbits 6 to 7 days of age. By 8 days, the EEG-behavior correlation described by

Vanderwolf became evident: Certain types of movement were always accompanied by theta activity (Type 1) whereas other types (including immobility) were accompanied by irregular activity. By 14 days of age, a second, lower-frequency theta (Type 2) developed.

Differences in the rate of development of the two types of theta have been attributed to possible differences in the ontogenesis of different neurotransmitter systems.

It has been shown by a number of investigators that injection of cholinergic agonists (ie. acetylcholine, eserine) produces trains of Type 2 theta in rats and further enhances the rabbit's natural Type 2 theta, without producing any behavioral activation (ie. without producing movement) (Monnier & Romanowski 1961, Sailer & Stumpf 1957, Vanderwolf 1975). The effects of eserine can be counteracted by an anticholinergic drug (ie. atropine SO_4). Atropine is thought to antagonize muscarinic receptors of cholinergic neurons, thus reducing neuronal activity. In both eserinized animals and those treated with atropine sulfate, Type 1 movement theta is still seen.

It has been demonstrated (Creery and Bland 1980) however, that in very young rabbits (between 8 and 15 days of age) atropine sulfate is nonselective in its effects,

abolishing both movement-correlated theta (Type 1) and immobility-related theta (Type 2).

Like rats, rabbits display RSA when they are anesthetized with urethane, ethyl alcohol, or volatile anesthetics. Such theta is readily abolished with atropine SO_4 administration (Kramis et al 1975, Vanderwolf, Kramis & Robinson 1978, Whishaw 1972). If atropine SO_4 is administered first, followed by a volatile anesthetic, theta ceases to appear about the same time as Type 1 movements cease. A combination of volatile anesthetic and atropine SO_4 thus abolishes all theta.

To summarize the characteristics of the two types of theta:

Type 1 - will occur in awake animals if and only if Type 1 movements occur:
- insensitive to atropinic drugs
- sensitive to volatile anesthetics
- frequency range 7 - 12 Hz

Type 2 - can occur during behavioral immobility in the awake animal or during anesthesia:
- sensitive to atropinic drugs
- resistant to volatile anesthetics
- frequency range 4 - 7 Hz

It is important to note that while the actual waveform of the two types of theta do not differ appreciably, there is a difference in frequency ranges. However, some overlap of frequency is possible (Bland and Vanderwolf 1972a).

Unit Recording Studies

In the majority of single-unit experiments, the strategy is to simply record extracellular spike potentials from neurons in the hippocampus and study the correlation between the firing of the neurons and the behavior of the animal.

In a study modelled after visual system receptive field experiments, Ranck (1973) looked at the behavioral correlate of the neuronal firing (the events in the behavior of the animal; or the afferent inputs which are associated with neuronal firing) as well as the firing repertoire of the neuron (patterns of firing which actually occur). He distinguished two basic neurons, the theta unit and the complex spike unit. These two neuron types differed in spike morphology and duration, firing rate and pattern, relation to hippocampal EEG, and relation to behavior. Theta cells are defined as cells which increase (approximately double) their rate of firing, if and only if, there is a slow wave theta rhythm in the hippocampus (Ranck 1973).

The defining characteristic of a complex-spike cell is that it sometimes has a spontaneously occurring burst of about 2 - 10 action potentials of decreasing amplitude and increasing duration recorded extracellularly, with very short (less than 5 msec) interspike intervals. It is likely that both of these types contribute to the generation of the extracellular theta rhythm (Ranck 1973), even though the activity of the complex spike cells does not have any simple relation to the presence or absence of theta.

Due to their relative proportions and locations, it has been suggested that the class of projection cells and the class of complex spike cells have a very large overlap and the class of interneurons and the class of theta cells have a very large overlap (Fox & Ranck 1975). Thus, complex spike cells, found usually in stratum pyramidal and granulosum may be pyramidal/granule cells. Likewise, theta cells may be interneurons as they are usually found outside stratum pyramidal and granulosum at sites where anatomical studies have revealed many interneurons. Recent studies (Fox & Ranck, 1981) found that theta cells and "basket" cells (Andersen et al 1964) have a very large overlap and are probably identical. However, they note that it cannot at present be concluded that their "theta" cells and Andersen's "basket" cells are inhibitory interneurons.

O'Keefe and Dostrovsky (1971) observed that the activity of some hippocampal cells were correlated with the animal's position in space. It was suggested that the hippocampus may have a role of a spatial map (O'Keefe 1976, O'Keefe & Conway 1978, Olton, Branch & Best 1978). Several studies suggesting that complex spike cells fire in relation to spatial position, termed such cells place units (O'Keefe 1976, O'Keefe & Dostrovsky 1971, Nadel et al 1975). However, Ranck (1973) and O'Keefe (1976) note that it is not clear whether all complex spike cells have spatial properties, as some seem to fire to activity regardless of spatial location. O'Keefe and colleagues (O'Keefe & Dostrovsky 1971, O'Keefe 1976, O'Keefe & Conway 1978) describe the units in the CA1 field of the hippocampus of the freely-moving rat as falling into two general classes: Place Units, as mentioned above, and Displace Units. The Displace Units are those whose firing pattern relates to the behavior of the animals regardless of the animal's position in the environment. As with Ranck's cells, the Place and Displace Units can be distinguished not only on the basis of their behavioral/physiological correlate, but also in terms of parameters such as firing rate, firing pattern, etc. Ranck's complex-spike cells show the same configuration as the Place Units, and the theta cells are clearly identical to the Displace Units (O'Keefe & Nadel 1978).

O'Keefe and Nadel (1977) suggest that the hippocampus plays an important function in the spatial orientation of animals. There are two spatial co-ordinate systems involved: The first system operates in an egocentric spatial framework, noting changes with respect to the environment whenever movement occurs. The second system is one of absolute co-ordinates, based on stable environmental features. It is assumed that place cells acquire information about the environment through sensory inputs via entorhinal cortex, while the theta-generating inputs ascending by way of the septal nucleus provides information about the animal's movement. It is the interaction of these two systems that generates the cognitive map of the environment. It is suggested that these maps provide a basis for objective spatial cognition for a variety of purposes including food and water gathering, navigation and territoriality. Additional support for the spatial hypothesis comes from the observed effects of hippocampal damage. The direct effects involve spatial deficit including the loss of novelty-detection/exploration and of place learning (Maut 1972, O'Keefe, Nadel, Keightley & Kill 1975, Black, Nadel & O'Keefe 1977, Olton, Branch and Best 1978).

Objectives

The hippocampus, because of its highly organized structure, serves as an excellent model system for electrophysiological and pharmacological study. The purpose of the present investigation is to study whether theta cells in the CA1 area participate in both types of theta, in the freely moving rabbit. More specifically, the following questions were asked:

1. Do the same cells participate in both Type 1 and Type 2 theta;
2. If the same cells participate in both kinds of theta, what is the relationship between unit discharges and phase of theta;
3. What is the relationship of theta cell discharge patterns to three behavioral conditions:
   Type 1 theta behaviors (voluntary motor patterns). These include walking,hopping, postural shifts, head movements and manipulatory movements using the paws.

   Type 2 LIA behaviors (automatic motor patterns). These include alert immobility, chewing, lapping, and grooming.

Type 2 Theta behavior (alert immobility with presentation of sensory stimuli). Sensory stimuli consisted mostly of pure auditory tones, with occasional hand claps, whistles, and hand waves.

The direction that this research has taken has involved two components. The first part consisted of observational studies of CA1 layer cell relationships to theta and behavior in the freely-moving, unanesthetized rabbit. The second part consisted of observational studies of such relationships under pharmacological manipulation in the freely-moving rabbit.

METHODS

Subjects and Surgical Procedure

The experimental subjects were 42 male and female juvenile Dutch-belted rabbits (Orcytolagus cuniculus) (2.2 - 2.8 kg). The animals were prepared for standard sterotaxic surgical procedures using sodium pentobarbitol (30 mg/kg, marginal ear vein), with supplemental doses administered interperitoneally, as required. A rectal probe temperature servo system monitored and maintained the body temperature at 37°C.

Electrolytically sharpened and kynar insulated tungsten microelectrodes (1u tip; 5 - 10 megohms at 100 Hz) were used for recording. The rabbit's head was held so that the plane between bregma and lambda was horizontal. A reference electrode was implanted in the dentate gyrus region with co-ordinates: Posterior bregma, 5.0 mm; lateral midline 5.0 mm; and ventral to dorsal surface 4.8 mm. This reference electrode was usually implanted in the right hippocampus. At the same posterior and lateral co-ordinates a 3 mm trephine hole was drilled in the contralateral side of the skull. A number 20 nylon nut was then placed over the hole to accommodate the moveable microelectrode drive system.

The ground electrode was a male Winchester subminiature connector soldered to a jeweller's screw and placed

in the skull near the midline, anterior to bregma. An uninsulated tungsten rod served as an indifferent electrode and was placed opposite to the ground anterior to bregma. The respective leads were attached to a female 9-pin amphenol plug and the entire assembly was fixed to the skull with #18-8 pan-head screws and dental acrylic.

Testing procedures were initiated after a minimum one-week post-operative period.

Electrical Recording

Units were isolated using a modification of Ranck's (1973) moveable microdrive system we have described previously (Bland, Sinclair, Jorgenson & Keen, 1980). The microdrive was placed in the well at each testing session. Once electrical contact was made at the dural surface, the number of complete revolutions (approximately 321μ/revolution) were monitored. Most CA1 layer units were recorded at 9 - 10 turns (and most dentate layer units at 15 - 16 turns). Phase relations of theta between the dentate reference electrode tip location during the experiment (see Bland & Whishaw, 1976). After the termination of a recording session, electrode locations were marked by passing a DC current (3 - 5 μA, 3 sec, electrode negative). At the end of the experiment, the animals were sacrificed with an overdose

of pentobarbital, perfused with saline and Formalin, and the brains removed. Microelectrode tip and track locations were reconstructed from unstained brain sections, prepared by the frozen technique.

Signals from the brain were passed through two dual field-effect transistors (2N5545) arranged in a circuit described by Rosetto and Vandercar (1972) and located in the male Amphenol plug assembly. They were led through a 9-pin electromechanical commutator and into a Grass Model P511 wide-band AC preamplifier, with the low filter set at 1 Hz and the high filter at 10 kHz. The signals from the reference electrode were then attenuated and displayed using a Grass Model 7B polygraph and a series 5100 Tektronix storage oscilloscope. The polygraph amplifiers were generally set at 1 Hz and 35 Hz for the ½ amplitude low and high filter settings, respectively. The signals from the moveable microelectrode were, in addition to this, led off from the P511 preamplifier to a Kronhite filter and then to the oscilloscope. Filter settings for the unit activity were narrow band (300 Hz to 10 kHz). Unit activity was also recorded on one channel of the audio input of a Sony Betamax Video recorder, while the slow wave activity from one of the microelectrodes was frequency modulated and recorded on the

other audio channel. All signals were stored on an Ampex PR2200 FM tape recorder for subsequent computer analysis.

Behavioral Observations

Behavioral testing was carried out in a large Faraday cage (122 x 65 x 61 cm), the front and top of which was clear lucite, screened on the inside with copper. Once a unit was isolated, 10 - 20 minutes were allowed to pass. If the unit was judged to be stable, testing began. Behavior was coded on the FM tape recorder by pressing two buttons which produced 1V, DC square waves of opposite polarity. For 10-minute periods, two randomly selected spontaneous behaviors were coded by an observer looking only at the animal. Behaviors were grouped into three categories:

1. Type 1 - Motor behaviors
2. Type 2 - LIA behaviors (including alert immobility)
3. Type 2 Theta behavior - alert immobility with sensory stimulii presented (see Vanderwolf & Leung, 1981.

Immobility was confirmed by a movement-sensing system described previously (Creery & Bland 1980, Leblanc & Bland 1979). The animal's behavior and corresponding unit activity were also recorded on a Sony Betamax Video recorder using a

split-screen technique. Sweep speed for this display was 5 msec/div. Sensory stimulii consisted mainly of pure tones presented through dual speakers mounted on either side of the box and prerecorded on a Sony tape deck. Occasionally, hand waves were presented as visual stimulii.

Pharmacological Manipulations

In a number of recording sessions, after unit stability was clearly demonstrated, pharmacological inquiry was undertaken with interperitoneal drug administration followed by behavioral observation. In some instances, atropine SO_4 and eserine were tested independently, while at other times administration of drugs occurred in sequence. Dosage levels, interperitoneally, were for eserine 1 mg/kg and for atropine SO_4 50 mg/kg.

ANALYSIS

Data from the three behavioral categories were analysed by a PDP 11-34 computing system. The procedures for theta-spike analysis can be broken down into a number of discrete steps, both conceptually and operationally. At each of the separate steps a variety of parameters (ie. time base for data display, number bins, etc.) may be specified or operations performed. Conceptually, the steps are as follows:

1. Data acquisition
2. Data selection
3. Trigger point selection
4. Display of raw data with trigger points
5. Normalize cycles; average and produce histograms
6. Cross-correlation of appropriate data

Specifically, slow wave signals were analogue to digitally converted, and in the case of theta waves, the positive peaks marked. Unitary discharges were first led into an Ortec single channel analyser (window discriminator) and then entered into the computer as a digital event. Seven data plots were put out on an X-Y plotter:

1. Raw data sample (1 sec) - slow wave activity with simultaneous unitary discharges;
2. Histogram of theta frequency;
3. Interspike interval histogram;
4. Normalized theta waves with standard errors of amplitudes;
5. Histogram of unit discharges plotted against phase of normalized theta;
6. Unit discharges plotted directly on normalized theta waves;
7. Cross-correlation of unit discharges and theta activity

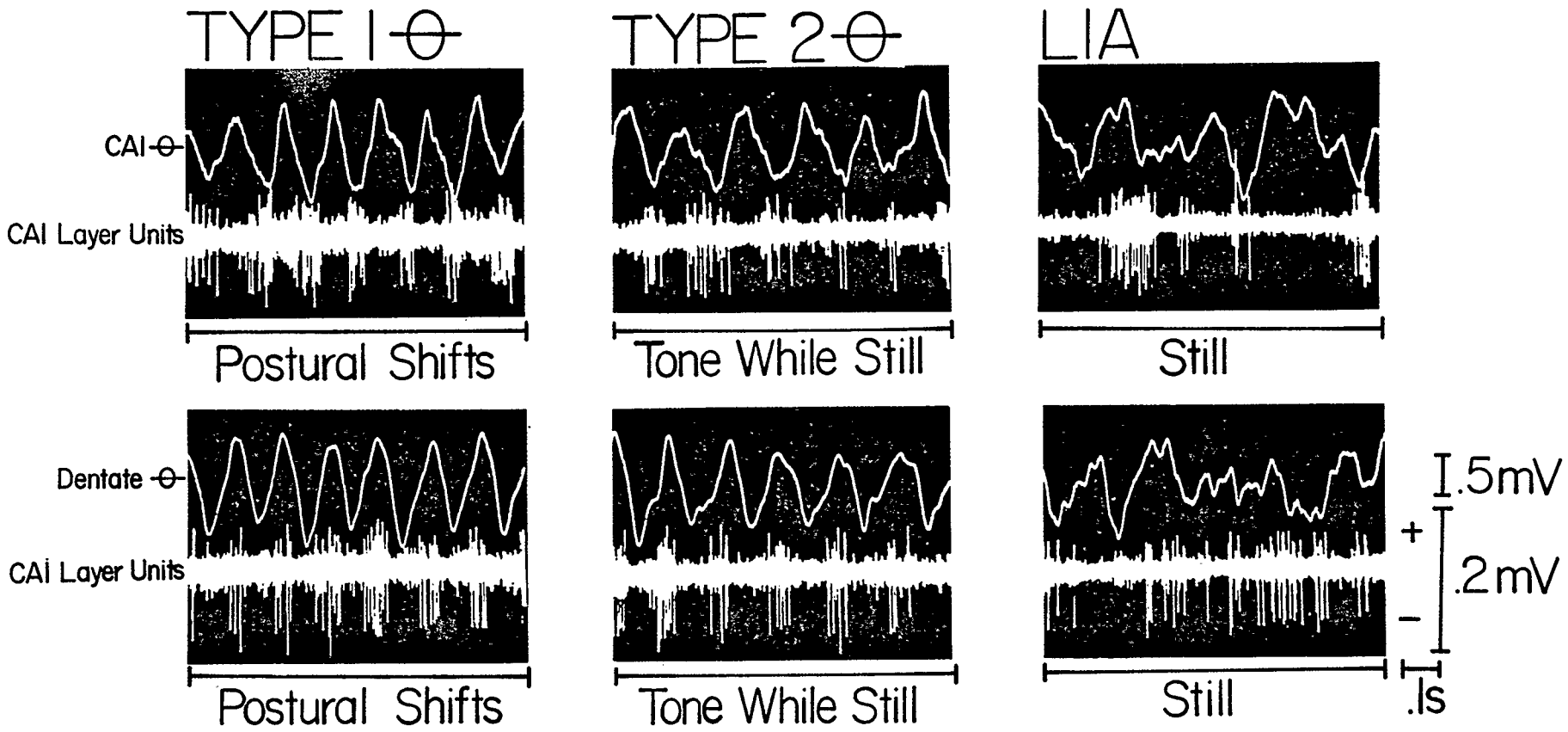
Results

A total of 54 theta unit groups were isolated and studied. Out of these, 19 CA1 layer unit groups were identified histologically and held long enough to collect data for all three behavioral categories. Theta cells were defined using Rancks (1973) criteria that they increase their firing rate if, and only if, theta slow wave activity was present.

CA1 Layer Theta Units

CA1 layer theta units had durations of .3 to .4 msec measured across the negative component of the spike (narrow band recording). The relationships of the firing repertoires of CA1 layer theta units to slow wave activity and behavior are shown in Figure 1. The upper panels illustrate the three behavior categories with the slow wave activity taken from the local electrode. The lower panels show the same behaviors with the slow wave taken from the dentate reference electrode. Note that the units began discharging just prior to the negative peak of the local theta wave and that there were fewer discharges per theta wave in the Type 2 condition. During alert immobility accompanied by large amplitude irregular slow wave activity, the units discharged in an irregular fashion, with a marked tendency to fire in a bursting fashion. When the same units were compared to theta recorded from the denate reference electrode, they discharged just before the positive peaks.

Fig. 1. CA1 layer theta units and simultaneous slow waves from the local (upper panels) and the dentate reference (lower panels) electrodes. Duration of behavior is indicated by the bars under each panel. Note that the units discharge rhythmically just prior to peak negativity of the local theta wave and just prior to peak positivity of the dentate reference theta wave. Units discharge irregularly when the slow wave is irregular.


TYPE 1
TYPE 2
LIA
CAI
CAI Layer Units
Postural Shifts
Tone While Still
Still
Dentate
CAI Layer Units
Postural Shifts
Tone While Still
Still
1.5mV
+
.2mV
−
.1s

Computer analysis of the Type 1 behavior category and units of CA1 layer theta cells compared to the local theta, is presented in Figure 2. Note the lower amplitude of the local CA1 theta in Figure 2A, recorded during the Type 1 behavior category. Figure 2B gives the histogram of the durations of theta for the total sample time of 15 sec. The modal frequency was 6.7 Hz and the mean frequency was 6.5 Hz. The first order interspike interval histogram of unit discharges is shown in Figure 2C. The peak interspike interval was 2 msec and the mean interspike interval was 10.1 msec. The total number of theta waves for the Type 1 category (93) are shown normalized with respect to time, in Figure 3A. Figure 3B illustrates the histogram of the occurrence of unit discharges (1409) in relation to the phase of the normalized theta waves. The mean phase of unit discharges was 162°. These data are also given in Figure 3C with the unit discharges represented as an actual point on the normalized theta waves. The result of the theta-spike cross-correlation is shown in Figure 3D. The maximum phase correlation of unit discharges occurred at 144° with a Rho value of .96.

Computer analysis of the Type 2 behavior category and units of CA1 layer theta cells compared to the local theta, is presented in Figure 4. Note the lower amplitude of the local CA1 theta in Figure 4A, recorded during the Type 2 be-

Fig. 2. Computer analysis of the Type 1 theta behavior category for the CA1 layer theta units and local theta from Figure 1. A. Raw Data Plot. Upper trace is the analogue to digital conversion of the slow wave activity. Lower trace is the unit activity converted to a digital event. Only 1 second of the total sample is shown. B. Histogram of the duration of movement theta for the total sample (15 sec.) (modal frequency = 6.7 Hz, $\bar{x}$ frequency = 6.5 Hz). C. First order interspike interval histogram of unit discharges (Peak ISI - 2 msec; $\bar{x}$ = 10.1 msec.).

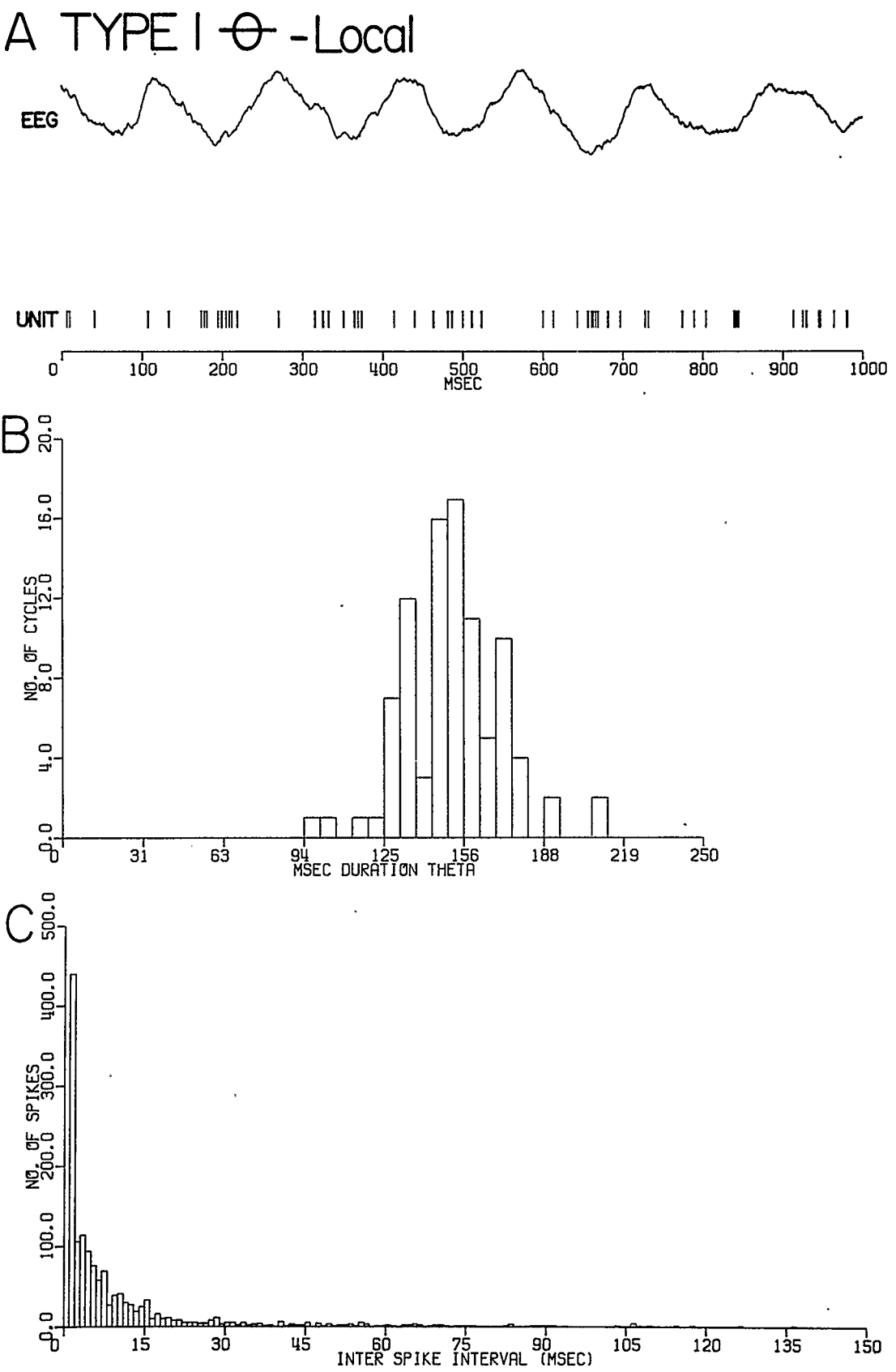
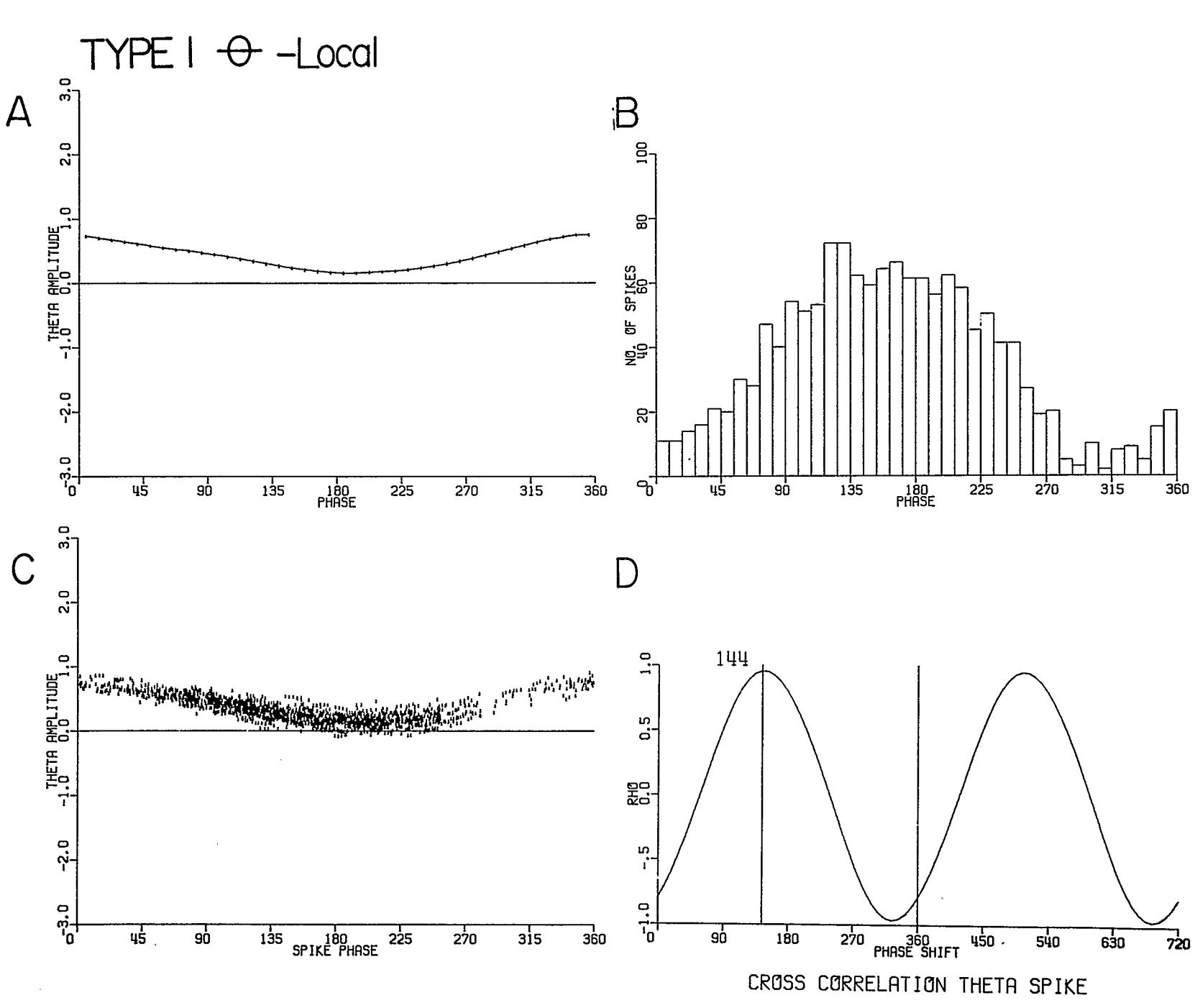

A TYPE I -Local
EEG
UNIT
0
100
200
300
400
500
600
700
800
900
1000
MSEC
B
NO. OF CYCLES
0.0
4.0
8.0
12.0
16.0
20.0
0
31
63
94
125
156
188
219
250
MSEC DURATION THETA
C
NO. OF SPIKES
0.0
100.0
200.0
300.0
400.0
500.0
0
15
30
45
60
75
90
105
120
135
150
INTER SPIKE INTERVAL (MSEC)

Fig. 3. Computer analysis of the Type 1 theta behavior category for the CA1 layer theta units and local theta from Figure 1. A. Total number of theta waves for the sample (93), normalized with respect to time. 0° and 360° represent positive peaks, with 180° representing peak negativity. Small vertical bars are the standard error of the amplitudes. B. Histogram of the occurrence of unit discharges (1409) plotted against the phase of the normalized theta waves ($\bar{x} = 162^{\circ}$). C. Same data as in B, except that unit discharges are represented as an actual point on the normalized theta waves. D. The result of cross-correlating A and B. The maximum phase correlation of unit discharges occurred at 144° with a Rho value of .96.


TYPE I -Local
A
THETA AMPLITUDE
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
0
45
90
135
180
225
270
315
360
PHASE
B
NO. OF SPIKES
100
80
60
40
20
0
PHASE
C
SPIKE PHASE
D
144
RHO
1.0
0.5
0.0
-.5
-1.0
90
450
540
630
720
PHASE SHIFT
CROSS CORRELATION THETA SPIKE

havior category. Figure 4B gives the histogram of the durations of theta for the total sample time of 12 seconds. The modal frequency of immobility theta was 6.5 Hz with a mean frequency of 6.0 Hz. The first order interspike interval histogram of unit discharges is shown in Figure 4C. The peak interspike interval of unit discharges was 2 msec with a mean interspike interval of 17.3 msec. The total number of theta waves for the Type 2 category (68) are shown normalized with respect to time, in Figure 5A. Figure 5B illustrates the histogram of the occurrence of unit discharges (582) in relation to the phase of the noramlized theta waves. The mean phase of unit discharges was 154°. These data are also given in Figure 5C with the unit discharges represented as an actual point on the normalized theta waves. The result of the theta-spike cross- correlation is shown in Figure 5D. The maximum phase correlation of unit discharges occurred at 144° with a Rho value of .89.

Computer analysis of the Type 1 behavior category and units of the CA1 layer theta cells, compared to the dentate reference theta, is presented in Figure 6. Note the larger amplitude of the dentate reference theta in Figure 6A, recorded during the Type 1 behavior category. Note from this figure that the units discharged close to the positive peaks of the dentate theta waves. Figure 6B gives the histogram

Fig. 4. Computer analysis of the Type 2 theta behavior category for the CA1 layer theta units and local theta from Figure 1. A. Raw data plot. Upper trace is the analogue to digital conversion of the slow wave activity. Lower trace is the unit activity converted to a digital event. Only 1 second of the total sample shown. B. Histogram of the duration of immobility theta for the total sample (12 sec) (modal frequency = 6.3 Hz; $\bar{x}$ frequency = 6.0 Hz). C. First order interspike interval histogram of unit discharges (peak ISI = 2 msec, $\bar{x}$ ISI = 17.3 msec).

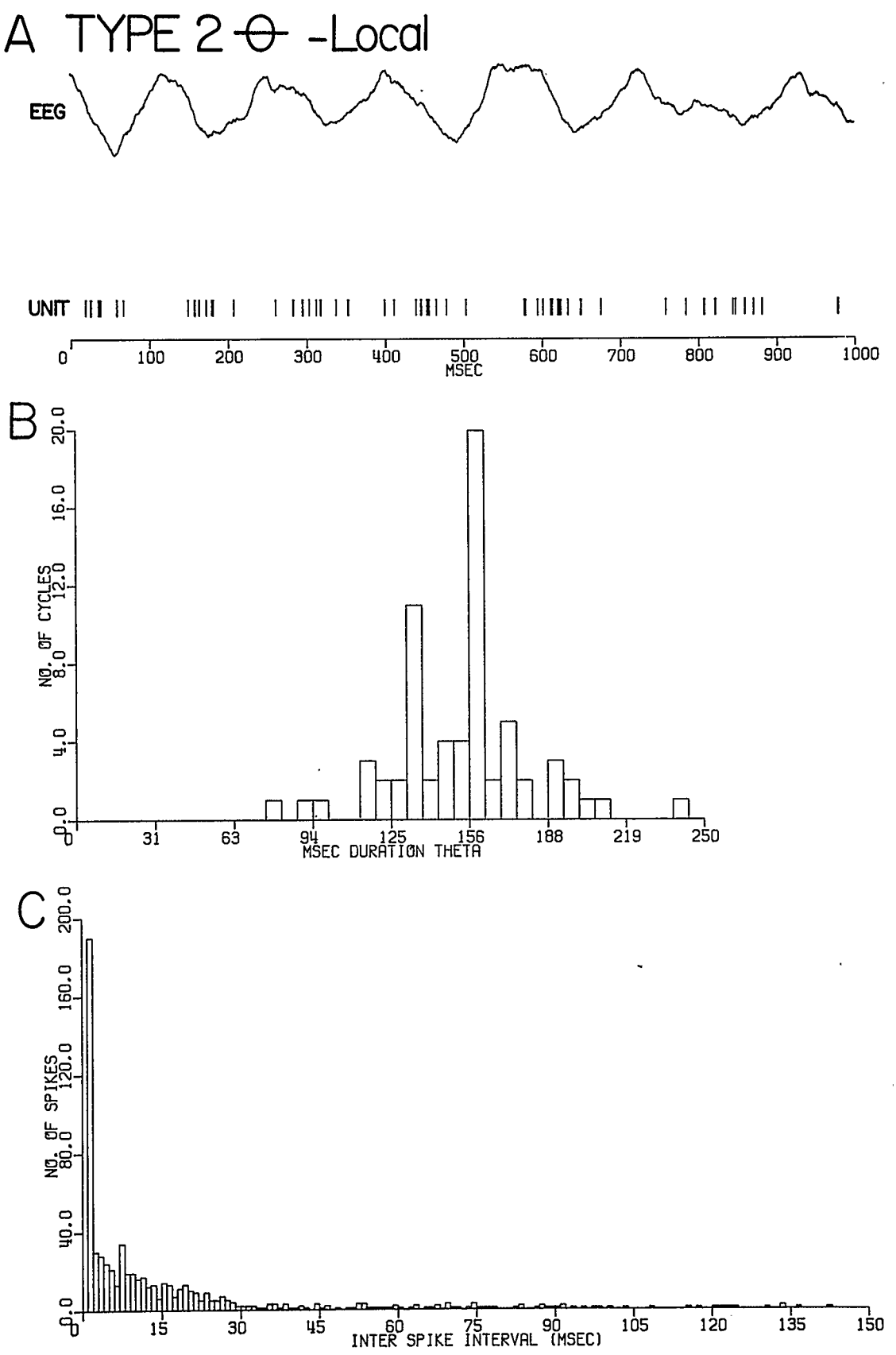

A TYPE 2 -Local
EEG
UNIT
0
100
200
300
400
500
MSEC
600
700
800
900
1000
B
NO. OF CYCLES
0.0
4.0
8.0
12.0
16.0
20.0
31
63
94
125
156
188
219
250
MSEC DURATION THETA
C
NO. OF SPIKES
0.0
40.0
80.0
120.0
160.0
200.0
0
15
30
45
60
75
90
105
120
135
150
INTER SPIKE INTERVAL (MSEC)

Fig. 5. Computer analysis of the Type 2 theta behavior category for the CA1 layer theta units and local theta from Figure 1. A. Total number of theta waves for the sample (68), normalized with respect to time. 0^{o} and 360^{o} represent positive peaks, with 180^{o} representing peak negativity. Small vertical bars are the standard error of the amplitudes. B. Histogram of the occurrence of unit discharges (582) plotted against the phase of the normalized theta waves ($\bar{x} = 154^{o}$). C. Same data as in B, except that the unit discharges are represented as actual points on the normalized theta waves. D. The result of cross-correlating A and B. The maximum phase correlation of unit discharges occurred at 144^{o} with a Rho value of .89.

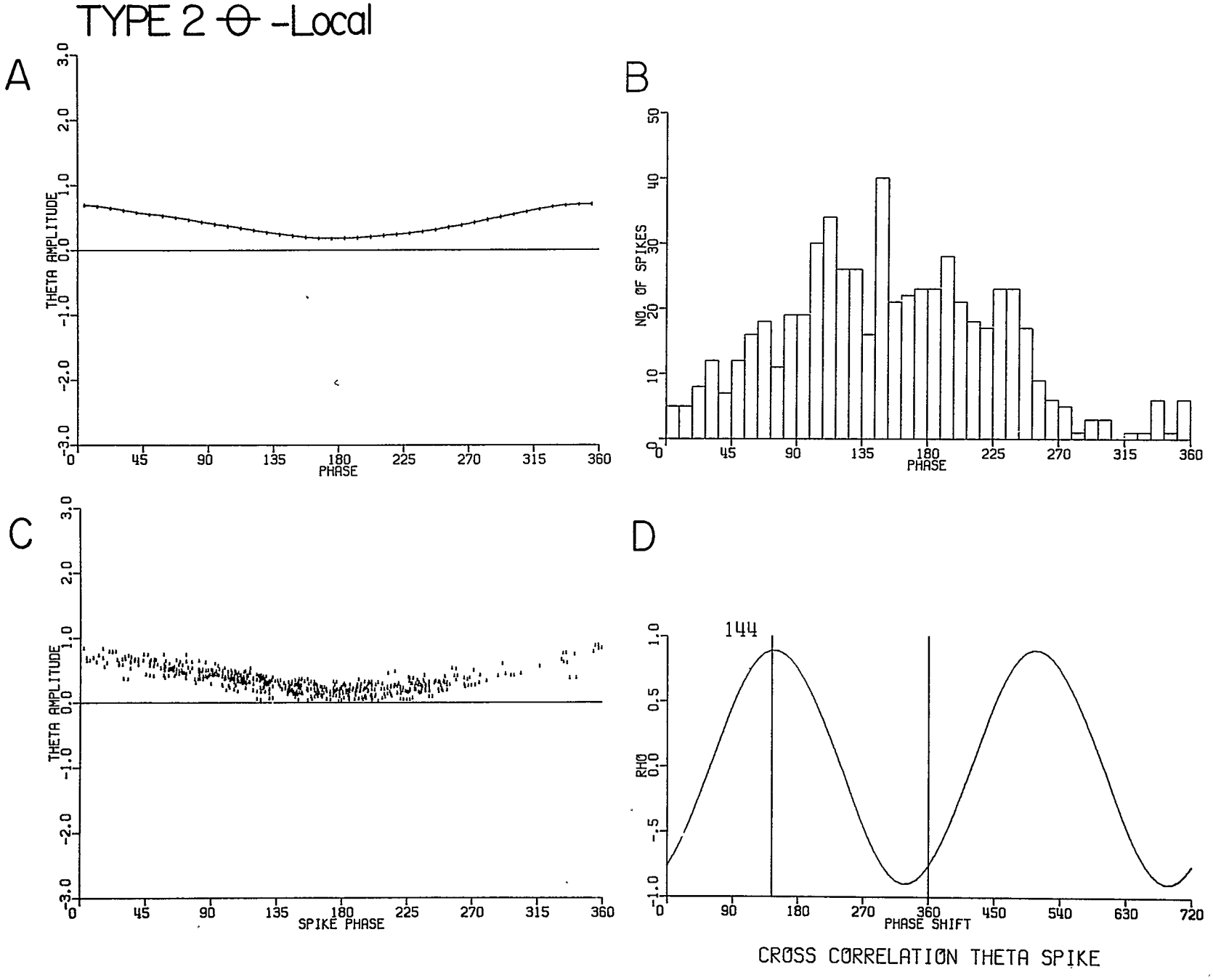

TYPE 2 θ -Local
A
THETA AMPLITUDE
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
0
45
90
135
180
225
270
315
360
PHASE
B
NO. OF SPIKES
50
40
30
20
10
0
PHASE
C
SPIKE PHASE
D
144
RHO
1.0
.5
0.0
-.5
-1.0
90
180
270
360
450
540
630
720
PHASE SHIFT
CROSS CORRELATION THETA SPIKE

Fig. 6. Computer analysis of the Type 1 theta behavior category for the CA1 layer theta units and dentate reference theta from Figure 1. A. Raw Data Plot. Upper trace is the analogue to digital conversion of the slow wave activity. Lower trace is the unit activity converted to a digital event. Only one second of the total sample shown. B. Histogram of the duration of movement theta for the total sample (22 sec) (modal frequency = 6.9 Hz; $\bar{x}$ frequency = 6.3 Hz). C. First order interspike interval histogram of unit discharges (Peak ISI = 2 msec; $\bar{x}$ frequency = 6.3 Hz). C. First order interspike interval histogram of unit discharges (Peak ISI = 2 msec; $\bar{x}$ ISI = 12.5 msec).

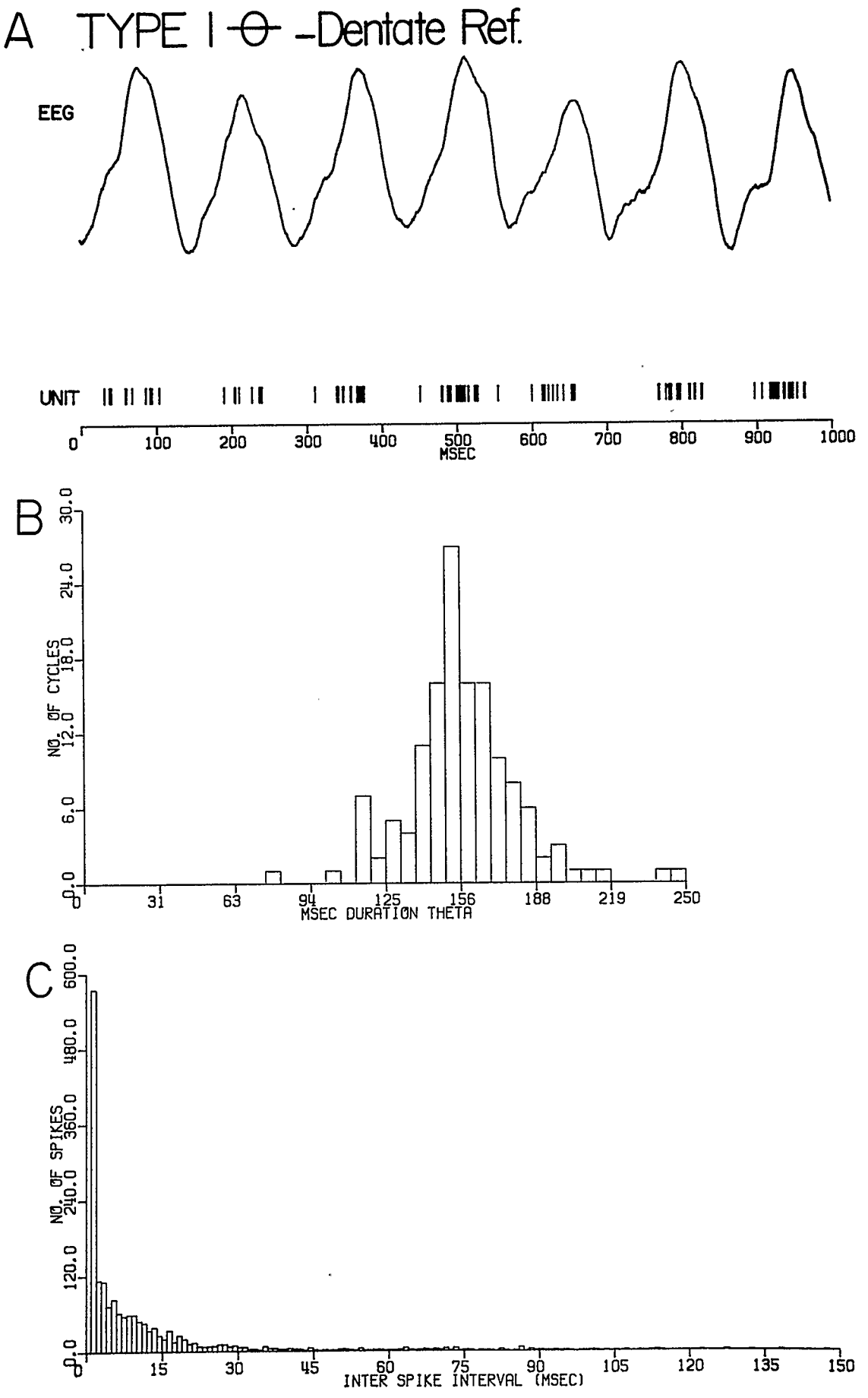
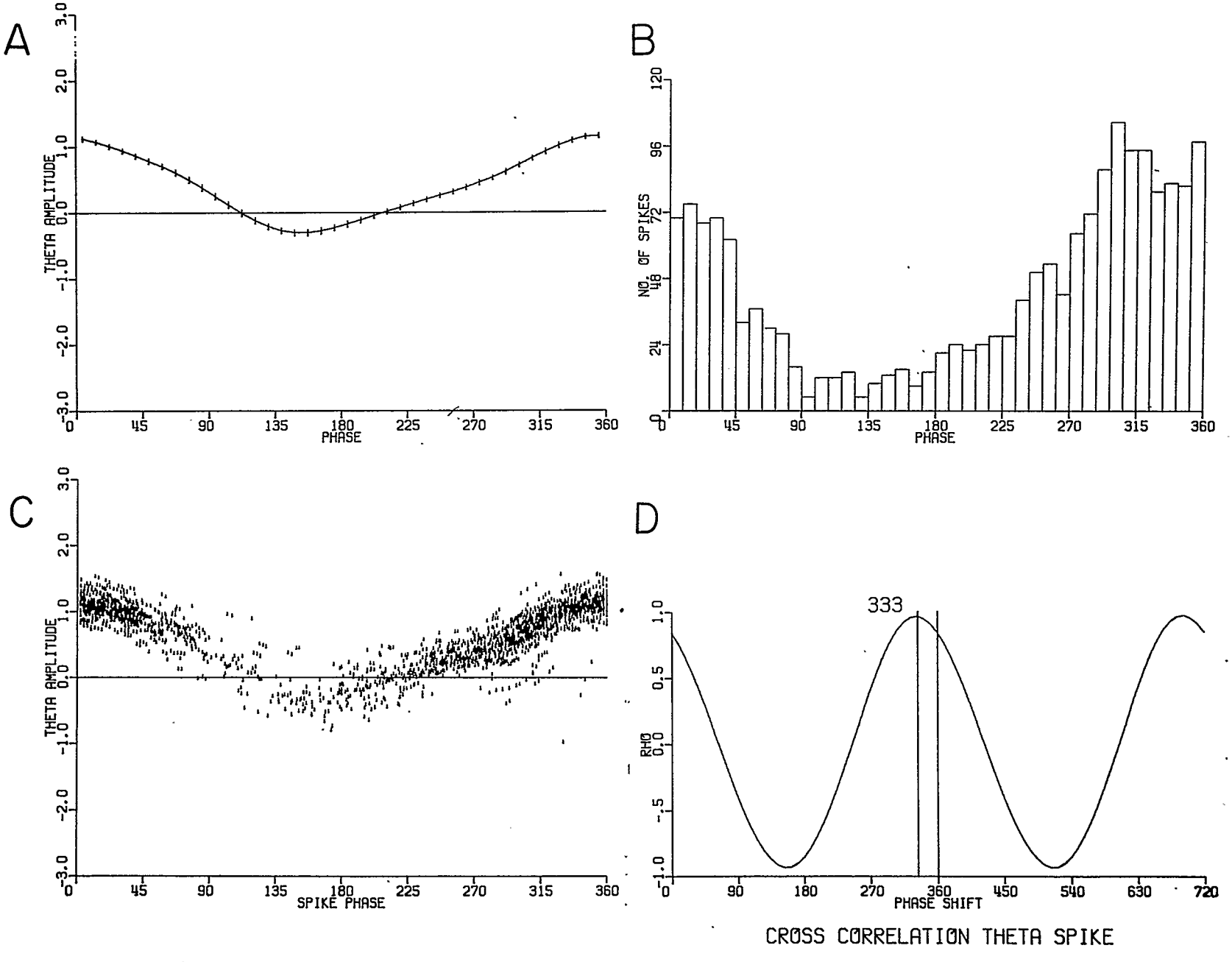

A TYPE I -Ø- -Dentate Ref.
EEG
UNIT
0
100
200
300
400
500
MSEC
600
700
800
900
1000
B
NO. ØF CYCLES
0.0
6.0
12.0
18.0
24.0
30.0
0
31
63
94
125
156
188
219
250
MSEC DURATIØN THETA
C
NO. ØF SPIKES
0.0
120.0
240.0
360.0
480.0
600.0
0
15
30
45
60
75
90
105
120
135
150
INTER SPIKE INTERVAL (MSEC)

Fig. 7. Computer analysis of the Type 1 theta behavior category for the CA1 layer theta units and dentate reference theta from Figure 1. A. Total number of theta waves for the sample (140), normalized with respect to time. 0^{o} and 360^{o} represent positive peaks, with 180^{o} representing peak negativity. Small vertical bars are the standard error of the amplitudes. B. Histogram of the occurrence of unit discharges (1780) plotted against the phase of the normalized theta waves ($\bar{x}$ phase = 213^{o}). C. Same data as in B, except that the unit discharges are represented as an actual point on the normalized theta waves. D. The result of cross-correlating A and B. The maximum phase correlation of unit discharges occurred at 333^{o} with a Rho value of .96.

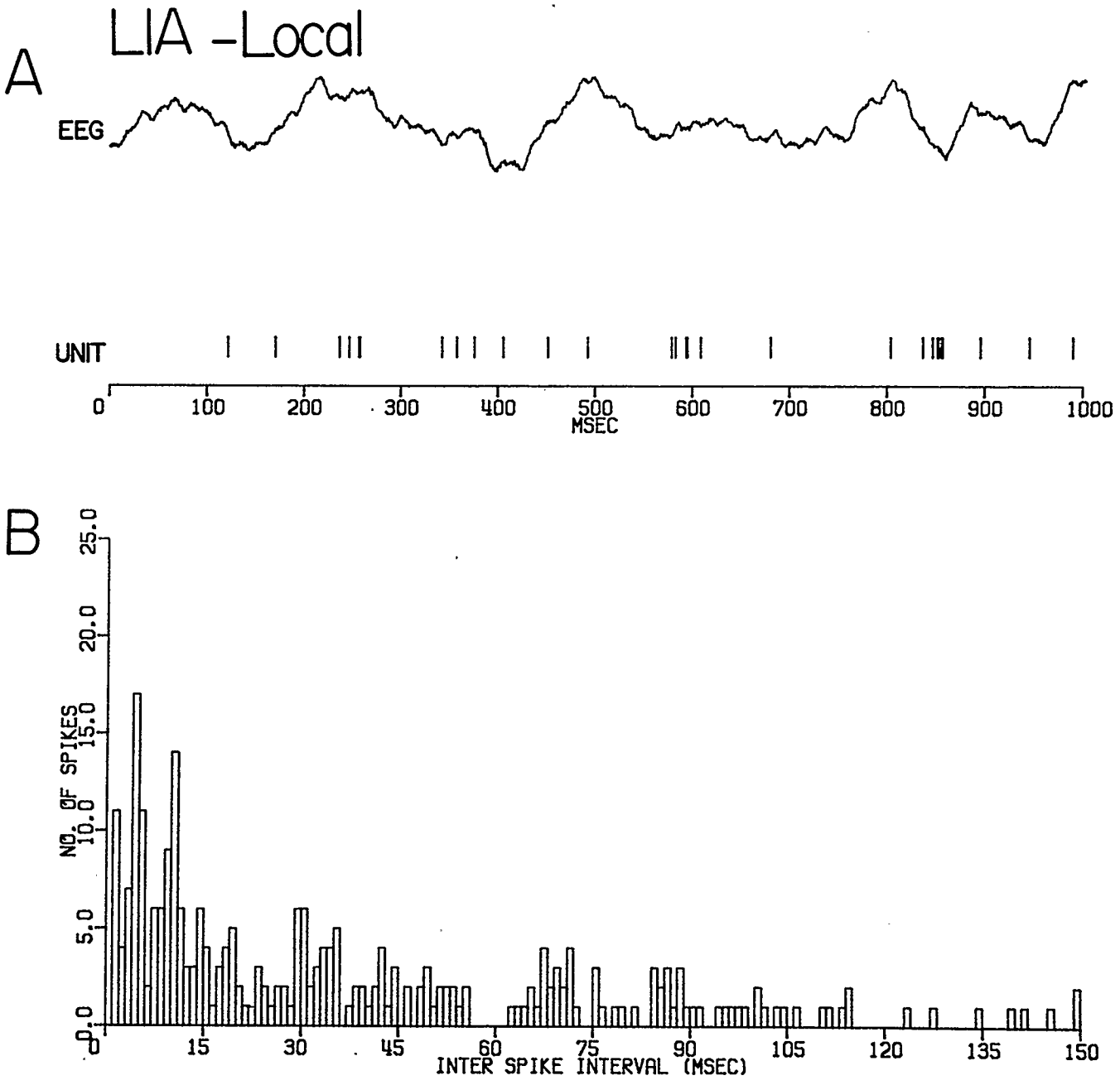
TYPE I -Θ- -Dentate Ref.



of the durations of theta for the total sample time of 22 sec. The modal frequency of movement theta was 6.9 Hz with a mean frequency of 6.3 Hz. The first order interspike interval histogram of unit discharges is shown in Figure 6C. The peak interspike interval was 2 msec with a mean interspike interval of 12.5 msec. The total number of theta waves for the Type 1 category (140) are shown normalized with respect to time, in Figure 7A. Figure 7B illustrates the histogram of the occurrency of unit discharges (1780) in relation to the phase of the normalized theta waves. The mean phase of unit discharges was 213°. These data are also given in Figure 7C with the unit discharges represented as an actual point on the normalized theta waves. The result of the theta-spike cross-correlation is shown in Figure 7D. The maximum phase correlation of unit discharges occurred at 333° with a Rho value of .96.

The computer analysis for the behavioral category of immobility accompanied by large amplitude irregular activity (from the local electrode), is presented in Figure 8. Figure 8A shows the irregular discharge pattern accompanying the large amplitude irregular slow wave pattern that can occur during alert immobility. The discharges had several peak interspike intervals, 2 msec, 10 msec, and 29 msec, respectively, with a mean interval of 9.2 msec. A summary of

Fig. 8. Computer analysis of the LIA behavior category for the CA1 layer theta units and local slow wave activity from Figure 1. A. Raw Data Plot. Upper trace is the analogue to digital conversion of the slow wave activity. Lower trace is the unit activity converted to a digital event. Only one second of the total sample (31 sec) shown. B. First order interspike interval histogram of unit discharges (Peak ISI = 2 msec, 10 msec, and 29 msec; $\bar{x}$ ISI = 9.2 msec).


A
LIA -Local
EEG
UNIT
0
100
200
300
400
500
600
700
800
900
1000
MSEC
B
NO. OF SPIKES
0.0
5.0
10.0
15.0
20.0
25.0
0
15
30
45
60
75
90
105
120
135
150
INTER SPIKE INTERVAL (MSEC)

the computer analyses for the remaining CA1 layer theta cells that had clear slow wave theta simultaneously occurring, is presented in Table 1.

Discharge Rate of CA1 Layer Theta Cells

The average discharge rates of CA1 layer theta units for the three behavior categories are presented in Figure 9. Note that the discharge rate was higher during Type 1 movements than during the Type 2 and LIA behavioral states. There were no significant differences in discharge rates between the Type 2 and LIA conditions.

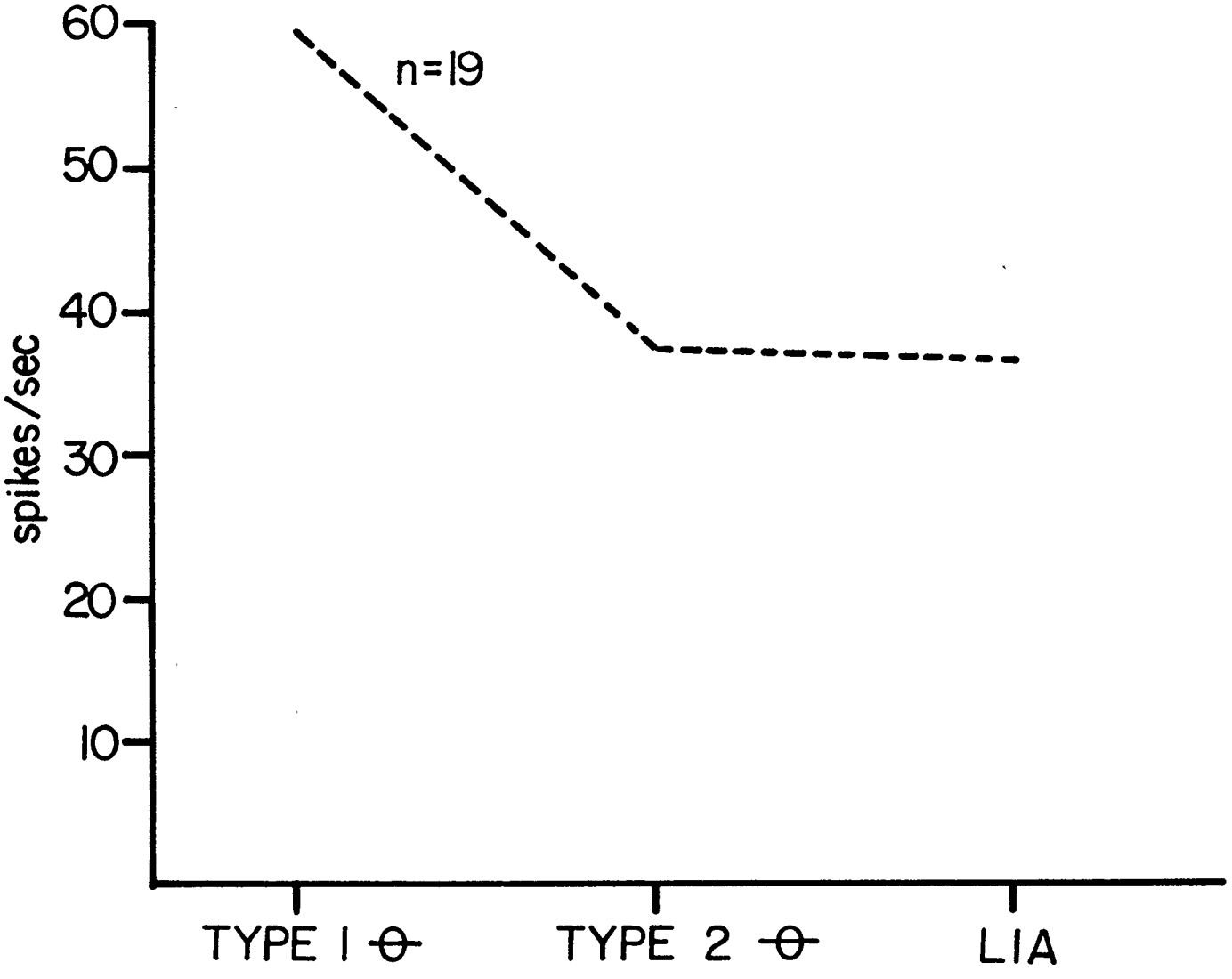
Analysis of Equivalent Theta Frequencies For Type 1 and Type 2 Theta Conditions

The computation of the average discharge rates from Figure 9 was made by collapsing across the frequency ranges of theta, for the Type 1 and the Type 2 theta conditions. Since there is an overlap in the physiological frequency ranges of Type 1 and Type 2 theta, an analysis was carried out on the discharge rates for equivalent (matched) frequencies. The analysis revealed that there were more discharges during Type 1 theta than during Type 2 theta for the same frequencies for the CA1 layer theta cells ($p < .001$; matched pairs sign rank test).

	cell no.	sample duration (sec)	mean phase of unit discharges	maximum phase corr. of unit discharges	Rho value
TYPE 1 THETA	5	20	137°	108°	.88
	10	23	167°	144°	.77
	14	20	157°	54°	.75
	16	18	175°	162°	.83
	20	35	184°	225°	.54
	23	24	173°	99°	.40
	25	26	188°	216°	.85
TYPE 2 THETA	5	13	137°	116°	.81
	10	21	168°	144°	.77
	14	21	171°	99°	.67
	16	16	179°	165°	.46
	20	28	185°	233°	.61
	23	27	182°	126°	.37
	25	34	192°	243°	.75

TABLE 1. Phase relations of CA1 layer theta cells (with good local theta) during Type 1 and Type 2 theta (maximum negativity = 180°)

Fig. 9. Discharge rates of CA1 layer theta cells, for the Type 1 and Type 2 theta behavior categories and the LIA behavior category.


60
50
40
30
20
10
spikes/sec
n=19
TYPE 1
TYPE 2
LIA

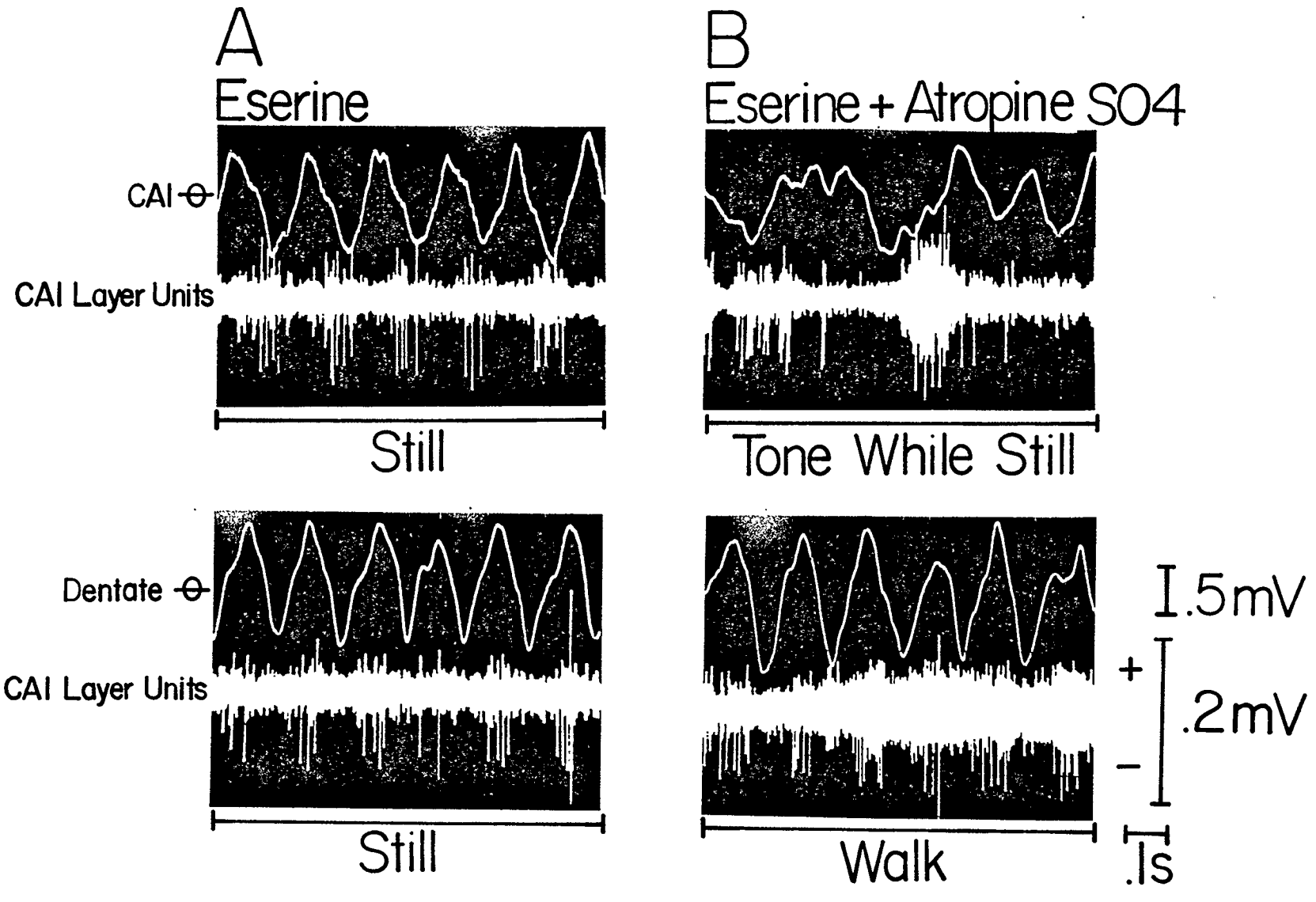
Timing of Theta Cell Discharges and Voluntary Movement

Due to limitations of the time-date-frame generator, only a frame-by-frame analysis could be carried out to assess the timing relationships of theta cell firing to behavior (a 33 msec epoch). A number of expert and totally naive observers were shown the video tapes and the only instruction given was to attempt to draw conclusions about the relationship between the cells' activity and the animals behavior. In addition, they were asked at the end of the session to express their confidence in their conclusions. The observers showed unanimity and high confidence on all CA1 layer theta cells analysed in the study. Theta cells were judged to increase firing rhythmically during both movement and the presentation of sensory stimuli (tones) while the animal was immobile. There was also complete agreement that cells increased their firing simultaneously with the onset of movement, but observers were less confident in saying that the cells increased firing prior to movement.

Pharmacological Effects of Eserine and Atropine SO_4 on Theta

The relationships of the firing repertoires of CA1 layer theta units to slow wave activity and behavior, in the eserine condition, is shown in Column A of Figure 10. The upper panel illustrates the firing of the units on the

Fig. 10. Effects of eserine (1.0 mg/kg IP) and eserine plus atropine sulfate (50 mg/kg IP) on the hippocampal electrical activity (CA1 and dentate EEG) and unit firing. Column A shows theta present with associated unit rhythmicity during eserine activation. Column B illustrates atropine administration following eserine. The top panel shows the Type 2 theta being abolished. Note the absence of any unit rhythmicity and the subsequent replacement by irregular unit activity. The bottom panel demonstrates the resistence of Type 1 movement theta to atropine SO_4. The addition of atropine SO_4 did not abolish the theta or unit rhythmicity associated with voluntary movement, in this case, walking.

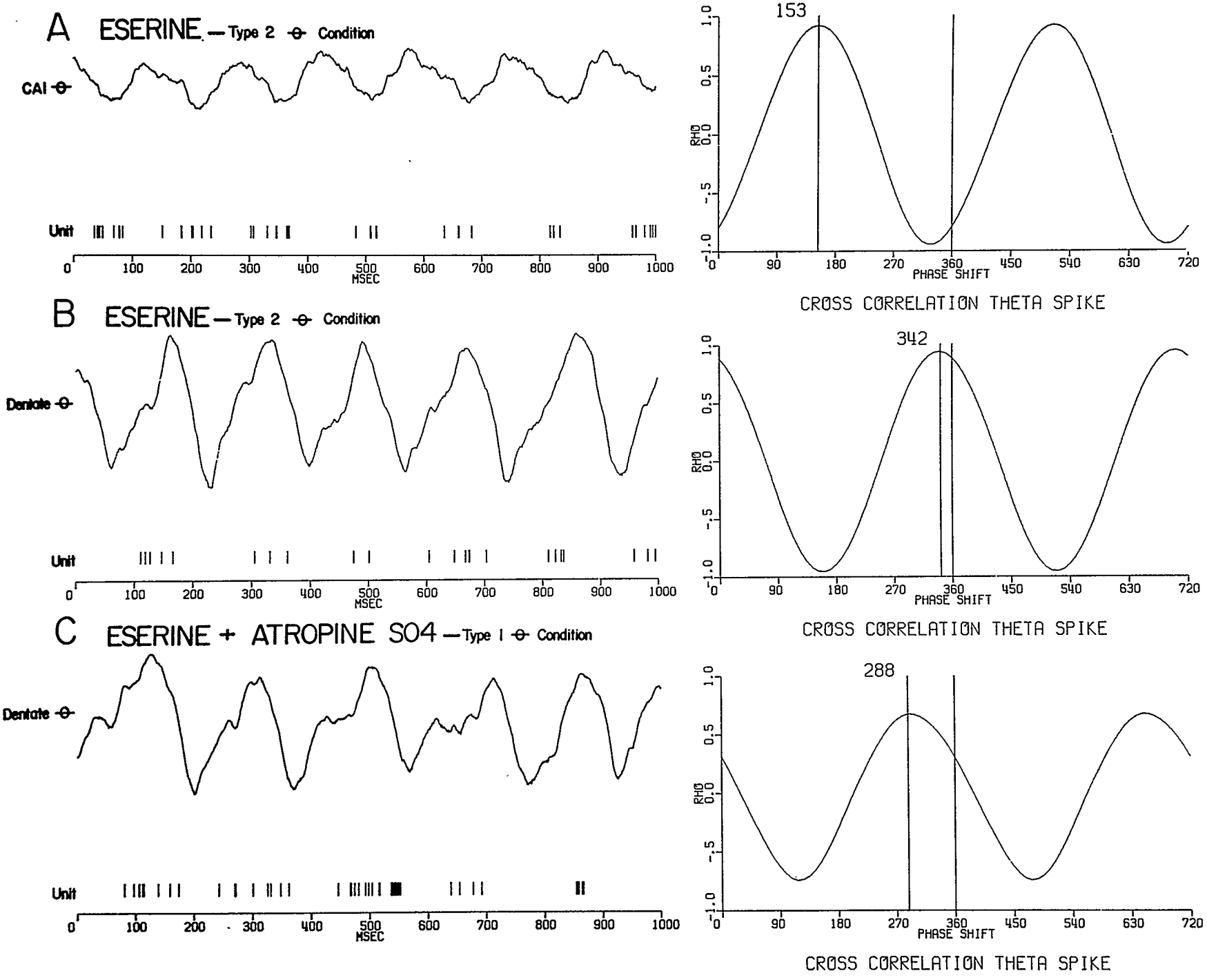

A
Eserine
CAI
CAI Layer Units
Still
B
Eserine + Atropine S04
Tone While Still
Dentate
CAI Layer Units
Still
Walk
1.5mV
+
.2mV
−
.1s

negative portion of the eserine-induced Type 2 local theta. The lower panel illustrates the firing of the units on the positive portion of the eserine-induced Type 2 theta taken from the dentate reference electrode. Column B of Figure 10 illustrates the effect of administration of atropine SO_4 on the eserinized preparation. The upper panel shows the abolishment of the eserine-induced Type 2 theta during immobility under atropine SO_4 application. Note the arhythmic firing of the associated unit activity. The lower panel illustrates, during the movement condition, that Type 1 theta has not been abolished. Note the associated unit firing is rhythmic and has not been noticably disrupted by the atropine SO_4.

Computer analysis carried out on the data from the drug manipulations is presented in Figure 11. The upper plots, A, illustrate the eserine-induced Type 2 condition. The left panel is the raw data plot showing the consistency of rhythmicity of units, with unit firing occurring on the negative component of the local Type 2 theta. The right panel, the theta-spike cross-correlation plot showed the maximum phase correlation of unit discharges to be 153° with a Rho value of .92. The middle plots, B, illustrate the eserine-induced Type 2 condition with the slow wave taken from the dentate reference electrode. The left panel is the raw data plot showing the consistency of rhythmicity of units,

firing on the positive component of the Type 2 theta taken from the dentate reference electrode. The theta-spike cross-sorrelation plot showed the maximum phase correlation of unit discharges to be 342^{o} with a Rho value of .94. The lower plots, C, illustrate the eserine plus atropine SO_4 condition for Type 1 theta taken from the dentate reference electrode. Note that whereas Type 2 theta and unit rhythmicity would be abolished under atropine SO_4 administration, Type 1 under such conditions is not abolished. The left panel is the raw data plot showing the consistency of rhythmicity of units under atropine SO_4 administration for the Type 1 condition, firing on the positive component of the dentate reference slow wave. The right panel, the theta-spike cross-correlation plot, illustrates the maximum phase correlation of unit discharges to be 288^{o} with a Rho value of .67.

Fig. 11. Analysis of slow wave and unit activity under eserine and eserine plus atropine SO_4 conditions. A. Eserine - Type 2 condition with local slow wave. Left side - Raw Data Plot. Upper trace is the analogue to digital conversion of the slow wave activity. Lower trace is the unit activity converted to a digital event. Only one second of the total sample shown. Right side - cross correlation with maximum phase correlation of unit discharges occurring at 153^{o} with a Rho value of .92. B. Eserine - Type 2 condition with dentate reference slow wave. Left side - Raw Data Plot, as in A. Right side - cross correlation with maximum phase correlation of unit discharges occurring at 342^{o} with a Rho value of .94. C. Eserine plus atropine SO_4 - Type 1 condition with dentate reference slow wave. Left side - Raw Data Plot, as in A. Right side - cross correlation with maximum phase correlation occurring at 288^{o} with a Rho value of .67.


A ESERINE — Type 2 Condition
CAI
Unit
0 100 200 300 400 500 600 700 800 900 1000
MSEC
153
RHO
1.0 0.5 0.0 -.5 -1.0
0 90 180 270 360 450 540 630 720
PHASE SHIFT
CROSS CORRELATION THETA SPIKE
B ESERINE — Type 2 Condition
Dentate
Unit
0 100 200 300 400 500 600 700 800 900 1000
MSEC
342
RHO
1.0 0.5 0.0 -.5 -1.0
0 90 180 270 360 450 540 630 720
PHASE SHIFT
CROSS CORRELATION THETA SPIKE
C ESERINE + ATROPINE SO4 — Type 1 Condition
Dentate
Unit
0 100 200 300 400 500 600 700 800 900 1000
MSEC
288
RHO
1.0 0.5 0.0 -.5 -1.0
0 90 180 270 360 450 540 630 720
PHASE SHIFT
CROSS CORRELATION THETA SPIKE

DISCUSSION

The present investigation yielded three major findings. First, it appears that the same cells are participating in both the Type 1 and Type 2 theta behavior conditions. Second, all theta cells discharged on the negative phase of their slow wave. More specifically, CA1 layer theta cells discharged just prior to peak negativity. And finally, a number of relationships of theta cell discharge patterns to the behavioral conditions were found. At the onset of Type 1 movement, all theta cell groups increased their firing and continued to fire rhythmically for the duration of the movement. During Type 2 LIA behaviors, theta cell firing was arhythmic, and during the Type 2 theta behavior condition, theta cell groups again fired in a rhythmic pattern for the duration of the theta slow wave activity (however, there were fewer rhythmic discharges per theta wave, compared to the Type 1 theta condition).

Behavioral Correlates of Theta Cells

Neuronal firing of CA1 layer cells was observed in relation to the rabbits' behavior, in order to better understand any relationships that might exist. Of particular interest to this study was the firing repertoires of CA1 layer theta cells and their behavioral correlates.

Ranck's (1973) definition of a theta cell requires some modification, at least for the rabbit. Theta cells were defined as cells which increase (approximately double) their rate of firing if, and only if, there is a slow wave theta rhythm in the hippocampus. This definition is still accurate for the Type 1 (voluntary) movement category for rabbits, but does not hold for the Type 2 (immobility-related) theta behavior category. That is, there were no significant differences in firing rate between the Type 2 condition and the LIA condition, at least for groups of theta cells. What appears to be the defining factor is the rhythmicity, or more specifically, a high degree (or modulation) of rhythmicity for every occurrence of theta. The latter part of this statement is important since almost all hippocampal neurons show some degree of rhythmicity for at least part of an epoch of slow wave activity (Bland, Andersen, Ganes, & Sveen, 1980). They noted that dentate granule cells have a higher modulation index than the CA1 pyramids, with CA3 cells and basket cells having a lesser tendency towards rhythmicity.

The behavioral correlates of theta cells for the Type 1 (voluntary) movement category were identical with the behavioral correlates of the slow wave theta rhythm (Vanderwolf, 1969, Vanderwolf, Kramis, Gillespie & Bland, 1975). This confirms the findings of Ranck (1973) and O'Keefe and

Dostrovsky (1971). Ranck (1973) noted that since theta cells increased their rate of firing if and only if there was a regular theta rhythm in the slow waves, the behavioral correlate of the rapid mode of firing of theta cells was identical to the behavioral correlate of the slow wave theta rhythm.

The present study is the first to demonstrate that the same theta cells participate rhythmically during the appearance of Type 2 theta. The behavioral correlates of Type 2 theta are at present not well understood, but the behavioral conditions where it appears suggests that it is associated with the processing of "phasic" sensory inputs. That is, in the immobile rabbit, Type 2 slow wave theta may be initiated by various types of olfactory, visual, auditory, somatic, and kinesthetic stimulii. Presumably, in each of these modalities there exists a background or "tonic" level of activity at any given moment. Type 2 theta appears with changes in the level of sensory activity, but habituates rapidly if this new level is maintained (that is, becomes tonic). Theta cell firing was irregular during behaviors correlated with large amplitude irregular slow wave activity. Winson (1972) has suggested that there may be a correspondence between the theta-correlated behaviors and an important mode of natural behavior of

various species. Thus, our finding that Type 2 theta in the rabbit is easily elicited by sensory input suggests this information about the environment may indeed be of survival value for that species.

Relations of Theta Cell Discharges to Frequency of Slow Wave Activity

Although we did not carry out an extensive analysis of these relationships, it would appear that a general statement might be that the lower the theta frequency, the fewer the theta cell discharges there were per wave. With respect to Type 2 theta, there may even by complete discharge failures for some waves of a low frequency theta sample. These results support the findings reported by Bland, Andersen, Ganes and Sveen (1980) for the acute rabbit. This statement may apply only over a limited range of frequencies for the two types of theta, however. An important observation we did make was that for equivalent theta frequencies, there were still significantly more discharges for Type 1 theta than for Type 2 theta. This may be physiologic evidence that the systems providing the afferent drive for the two types of theta are indeed different.

It is interesting to note that theta cell discharges did not decrease in frequency during large amplitude irregular slow wave activity compared to the Type 2

theta condition, but still differed dramatically in the sense that they were totally arhythmic.

Coupling of Theta Cell Discharges to the Phase of Slow Wave Theta Activity

The general finding reported in the present study was that all CA1 layer theta cells discharged on the negative phase of their local theta wave. More specifically, CA1 layer theta cells tended to discharge just prior to peak negativity and end at the peak. Bland, Andersen, Ganes, and Sveen (1980) previously studied the participation of physiologically identified hippocampal formation neurons in spontaneously and hypothalamically-induced Type 2 theta activity in rabbits lightly anesthetized with urethane. Computer analysis of that data indicated that virtually all CA1 pyramidal cells were phase-locked to the negative portion of the theta waves recorded from the corresponding region. The situation appears different for the rat. Wolfson, Fox, and Ranck (1979) reported that for rats, walking on a treadmill (Type 1 theta), pyramidal cells and interneurons in the CA1 all tended to fire on the positive phase of dentate theta. However, for the urethanized rat (Type 2 theta), Wolfson, Fox and Ranck (1981) report that CA1 interneurons fire on the negative peak of dentate theta activity. Buzsaki and Eidelberg (in press) have recently studied rats anesthetized

with urethane, using the spike-triggered averaging method. They report that projection cells in the CA1 fire with the highest probability on the negative phase of their local theta. Interneurons in the CA1 region discharged on the negative portion of the dentate theta wave. Clearly there are some important differences between these studies, and their resolution awaits more detailed study of the mechanisms underlying Type 1 and Type 2 theta as well as positive identification of theta cells.

Identification of Theta Cells

In this study we did not attempt to identify theta cells, but localized them to the region of the CA1 layer. Theta cells could morphologically be projection cells, interneurons, or some of each. However, the electrophysiological evidence we do have supports the suggestion of Fox and Ranck (1975, 1981) that most theta cells are interneurons. The duration of the extracellular negative spike for all theta cells was .3 to .4 msec and they all fired with single as opposed to complex patterns. The maximum firing rates of theta cells were also within the range of 30-100/sec, sustainable for many minutes, as reported by Fox and Ranck (1981). Buzsaki and Eidelberg (in press) have specifically proposed that septal theta "pacemaker" cells directly

excite hippocampal interneurons which in turn rhythmically inhibit a number of projection cells. There still exists the possibility that in the rat there are several subclasses of theta cells taking part in Type 1 and Type 2 theta, respectively. To our knowledge, there are no studies in the rat equivalent to the present one in the rabbit, where the same theta cells were studied during the two types of theta.

Timing of Theta Cell Discharges and Voluntary Movement

All theta cells in the CA1 layer were judged to discharge in a rhythmic fashion simultaneous with the onset of voluntary movement. Technical limitations and the behavioral paradigm used did not permit any sure judgements about whether cell firing preceded movement.

Our previous work, together with the present study, has provided evidence that the hippocampal formation is active in the theta mode during two conditions in the rabbit. In one condition, the rabbit is immobile and responding to changes in sensory level. We suggest that this Type 2 theta system is responsible for providing a "priming" input to the hippocampal formation. That is, this system signals phasic changes in level of sensory input from a tonic background and puts the hippocampal motor program selector in a "primed" or alert mode. If a program is not activated,

this system habituates (with repetitions of the stimulus, it becomes tonic). In the other condition, the rabbit is involved in making a class of movements we have termed voluntary. This Type 1 theta system indicates that the hippocampal formation has received the input to activate the selection of appropriate motor programs. We do not mean to imply that the hippocampal formation is the main loci for motor program selection, only that it is one structure providing such input. The nature of the input may involve the spatial or temporal patterning of motor programs, or the intensity of their activation. The relation of theta cell spike train dynamics to the frequency of slow wave theta and accompanying movement suggests the hippocampal formation may be involved in intensity of activation. Finally, the large amplitude irregular activity occurring during Type 2 LIA (automatic) behaviors may indicate that the hippocampus is not actively involved in their organization.

Pharmacological Effects

As previously mentioned, eserine administration was seen to enhance the rabbits' natural Type 2 theta, while atropine SO_4 abolished Type 2 theta and the associated unit rhythmicity. Atropine SO_4 also produced decreased discharge rates of units associated with Type 1 behavior. There

seems to be at least two ways to explain the results:

1. Nonspecific effect of atropine,
2. Specific effect/evidence for two ascending systems

The first idea, the nonspecific effect, sees theta as a continuum. This explanation rejects the concept of two types of theta, assuming rather that theta frequency is related to the level of activity and that the associated unit activity responds in a simple recruitment manner. That is, as intensity of movement increases, the number of unit discharges increases. Nonspecificity relates to the effect of atropine on the cells involved with the theta. With the administration of atropine, cells are "knocked-out" (ie. a local anesthetic effect), so that with lower levels of activity where few cells normally participate, under the drug condition, the cells are essentially all "quiet" (little activity is seen). In higher levels of activity (ie. Type 1 behaviors), where relatively more cells are normally involved (nondrug), the atropine tends not to effect all the cells, thus some remain active. But, this decrease in total normal cell activity results in the lowered firing rates seen in the atropine Type 1 condition.

The second idea, the specific effect, sees theta as being generated by at least two separate pharmacological

ascending systems. The evidence - including eserine enhancement of Type 2 theta as well as atropine abolishment of Type 2 theta - is interpreted as there being a separate cholinergic system mediating this immobility slow wave. The hippocampus, when the animal receives a sensory input - generates Type 2 theta. It may be that this Type 2 theta is indicative of a preparatory mode of hippocampal function, as previously mentioned. Type 1 theta appears during behaviors associated with voluntary movement. Due to the virtually ongoing nature of sensory processing, it may be possible that Type 1 theta (in the nondrug condition), is a composite phenomena which includes Type 2 theta (must be present because sensory feedback and processing is required during movement). The remaining contributor(s) to the Type 1 theta rhythm cannot be clearly seen in the nondrug state - so, by using atropine sulfate to abolish Type 2 theta, we should be able to isolate the additional contributor to the Type 1 theta rhythm. During Type 1 behavioral conditions, in the drug state (atropine), a decreased rate of unit discharge is seen. That is, Type 1 theta unit discharge rates are lower in the drugged condition than in the nondrug condition. This result may possibly be due to the blockade (via atropine action) of the pharmacological input to the Type 1 system. What is then seen after the abolishment of

the Type 2 system activity would then be due to at least one other pharmacological input to the hippocampal formation. Therefore, Type 1 in the nondrug condition represents the summation effects from the different ascending pharmacological systems (Note: This explanation presupposes the functional presence of Type 2 during Type 1 theta in the nondrug state).

Conclusion

To conclude, this thesis described the relations of theta cells in the CA1 layer of the hippocampal formation to slow wave activity and motor behavior in the freely moving rabbit. The same cells were found to participate in both Type 1 and Type 2 theta, with all theta cells discharging on the negative phase of their local slow wave. Specifically, CA1 layer theta cells discharged just prior to peak negativity. Relationships were found to exist between theta cell discharge patterns and the rabbits' behaviors. At the onset of Type 1 movement, all theta cell groups increased their firing and continued to fire rhythmically for the duration of the movement. During Type 2 LIA behaviors, theta cell firing was arythmic, and during the Type 2 theta behavior condition theta cell groups again fired in a rhythmic pattern for the duration of the theta slow wave activity (however,

there were fewer rhythmic discharges per theta wave, compared to the Type 1 theta condition). And finally, the findings suggest that the cells in the CA1 layer that are rhythmically coupled to both types of theta activity may receive two separate ascending inputs for theta, the one mediating Type 2 being cholinergic.

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