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Anticholinergic Manipulation of Hippocampal Theta Rhythm and Theta Cells in Freely Moving Rabbits

by

Sylvie Suzanne Roquet

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Anticholinergic manipulation of hippocampal theta rhythm and theta cells in freely moving rabbits", submitted by Sylvie S. Roquet in partial fulfillment of the requirements for the degree of Master of Science.

Dr. Brian H. Bland
Supervisor
Department of Psychology

Dr. Roderick M. Cooper
Department of Psychology

Dr. Timothy B. H. Rogers
Department of Psychology

Dr. Merle E. Olson
Department of Biology

Date NOVEMBER 6, 1987

ABSTRACT

Hippocampal EEG and extracellular discharges of theta cells were recorded from 10 awake Dutch belted rabbits before and after a systemic administration of atropine sulfate (50 mg/kg, ip), an anticholinergic agent. Before the drug treatment, three behavioral categories were characterized by three EEG patterns: movement and atropine-resistant theta; immobility concurrent with tactile and auditory stimulation and atropine-sensitive theta; and immobility and irregular large amplitude activity. Fourteen theta cells, their name deriving from their rhythmical discharges phase-locked to theta waves, were isolated with tungsten microelectrodes. Theta cells' discharge frequency was positively related to slow wave theta frequency. During movement, mean theta frequency was higher than during sensory elicited theta. The mean firing rate of theta cells was also highest during movement and higher than in the other two behavioral categories. Even at equivalent slow wave theta frequencies their discharge rate was higher during movement than during immobility with sensory stimulation.

For seven out of eight neurons studied in a post-

atropine testing condition, the anticholinergic drug selectively abolished slow wave theta normally elicited by sensory stimulation and these waves were replaced by irregular activity. The effect on movement related slow wave theta was a decreased average frequency. Theta cells decreased their firing rate during movement whereas no change was detected during sensory processing behaviors. An unexpected finding was the decreased mean discharge rate of theta cells in the LIA behavioral category.

The rhythmic field and cellular activities recorded in the dorsal hippocampal formation of the rabbit had the same behavioral correlates, sensory processing and movement. The anticholinergic drug, atropine, blocked the sensory related slow wave theta only. Blocking of this input resulted in decreased firing rate of theta cells and decreased theta rhythm frequency only during movement, a behavior where both sensory and motor components are normally coactive. This research supports the sensorimotor integration functional model proposed by Bland (1986).

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INTRODUCTION

The study of sensory and motor correlates of the hippocampal theta rhythm was performed by recording the electrical field potentials and extracellular potentials in the hippocampal formation of freely moving rabbits before and after a systemic injection of atropine sulfate, a cholinergic blocker. Electrophysiological activity was analyzed with respect to three behavioral categories: movement, immobility and immobility with concomitant sensory stimulation. In order to provide a substantial background for the research hypotheses, the following sections will cover the anatomy, electrophysiology, pharmacology and behavior related to the hippocampal theta rhythm.

Although species differences exist among small mammals they are outnumbered by similarities. The vast majority of research in this field was done on the rat and when comparable studies on the rabbit are available, they are reported. It may be stated at this point that the choice of the rabbit as an experimental subject was based on electrophysiological and behavioral considerations. The first multispecies research on hippocampal theta rhythm (Green & Arduini, 1954) demonstrated that theta could be

elicited by sensory stimulation most effectively in the rabbit. Sensory related theta was later shown to be cholinergic (Kramis, Vanderwolf and Bland, 1975; Vanderwolf, 1975), a finding which is central to the present research hypotheses. Another advantage is the behavioral docility of the rabbit. Experimentation involved the manual use of a microdrive to search for theta cells hence it was important that the animal be docile and prone to immobility.

Anatomy

The hippocampal formation is a curved structure which lies alongside the lateral ventricles. In the forebrain, it approaches the midline anteriorly, ventral to the corpus callosum, and adjacent to the contralateral hippocampal formation and caudal to the septum to which it is connected by the fimbria-fornix. Posterolaterally it curves toward the occipital cortex. The most posterior portion is referred to as the occipital bend. Ventral to the occipital bend the hippocampus again turns in an anteromedial direction toward the temporal cortex and the amygdaloid area. The deep surface, consisting of white matter (alveus), forms part of the postero-medial wall of the lateral ventricles. The direction toward the anterodorsal pole and septal nuclei along the longitudinal axis of the hippocampus is termed septal whereas the direction toward the anteroventral pole is termed temporal (Blackstad, Brink, Hem, & Jeune, 1970).

The hippocampal formation is composed of the hippocampus, the dentate gyrus and the subiculum. The hippocampus was first subdivided along the vertical axis into regio superior and regio inferior by Cajal (1911), and

later along the longitudinal axis (Lorente de No, 1934) into four fields. Only two of these fields are considered to be distinct (Blackstad, 1956). A common combined terminology refers to the smaller cell bodies in regio superior as field CA1 and to the giant pyramids of regio inferior as field CA3.

The microscopic anatomy of the hippocampal structure, as drawn by Brodal, is reproduced in Figure 1. In the hippocampus proper, the following layers are distinguished. The alveus contains axons originating in the subiculum, the entorhinal area and the hippocampus. The basal dendrites of the pyramidal cells extend toward the ventricular surface in the layer oriens. The cell bodies lie along the pyramidal layer. Apical dendrites emerge through the radiatum layer while the distal segments are found in the lacunosum-molecular area. The layer lucidum contained within layer radiatum is formed by the mossy fibers linking the dentate gyrus and field CA3.

The dentate area is composed of the fascia dentata and the hilus fasciae dentatae (Blackstad, 1956). In the fascia dentata, the granular layer is composed of granule cell bodies whose dendrites extend into the molecular layer. The hilus contains polymorph cells and axons of the granule

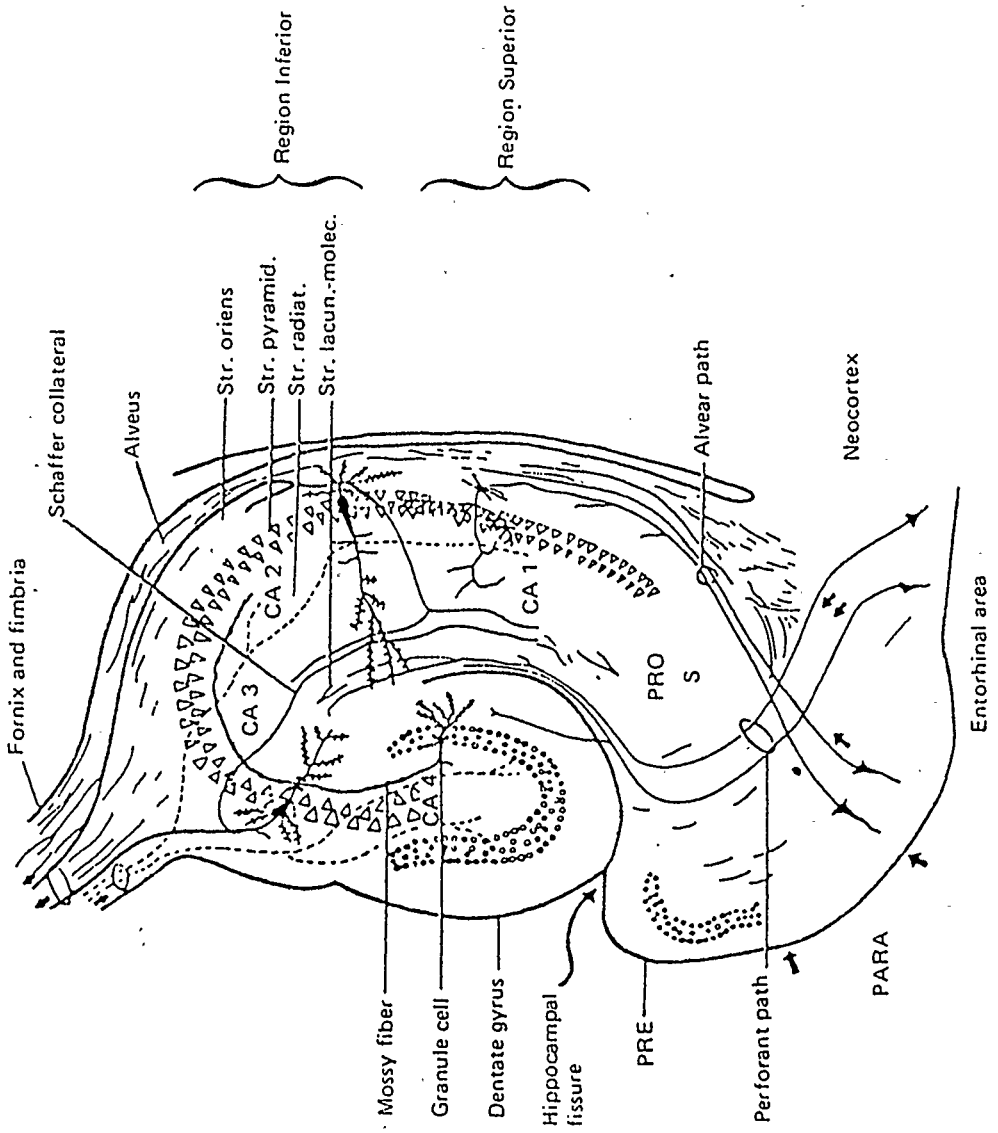


Figure 1. Hippocampal finer structure drawn by Brodal (1947).

cells. In a transverse section from the occipital bend, the fascia dentata has a characteristic U-shape. The two limbs, the medial and the lateral blades, join together posteriorly at the dentate crest.

The subiculum is one of four parts of the subicular complex. It lies continuous with field CA1 of the hippocampus. The subiculum has one single cell layer, as the hippocampus and the dentate gyrus have, composed of large loosely arranged pyramidal cells. The subicular complex also includes the prosubiculum, the presubiculum and the parasubiculum which are all neighboring areas (Blackstad, 1956).

Meibach and Siegel (1977) have used the term dorsal fornix to represent the bundle of fibers which run in the medial part of the fornix between the dorsal and ventral hippocampal commissures. In rostral sections of the hippocampal formation, the dorsal fornix is situated immediately adjacent to the fimbria (both originate in the alveus). The ventral hippocampal commissure is the commissural component of the fimbria-fornix system. The part of the fornix which projects anterior to this commissure is known as the precommissural fornix whereas another part, the postcommissural fornix, projects

posteriorly to the commissure towards the hypothalamus.

The septum, a mass of grey matter lying between the anterior horns of the lateral ventricles, has been parcellated into lateral, medial, posterior and ventral parts. According to Swanson and Cowan (1979), the lateral part consists of the lateral septal nucleus; the medial part contains the medial septal nucleus (dorsal) and the nucleus of the diagonal band of Broca (ventral); the posterior part includes the septofimbrial and the triangular nuclei; finally, the ventral part refers to the bed nucleus of the stria terminalis.

Neuronal connection of the hippocampal formation with other structures in the brain involves mainly the entorhinal cortex and septal nuclei. The crucial role of the septum in theta generation (Green & Arduini, 1954) makes these paths of particular interest. The internal organization of fibers follows a laminar distribution. Hippocampal efferents are directed towards the septum, subicular projections also reach other targets such as the hypothalamus.

Entorhinal projections

The entorhinal cortex, located adjacent to the subicular complex, has three afferent routes to the hippocampus (Cajal, 1911 in Raisman, Cowan, & Powell, 1965). The perforant path is the major extrinsic input to the hippocampus and dentate area. Perforant fibres arise from the lateral part of the entorhinal cortex forming discrete fasciculi which cross the subiculum and enter the layer lacunosum-moleculare of the hippocampus where they contact the peripheral parts of the apical dendritic shafts of the pyramidal cells in CA1 and further reach the molecular layer of the dentate gyrus. Blackstad (1958) reported that fiber termination was limited to the outer part (away from the cell body) of the molecular layer in the dentate region. Hjorth-Simonsen (1972) has reported an additional medial origin to the perforant path with an identical course to that of lateral origin. Fibres were traced from the medial entorhinal area to the middle zone of the dentate molecular layer and to the deep part of the molecular layer in the hippocampal field CA3. Lateral and medial entorhinal origins were confirmed by Segal and Landis (1974). Hjorth-Simonsen and Jeune (1972) have described the topical

organization of these paths: dorsal entorhinal lesions evoke terminal degeneration in the septal parts (i.e. toward the septum) of the hippocampus while ventral lesions produce degeneration in increasingly temporal segments of the hippocampus.

Secondly, the crossed perforant path is referred to by Blackstad (1956) as a sparse contralateral projection of entorhinal fibres through the dorsal hippocampal commissure to the lacunosum-moleculare layer of CA1 rather than to the presubiculum (Raisman et al., 1965). Swanson and Cowan (1977) using the autoradiographic method observed entorhinal fibres travelling via the dorsal hippocampal commissure to the contralateral CA1 and to the outer two thirds of layer molecular in the dentate gyrus.

Blackstad (1956) has reported crossed fibres in the dorsal hippocampal commissure (some of unknown origin) terminating in the superficial plexiform or molecular layer of the subiculum and others originating in the entorhinal cortex and distributed in the outer part of the molecular layer of the subiculum. The ventral subiculum is known to receive a substantial projection from the amygdala (Krettek and Price, 1974).

The third entorhinal projection, the alvear tract,

leaves the medial entorhinal area, enters the hippocampus on it's ventricular surface and is conveyed for a short distance in the alveus. The alvear fibres make contact with the basal dendrites of the pyramidal cells in the subiculum and in the layer oriens of CA1 of the hippocampus. Swanson and Cowan (1977) also described a temporoalvear bilateral tract originating in the lateral and medial parts of the entorhinal area with some fibres joining the alveus and terminating in the hippocampus proper. This report confirmed the earlier findings of Raisman et al. (1965) who traced ipsilateral degeneration from the medial entorhinal cortex to the layer oriens of CA1 and degeneration across the dorsal hippocampal commissure to the entorhinal area and hippocampus of the opposite side.

Septal efferents and afferents

Septal efferent fibres are known to leave the septum via three routes, the dorsal fornix-fimbria to the hippocampus, the medial forebrain bundle to the hypothalamus and the stria medullaris to the habenular nuclei (Meibach & Siegel, 1977). Telencephalic efferents are directed principally to the hippocampus.

Septal efferents to the hippocampus can be traced after lesion of the septum by a diffuse type of degeneration appearing in silver impregnated sections, mainly in CA3 (oriens and radiatum) and hilus of the dentate area (Hjorth-Simonsen, 1973; Mellgren & Srebro, 1973). Transection of the fimbria-fornix causes retrograde cell changes in the medial septum and diagonal band nuclei (Gage, Wictorin, Fischer, Williams, Varon, & Bjorklund, 1986). Lesioning the medial septum and staining with a silver impregnation method results in a similar laminar degeneration distribution in the hippocampus (Raisman, 1966; Mosko, Lynch, & Cotman, 1973). By means of retrograde transport of horseradish peroxidase (HRP), Segal and Landis (1974) and Meibach and Siegel (1977) traced the medial septum projection to the cellular layers of all CA fields, dentate area and subiculum. In an autoradiographic study of the septo-hippocampal projection, Swanson and Cowan (1979) reported a projection to most parts of the hippocampal formation including the hilus of the dentate area with peak grain density occuring close to the cellular layers. The innervation patterns of hippocampal afferentation from the medial septum and vertical limb of the diagonal band were recently described with the sensitive PHA-L anterograde

intra-axonal marker (Nyakas, Luiten, Spencer & Traber, 1987). These authors reported innervation of layer pyramidal, molecular-radiatum transition zone, the molecular layer close to the alveus, and layer oriens in the CA1 subfield. In the dentate gyrus, afferent fibers were restricted to the middle third of the molecular layer, granular layer and hilus region. CA3 contained termination fibers in layers oriens and pyramidal. The more detailed afferentation which resulted may be due to tracing of finer fibers undetected with the use of degeneration and autoradiographic techniques.

The septal diencephalic efferents are more extensive than the corresponding afferent inflow. The lateral septal nucleus projects to the lateral hypothalamus via the medial forebrain bundle (Meibach & Siegel, 1977; Swanson & Cowan, 1979). The diagonal band nucleus also projects through the medial forebrain bundle to the lateral hypothalamus (Raisman, 1966; Swanson & Cowan, 1979). Both septofimbrial and triangular nuclei of the posterior division have ascending fibres to the medial habenular nucleus (Raisman, 1966). Raisman also noted a projection from this and the ventral divisions through the medial forebrain bundle to the lateral hypothalamus. The bed nucleus of the ventral

division sends fibres via the stria terminalis to the habenular nuclei (Meibach & Siegel, 1977).

With regard to the telencephalic afferents, the septum receives a projection from the hippocampus, a projection from the pyriform cortex (and possibly the amygdala) to the horizontal limb of the diagonal band nucleus and a specific projection from the amygdala to the bed nucleus of the stria terminalis (Raisman, 1966).

According to Raisman (1966), the septal diencephalic afferents are entirely conveyed in the medial forebrain bundle. The lateral septal nucleus receives fibers from the hypothalamus and from the ventral tegmental area (Swanson & Cowan, 1979). The hypothalamus and midbrain project to both the medial septal and diagonal band nuclei (Raisman, 1966). Swanson and Cowan (1979) also described fibres projecting from the lateral preoptic area to the medial septal diagonal band complex and fibres from the medial forebrain bundle to the medial septal nucleus. In the ventral division, the bed nucleus receives fibres from the ventral tegmental area and the medial forebrain bundle.

Intrinsic organization

Afferent lamination is an organizing principle in the hippocampus. Contained within the sheets of cells is a functional trisynaptic circuit. The circuit is oriented transverse to the longitudinal axis of the hippocampus. Thus, the perforant path to the granule cells, the granule cell projection onto CA3 pyramidal cells, the CA3 pyramidal projection to area CA1 via the Schaffer collaterals and the efferents of CA1 pyramidal cells into the alveus have a transverse, or lamellar orientation (Andersen, Holmqvist & Voorhoeve, 1966; Blackstad et al., 1970; Hjorth-Simonsen, 1973). The blood supply from the posterior cerebral artery also follows the lamellar organization of the hippocampal cortex in the rabbit (Nilges, 1944). The three-synapse chain can be functionally preserved in a 400 micron slice in vitro (Skrede & Westgaard, 1971). The hippocampal longitudinal association fibers (Zimmer, 1971; Hjorth-Simonsen, 1973) are thought to be involved in interlamellar communication.

Field CA1 appears on connectional grounds to be divisible into septal and temporal subfields, the former projecting through (perhaps to) the subiculum, the

parasubiculum and the deeper layers of the perirhinal area, while the latter projects only to the subiculum (Swanson & Cowan, 1977). Axons of CA1 pyramids in the alveus terminate on the ipsilateral apical dendrites of the subicular pyramidal cells, specifically in the plexiform layer (Hjorth-Simonsen, 1973; Swanson & Cowan, 1977; Tamamaki, Abe & Nojyo, 1987). Field CA3 sends a limited ipsilateral projection to the presubiculum, the parasubiculum, the medial and lateral entorhinal cortex (especially layer IV) and to the cingulate area (Swanson & Cowan, 1977). Field CA3 projects ipsilaterally to the subiculum molecular layer (Swanson & Cowan, 1977). The subiculum, like field CA1, is one of the few areas in the hippocampal region which does not contribute a crossed projection to the hippocampal formation of the other side (Swanson & Cowan, 1977).

The dentate gyrus does not project outside the hippocampus. Many authors confirm the projection of the granule cells to the lucidum layer in CA3, mossy fibres (Blackstad et al.,1970; Swanson & Cowan, 1977; Gaarskjaer, 1986). Gaarskjaer's review (1986) provides the following description: the mossy fibres originate in the dentate granular layer and the few granule cells found scattered in the dentate molecular layer and hilus. Via a complex system

of collaterals the mossy fibres terminate on several types of neurons in the hilus, e.g. the basket cells and the mossy cells (Amaral, 1978). Some interneurons are designated basket cells because their axons form basketlike plexa around the bodies of the pyramidal or granule cells. Typically, their axons first ascend in the apical dendritic layers and then descend, branching profusely. They are distributed througout the hippocampus. Upon leaving the hilus, the mossy fibres form the lucidum layer which terminates on the proximal part of the apical and basal dendrites of CA3 pyramidal and basket cells.

The inner third (close to the cell body) of granule cell dendrites receive a massive input from the ipsi- and contralateral hilus via the ventral hippocampal commissure (Blackstad, 1956; Swanson & Cowan, 1977). According to Berger, Semple-Rowland and Basset (1980), the existence of association and commissural afferents to the dentate gyrus has been reported for a number of species (rat, rabbit, guinea pig). Fibres of both pathways terminate in the inner third of the dentate molecular layer. Using axonal transport techniques (HRP and autoradiographic) they identified the cells of origin (bilateral) as the polymorph neurons within the temporal dentate hilar region.

Ipsilateral and commissural (via the fimbria and the ventral hippocampal commissure) fibres from CA3 pyramidal cells terminate just above the mossy fibres in layer radiatum and below the cell bodies in layer oriens of the hippocampus (Blackstad, 1956; Hjorth-Simonsen, 1973; Raisman et al., 1965; Zimmer, 1971). Part of the ipsilateral projection constitutes the Schaffer collaterals which innervate layer radiatum of CA1 (Hjorth-Simonsen, 1973).

In summary, the different layers of the hippocampus receive fibres of different origin: the molecular layer is particularly innervated by exogenous fibres, the middle layers, layers radiatum and lacunosum, especially by endogenous fibres or Schaffer collaterals and commissural fibres (Blackstad, 1956).

Hippocampal efferents

The hippocampal formation projects to the septum by way of the precommissural fornix. Segal and Landis (1974) have demonstrated that horseradish peroxidase injected into the dorsal hippocampus stains cells in the medial aspect of the medial septal nucleus while a similar injection into the ventral hippocampus reveals cells in the lateral portion of

the medial septal nucleus. Raisman (1966) described the reciprocal septo-hippocampal connections as reentrant pathways. These involve projections from CA1 to the medial septal diagonal band complex which sends fibres to the fields CA3 and CA4. CA3 Schaffer collaterals terminate in CA1 and other collaterals from CA3 project to the lateral septal nucleus cells which, completing the loop, project to the medial septal nucleus.

Swanson and Cowan (1977; 1979) have extended earlier findings and reported that field CA3 projects bilaterally, crossing the midline in the ventral hippocampal commissure, to the lateral septal nucleus. Field CA1 and the dorsal subiculum have an ipsilateral efferent terminating further rostrally than the CA3 projection in the lateral septal nucleus. These authors also observed fibres leaving the lateral septal nucleus to innervate the medial septal diagonal band complex. The precommissural projections of the ventral subiculum pass through the septofimbrial nucleus, other fibres continue into the bed nucleus of the stria terminalis, and further ventrally into the medial parts of the nucleus accumbens, and to the deeper layers of the infralimbic cortex, to the posterior and medial parts of the anterior olfactory nucleus, and to the molecular layer

of the taenia tecta.

In addition to the subiculo-septal projections described previously, Swanson and Cowan (1977) reported that the dorsal part of the subiculum projects bilaterally by way of the fimbria to the pars medialis and pars posterior of the medial mammillary nucleus. The dorsal subiculum also projects to the perirhinal area. The most ventral part of the subiculum gives rise to the medial cortico-hypothalamic tract which runs in the lateral fimbria (Raisman et al., 1965) and terminates in the capsular zone surrounding the ventromedial nucleus of the hypothalamus. Through it's links with the various basal forebrain and hypothalamic areas the subicular complex is in a position to modulate the activity of most of the structures contributing to the rostral part of the medial forebrain bundle, and beyond them to important brainstem structures.

Swanson and Cowan (1977) summarize the fibre organization of the hippocampal formation as follows: the dentate gyrus occupies a pivotal position in the processing sequence since it is the recipient of the major extrinsic input to the hippocampus from the entorhinal cortex (Hjorth-Simonsen, 1972) and it projects upon fields CA3 and CA4 by way of the mossy fibre system (Blackstad et al., 1970).

Field CA4 feeds back upon the dentate gyrus of both sides while CA3 not only provides a major hippocampal outflow to the septum but also projects massively to CA1 through it's Schaffer collaterals, as well as to fields CA1 and CA3 of the contralateral side.

Pharmacology

It has been a long standing belief that acetylcholine is a neurotransmitter in the brain. Still no cerebral pathway has been conclusively shown to act by the release of acetylcholine. However, there has been much evidence accumulating in favor of it's involvement in the hippocampal formation.

Acetylcholine (ACh) is synthetized from acetyl-CoA and choline in a reaction catalyzed by the enzyme choline acetyltransferase (ChAT). After ACh is released from the nerve terminal it is broken down into acetate and choline by the enzyme acetylcholinesterase (AChE). The regional distribution of ChAT follows that of ACh fairly closely. Therefore, ChAT is a reliable marker for cholinergic structures. In comparison choline is a poor marker because it has a widespread distribution. Similarly, AChE can be found in non-cholinergic neurons but it's distribution is often correlated with that of ACh in the hippocampus. ACh receptors with muscarinic, nicotinic and mixed properties have been described. (See review by Storm-Mathisen, 1977.)

The only cholinergic afferents to the hippocampal formation arise in the septum (Lewis & Shute, 1967). The

findings, which formed the main basis for the concepts of the cholinergic septo-hippocampal pathway, are as follows. After transection of the fimbria, AChE (Shute & Lewis, 1961) and ChAT (Lewis, Shute, & Silver, 1967) almost completely disappear from the hippocampal region. Staining for AChE shows accumulation of reaction product in some of the stained nerve fibres on the septal side of a transection of the fimbria, whereas practically all staining is lost on the temporal side. Also, ChAT accumulates on the septal side of a transection of the fimbria. During ontogenesis AChE staining in the hippocampal formation appears earliest in the septal part and spreads toward the temporal portion. After AChE inhibition, recovery of the enzyme activity in the septum precedes that in the hippocampus. (See review by Storm-Mathisen, 1977.) The fine medial septum fiber system innervating the hippocampus was shown to have a remarkably similar distribution to that of cholinergic marker enzymes (Nyakas et al., 1987).

By placing a small cup filled with artificial cerebro-spinal fluid on the alvear surface of the dorsal hippocampus it was possible to show that ACh is spontaneously released, and that the release could be increased several times by electrical stimulation in the medial septum (Dudar, 1975;

1977). The latter effect was abolished by sectioning the fimbria (Dudar, 1975).

The distributions of ACh, ChAT and AChE in the hippocampal formation were compared and a very close correspondence was found (Fonnum, 1970; Gage et al., 1986; Kuhar, Sethy, Roth & Aghajanian, 1973). All tended to diminish in parallel after lesion of the presumed cholinergic input (Kuhar et al., 1973; Lewis et al., 1967). Lesions restricted to the medial part of the septum induced the loss of up to 90% of the hippocampal ChAT, whereas lesions in the lateral parts of the septum were ineffective (Oderfeld-Nowak, Narkiewicz, Bialowas, Dabrowska, Wieraszko, & Gradkowska, 1974). The loss of 60% of the high affinity choline uptake (sodium dependent) activity from the hippocampus after lesions of the medial septum has been reported by Kuhar et al. (1973). The high affinity choline uptake was a valuable marker for ACh and ChAT although there remained some background uptake resistant to septal lesions. Hemicholinium-3 is a powerful inhibitor of the high affinity choline uptake but it's use as a marker has proved to be unspecific (see review Storm-Mathisen, 1977).

When applied by iontophoresis ACh produces mostly slow excitation in the hippocampus (Bland, Kostopoulos, &

Phillis, 1974; Segal, 1978). According to most reports this is antagonized by atropine, indicating that muscarinic type receptors are involved. Petsche and Stumpf (1960) demonstrated that systemic injection of physostigmine elicited hippocampal theta. Microinfusions of cholinergic agents in the hippocampus elicited theta in urethane anesthetized rats when applied in layers oriens and radiatum of CA1 and CA3, layers granular and molecular of the dentate area and in the infragranular region of the hilus (Rowntree & Bland, 1986).

Lynch, Rose and Gall (1977) compared the distribution of AChE-stained cell bodies in the medial septal and diagonal band nuclei to the distribution of cells containing HRP reaction product and concluded there was a large amount of overlap. They mentioned the possibility that some cells stained by HRP did not stain for AChE. Baisden, Woodruff and Hoover (1984) have extended these findings and reported that less than 50% of HRP-labelled cells in the medial septal diagonal band also contain AChE. The majority of cholinergic terminals contacting hippocampal neurons most likely arise from cholinergic cells in the medial septal diagonal band complex. However, some immunocytochemical studies employing different antibodies against ChAT have

recently described a few cholinergic neurons in the hippocampus and fascia dentata that might contribute to the hippocampal cholinergic innervation. Frotscher, Schlander and Leranth (1986) reported that ChAT-immunoreactive cells were rare but were observed in all layers of the hippocampus and fascia dentata with a preponderance in zones adjacent to the fissure and in the part of CA1 bordering the subiculum. All the neuron types showing this activity were considered to be non-pyramidal neurons.

After lesions of the medial septum, Mellgren and Srebro (1973) reported an almost complete loss of AChE in all layers of the hippocampal formation except in the moleculare layer of the subiculum and CA1. Fonnum (1970) localized the highest ChAT activity to the infrapyramidal (oriens) layer and described a gradual decrease of this activity with increasing distance from the pyramids. In the dentate gyrus, the highest ChAT activity was found in the polymorph and molecular layers (Fonnum, 1970). AChE is predominant in these two layers (Mosko et al., 1973). They show high density following autoradiographic labelling and medial septal lesions (Lynch et al., 1977).

Fonnum (1970) and Rotter, Birdsall, Burgen, Field, Hulme and Raisman (1979) reported a high density of

muscarinic receptor binding sites in layers oriens and radiatum of the hippocampus and the molecular layer of the dentate area (Kuhar et al., 1973; Rotter et al., 1979). Kuhar and Yamamura (1975) found the label ^{3}H-quinuclidinyl benzilate (QNB, muscarinic antagonist) to be mainly associated with regions containing dendrites, and therefore possibly synaptic receptors. The grain densities were high in the oriens, radiatum and molecular layers. Most of the QNB label in the hippocampal region could be displaced by pretreating the animals with atropine. Aguilar, Jerusalinski, Stockert, Medinia, and De Robertis (1982) reported a loss of 44% of ^{3}H-QNB binding sites in the hippocampus following selective destruction of pyramidal cells in CA3. A high density of nicotinic receptor binding sites was localized in layer oriens of CA1 and the polymorph region of the hilus with the use of alpha-bungarotoxin, an irreversible nicotinic antagonist (Hunt & Schmidt, 1979). In a review article of putative neurotransmitters in the hippocampus, Storm-Mathisen and Ottersen (1984) reported that muscarinic binding sites were concentrated in layers oriens and radiatum and in the inner part of the molecular layer of the dentate area whereas nicotinic binding sites were concentrated in the hilus and the layer oriens. The

majority of nicotinic and muscarinic receptors in the hippocampus are located postsynaptically (Dudai & Segal, 1978).

The specificity of postsynaptic cells, the different distribution of muscarinic and nicotic receptors and the common cholinergic source of afferents (the medial septal diagonal band complex) together suggest that granule cells are muscarinic and some polymorph cells are nicotinic (Fibiger, 1982). This hypothesis gained support from Wheal and Miller (1980) who orthodromically activated granular cells by medial septal stimulation. This activation was antagonized by atropine sulfate (muscarinic antagonist). These findings were in partial agreement with the stratified distribution of ChAT and AChE in the granule cell region.

Stimulation studies have shown that fimbrial (Krnjevic, Reiffenstein, & Ropert, 1981), septal (Krnjevic & Ropert, 1981) and entorhinal (Ben-Ari, Krnjevic, Reinhardt & Ropert, 1981) inputs to the hippocampus are excitatory. At the level of the pyramidal cells, the response is a field positive wave which has been shown to correspond to the occurence of inhibitory postsynaptic potentials (IPSPs) in pyramidal cells (Andersen, Eccles, & Loyning, 1964). A local application of ACh by iontophoresis induced a

reduction of the amplitude of the positive wave and the appearance of population spikes (Krnjevic et al., 1981). Similarly, the application of muscarinic agonists, in particular, and nicotinic agonists induced population spikes in layer pyramidal of CA1 (Ropert & Krnjevic, 1982).

The facilitatory action of ACh in the hippocampus has been shown to work through at least two mechanisms: first, a direct postsynaptic action on the pyramidal cells (Ben-Ari et al., 1981) secondly, a presynaptic disinhibitory action (Ben-Ari et al., 1981; Krnjevic et al., 1981). Part of the action of ACh may be mediated by a reduction of the release of inhibitory transmitter (Ben-Ari et al., 1981; Valentino & Dingledine, 1981).

The depolarizing effect of ACh on CA1 pyramidal cells results from decreases in a voltage-dependent potassium stabilizing conductance (Brown, 1983), and a calcium activated potassium conductance (Benardo & Prince, 1982a; 1982b; Cole & Nicoll, 1983). The reduction in potassium conductance produces a slow depolarization by allowing voltage dependent sodium and calcium conductances in presynaptic terminals to dominate (Benardo & Prince, 1982b). In addition to this depolarizing action, ACh has been found to transiently block afterhyperpolarizations that usually

follow depolarization (Benardo & Prince, 1982a; Cole & Nicoll, 1983). This effect is believed to result from the blockade of a calcium activated potassium conductance (Cole & Nicoll, 1983). Thus, ACh appears to produce direct excitation as well as long term modulation of the excitability of pyramidal cells (Benardo & Prince, 1982a).

In contrast to the somatic effect of ACh, local application of ACh in the dendritic region reduces the field EPSP (excitatory postsynaptic potential) and the somatic population spike (in the slice: Valentino & Dingledine, 1981; in vivo: Rovira, Ben-Ari, & Cherubini, 1983), an effect which is also probably mediated presynaptically (i.e. a reduction of excitatory transmitter release, Valentino & Dingledine, 1981). Similar to the action of ACh, electrical stimulation of the medial septum enhanced the population spikes produced in the pyramidal layer by commissural stimulation and depression of the field EPSP recorded in the apical dendrites (Rovira, Cherubini, & Ben-Ari, 1983). Both effects could be blocked by systemic and local (Krnjevic & Ropert, 1981; Rovira, Cherubini & Ben-Ari, 1983) administration of muscarinic antagonists.

Rovira, Cherubini and Ben-Ari (1983) showed that both muscarinic and nicotinic receptors were involved in the

facilitatory action of cholinomimetics at the cell soma, whereas muscarinic and nicotinic agents had opposite actions on the dendritic field EPSPs. Nicotine enhanced the depression of the field EPSP. Interestingly, nicotinic agonists were more potent than muscarinic ones in depressing the soma field potential which may indicate that nicotinic agents have a purely disinhibitory action whereas a double mechanism would underline the muscarinic action, i.e. presynaptic reduction in GABA release as well as postsynaptic facilitation (Ropert & Krnjevic, 1982). Nicotinic receptors seem to produce excitation in a classical way by increasing the permeability to positively charged molecules, whereas muscarinic receptors may produce excitation (the dominant effect) by a decrease in the potassium conductance, and inhibition by an increase in the potassium conductance (see review by Krnjevic, 1974).

Many if not all afferents to hippocampal neurons terminate on both principal cells and nonpyramidal neurons (Leranth & Frotscher, 1987). Cholinergic fibres terminate on pyramidal neurons and granule cells and GAD (GABA synthetizing enzyme glutamic acid decarboxylase) and coexisting somatostatin (neuroactive peptide) are immunoreactive on nonpyramidal neurons in the hilar region

(Leranth & Frotscher, 1987). Binding sites for GABA are concentrated in the molecular layers of area dentata and CA1 (Storm-Mathisen & Ottersen, 1984). Most nonpyramidal neurons in the hippocampal formation may be immunostained with antibodies against GAD. These neurons are likely to be inhibitory because GABA has been found to be an inhibitory transmitter in the hippocampus and fascia dentata (Storm-Mathisen & Ottersen, 1984). GABA is very probably the main inhibitory agent released by inhibitory pathways in the hippocampus (Storm-Mathisen, 1977; Ben-Ari et al., 1981). Andersen, Bie, and Ganes (1982) demonstrated in the transverse hippocampal slice the inhibitory action of GABA on CA1 pyramidal cell single units and population spikes, the most effective site of action being the soma. The results of double labeling experiments combining GAD and SS immunostaining with retrograde HRP tracing provide evidence that at least some GABAergic neurons in the hilar region are projection (commissural) cells (Leranth & Frotscher, 1987). Similarly, double-labelled, GAD-positive and HRP-labelled commissural neurons in the hilus and CA3 have been observed (Ribak, Seress, Peterson, Seroogy, Fallon, & Schmued, 1986).

The presynaptic action of ACh has been explained by both feedforward and feedback inhibitory mechanisms.

Feedback inhibition by means of muscarinic receptors located on cholinergic axons or terminals, involved in the autoregulation of ACh release in the hippocampal slice, was proposed by Hadhazy and Szerb (1977). They demonstrated that atropine not only prevented the depressant effect of muscarinic agonists on ACh release but, when given alone, significantly potentiated the evoked release of ACh. Feedforward inhibition was suggested to operate through excitation of inhibitory interneurons (Benardo & Prince, 1982a).

Besides ACh and GABA, other putative neurotransmitters have been localized in the hippocampal formation. Histochemical and biochemical investigations have demonstrated a projection of norepinephrine and serotonin (5-HT) containing fibres to the hippocampus projecting via the medial forebrain bundle and partly through the lateral septum from the locus coeruleus and the raphe nuclei, respectively (see review by Storm-Mathisen, 1977). Axons from locus coeruleus have been traced to the hippocampal formation and other forebrain regions by autoradiographic techniques (Segal, Pickel, & Bloom, 1973). The grain density was highest in the hilus and layer oriens of the CA3 region. This projection has been confirmed by retrograde

transport of horseradish peroxidase (Segal & Landis, 1974).

Segal and Landis (1974) also reported hippocampal afferents deriving from the raphe nuclei. Hippocampal innervation was localized in the infragranular zone of the hilus and layer lacunosum-molecular of CA1 (Moore & Halaris, 1975). Lombardi, Gandolfi, Dall'oio, Pellegrini-Giampietro, Beni, Carla, Consolazione, and Moroni (1987) have reported a significant loss of 5-HT content, greatest in the dorsal hippocampus and less in the ventral hippocampus, following unilateral transection of the fimbria-fornix and the cingulate cortex. These transections interrupt fibres originating in the raphe medialis.

The content of histamine in cortical regions was extremely low (Storm-Mathisen, 1977). Aromatic amines more commonly produce inhibition in the brain (Krnjevic, 1974). Like choline, the amino acids glutamate and L-aspartic acid are involved in general metabolism which makes their localization in neuronal populations difficult. The input to the layer radiatum from CA3 axons is thought to utilize glutamate (Storm-Mathisen, 1977).

Electrophysiology

Electrical field recording in the hippocampus reveals a theta rhythm with a frequency band width between 4 and 12 hertz (Hz) in the rabbit. The theta rhythm has a quasi-sinusoidal waveform of varying amplitude. Hippocampal slow wave activity can also appear irregular in the polygraph recording of the electroencephalogram (EEG) consisting of either large amplitude irregular activity (LIA) or small amplitude irregular activity (SIA) of varying frequency. Reports on the behavioral correlates of hippocampal EEG activity have focused on the rhythmic characteristics and irregular activity termed LIA (Vanderwolf, Kramis, Gillespie & Bland, 1975).

Gross physiological phenomena such as hippocampal theta have been the object of much research into the fundamental mechanisms of EEG activity in the nervous system. Three procedures are necessary for the localization of spontaneous theta: recording of depth profiles in comparison to a reference point, correlation of rhythmical slow wave theta with the activity of identified single units, and correlation of theta with intracellular subthreshold membrane phenomena (Andersen, 1980).

First, consider the profile procedure. The source of theta generation has been associated with maximal amplitudes recorded in depth profiles of the hippocampus and dentate region. In urethane anesthetized, curarized or freely moving animals, theta of maximal amplitude has been located in layer oriens close to the pyramidal cell layer in CA1, confirming previous observations (Green & Arduini, 1954; Petsche & Stumpf, 1960), and layer molecular of the dentate gyrus. In layer radiatum of CA1 a null zone and a phase reversal of approximately 180 degrees were detected between the two generators (Green, Maxwell, Schindler & Stumpf, 1960; Bland, Andersen & Ganes, 1975; Bland & Whishaw, 1976; Winson, 1976). Theta recorded in the CA1 generator has a maximal amplitude of one millivolt and twice that amplitude may be recorded in the dentate generator (Bland et al., 1975). Another profile has been reported which shares the phase shift of approximately 180 degrees in the radiatum layer of CA1 but in addition, also shows a second phase reversal in the molecular layer of the lower blade of the dentate gyrus of the rat (Holsheimer, Boer, Lopes da Silva & van Rotterdam, 1982). They proposed a double dipole model representing two linearly coupled, approximately synchronous, sources of theta activity (CA1 pyramids and

dentate granule cells).

A third generator has been described more rostrally in field CA3 of the curarized rabbit by Petsche and Stumpf (1960). However, Bland and Whishaw (1976) reported weak theta in layer oriens of CA3 of urethane anesthetized rats while Green and Rawlins (1979) reported no theta in CA3 of urethane anesthetized rats. Bland et al. (1975) also reported finding no theta in CA3 of urethane anesthetized rabbits. It has been argued that theta recorded outside the CA1 and dentate generators is volume conducted (Bland & Whishaw, 1976).

Some experimental manipulations have attempted to demonstrate the independence of the generators. Bland et al. (1975) were able to abolish theta in CA1 by surface cooling, lesions and local anesthesia without affecting theta in the dentate area. However, they could not selectively remove dentate theta without removing it also from CA1. Sainsbury and Bland (1981) found that lateral septal lesions disrupted theta in field CA1 only and medial septal lesions abolished theta in both generators suggesting a specific afferent to CA1. Theta persisted in the dentate area following irradiation and destruction of as much as 92% of granule cells (Whishaw, Bland, & Bayer,

1978).

Second, consider the extracellular procedure. Hippocampal theta receives a major afferent drive from the medial septal diagonal band complex. In a topographic study of theta propagation in the cerebral cortex of the rabbit, Petsche and Stumpf (1960) found that hippocampal theta did not arise in the septum but in the hippocampus itself. They replicated Green and Arduini's (1954) finding by showing that after dorsomedial septal lesions no theta was found in any part of the brain. In conclusion, they proposed that hippocampal theta waves were governed by impulses arising in the septum and that the reticular ascending pathway acted as a pacemaker for the septum.

The idea of a septal pacemaker function for hippocampal theta rests on several observations. Besides the fact that the septum is necessary for the occurence of hippocampal theta, it has been demonstrated that damage to the feedback efferents to the lateral septum did not abolish slow wave theta (Rawlins, Feldon & Gray, 1979). Some cells in the medial septum and diagonal band nuclei fire in bursts, each locked to the same phase of the theta wave (Gaztelu & Buno, 1982; Macadar, Roig, Monti & Budelli, 1970; Ranck, 1973, 1976; Vinogradova, Brazhnik, Karanov & Zhadina, 1980).

Gogolak, Stumpf, Petsche and Sterc (1968) have shown that superimposed time histograms of these bursts could result in a theta wave. The independence of these bursts from the hippocampal activity was demonstrated by Petsche, Stumpf and Gogolak (1962) who were able to record bursting cells in the absence of theta rhythm. Andersen (1980) has formulated an alternative hypothesis taking into account the fact that the hippocampal theta waves are not driven cycle by cycle by uniformly phase-locked inputs from the septal region. Andersen states that the medial septal cells, although driving cyclic activity, provide the hippocampal neurones with a more graded or tonic influence which is promoting theta occurence.

The modulation of hippocampal theta can be traced back to the brainstem. After septal undercutting (including the medial forebrain bundle), the mean frequency of bursts was lowered in cells of the medial septal nucleus and rhythmic bursts were preserved. In this condition, low frequency slow wave theta was present in the hippocampal EEG of unanesthetized rabbits (Vinogradova et al., 1980; Brazhnik & Vinogradova, 1986). This deafferentation produced a two-fold increase of the spontaneous activity of medial septal cells indicating that the transected fibers exerted an

inhibitory influence (Vinogradova et al., 1980). Green and Arduini (1954) reported that hippocampal theta could be induced by electrical stimulation of the midbrain reticular formation, intralaminar thalamic nuclei, hypothalamus, septum, lateral and medial geniculate bodies. Stimulation of the brainstem, hypothalamus and septum with increasing intensity elicits hippocampal theta of increasing frequency (Bland & Vanderwolf, 1972; Robinson & Vanderwolf, 1978; Kramis & Vanderwolf, 1980; Brazhnik & Vinogradova, 1986).

In a review of the brainstem generation of hippocampal EEG, Vertes (1982) concluded that hippocampal synchronization (theta) was most effectively elicited with stimulation of the nucleus pontis oralis, and that hippocampal desynchronization was very effectively produced by stimulation of the median raphe nucleus. The effects of the brainstem on the hippocampus are mediated by the medial septal diagonal band nuclei. A desynchronizing system originating from the median raphe was shown to ascend through the region of the medial forebrain bundle and presumably reached the medial septum through this route. A synchronizing system was found to course rostrally from the pontine reticular formation through the supramammillary nucleus and probably reached the septum by a polysynaptic

pathway (Vertes, 1986).

Since Green and Machne (1955) reported a correlation between some hippocampal unit bursts and regular slow waves, Ranck and colleagues (Ranck, 1973; Fox & Ranck, 1975; 1981) and subsequently others (O'Keefe, 1976; Bland, Andersen, Ganes, & Sveen, 1980) have shown that a large percentage of cells throughout the hippocampus discharge in phase with hippocampal theta.

When theta activity occured spontaneously or following dorsomedial-posterior hypothalamic stimulation, CA1 pyramidal and dentate granule cells discharged during extracellular negativity (Bland et al., 1980). From phase histograms, it was found that almost all granule and CA1 pyramidal cells were modulated or phase-locked to theta activity. Further, it was reported that during hippocampal desynchronization dentate granule and CA1 cells discharged irregularly and at much lower rates than during theta.

Third, consider the intracellular procedure. There is no direct proof that the theta wave or any other EEG pattern is caused by synaptic potentials, but there is much circumstantial evidence for this point of view (Green et al., 1960). Recording intracellularly from identified CA1 pyramidal neurons in the rabbit, Fujita and Sato (1964)

observed that cells displayed rhythmic intracellular oscillations: membrane fluctuations in synchrony with, and of opposite polarity to, the locally generated theta rhythm. Pyramidal cells generally discharged two-spike bursts with each depolarizing wave. It was suggested that the EEG theta rhythm was formed by the extracellular field current driven by the intracellular rhythm and thereon superimposing spike potentials. Andersen (1980) replicated these findings in CA1 pyramidal and dentate granule cells. He concluded that both EPSPs and IPSPs were involved in the production of intracellular theta rhythm. Thus, the electromotive force of the EEG is seen partly as a summation of slightly asynchronous synaptic potentials. In addition, large depolarizing waves associated with burst discharges and the following post-activation hyperpolarization were thought to contribute, particularly at high levels of activation.

In addition to intracellular reports, it has been stressed that the recurrent inhibitory circuit is also involved in the genesis of the theta rhythm. A powerful and widespread recurrent inhibition of the pyramidal cells is ascribed to the axo-somatic synapses of the basket cells (Andersen, Eccles & Loyning, 1963). Thus the field potentials indicate the pyramidal IPSP is generated in the

region of the soma. After activation of pyramidal cells, EPSPs with repetitive discharges can be intracellularly recorded from neurons in layer oriens. It is assumed that interneurons probably of the basket type are activated via pyramidal axon collaterals (Andersen et al., 1964).

The alignment and apposition of pyramidal and granule cells were postulated to promote synchrony. There is evidence of electrotonic coupling between pyramidal cells (MacVicar, Ropert & Krnjevic, 1982) and between granule cells (MacVicar & Dudek, 1982).

It has been suggested that recurrent inhibition controls the spread of synchronous burst firing through recurrent excitatory pathways (Miles & Wong, 1987). Indeed, the application of picrotoxin, a GABA antagonist, on the CA3 area in hippocampal slices caused the discharge of pyramidal cells to become synchronized. The appearance and growth of rhythmic depolarizations spread via collaterals gradually with time.

Behavior

The theta rhythm has gained importance in behavioral studies ever since the report by Green and Arduini (1954). They manipulated various stimuli (sensory, electrical and pharmacological) in order to elicit hippocampal theta in acute and conscious animals. They concluded that the theta response resulted from sensory activation of the midbrain reticular formation.

Theta behavioral correlates have led to the postulation of two distinct types of theta. Vanderwolf (1969) first described movement-related theta, later called type 1 theta. Sensory related theta was termed type 2 theta (Kramis et al., 1975). The overall frequency of theta is higher when an animal is moving than when it is immobile and subjected to sensory stimulation, although there is some overlap in the two respective frequency ranges. During the phasic episodes of paradoxical sleep, theta is present in EEG records with a frequency much like that accompanying walking and rearing in the rat (Vanderwolf, 1969). In the rabbit, Harper (1971) found long trains of theta occurring during tonic stages of sleep and theta of higher frequency during phasic stages (similar to that seen during walking periods),

confirming the observations of Ranck (1973) on the rat. Theta types are further differentiated on the basis of cholinergic sensitivity and ontogeny.

Recording of hippocampal theta in newborn animals has shown that atropine-sensitive and atropine-resistant theta appear at different ages. Vanderwolf et al. (1975) reported that in rat and rabbit type 1 theta appeared at 12-14 days and type 2 theta at 20-22 days. In the guinea pig, a precocious species, both types of theta were present at birth. Leblanc and Bland (1979) recorded slow wave theta during movement and REM sleep in the rat at about 10 days of age. Cholinergic sensitive theta could be elicited and abolished in rats of the same age. Both types of theta were said to develop in parallel in the CA1 and dentate generators. In the rabbit, Creery and Bland (1980) observed that movement related theta appeared at 8 days and theta elicited by auditory stimuli could be recorded at 14 days and abolished by atropine at 23 days.

Cholinergic manipulations were performed on adult freely moving animals by systemic injections. In immobile rabbits, type 2 theta in response to sensory stimulation was abolished with antimuscarinic agents, atropine and scopolamine, while mobility related type 1 theta was

unaffected (Kramis et al, 1975; Vanderwolf et al., 1975; 1978; Bland, Seto, Sinclair & Fraser, 1984). Immobile rats rarely produce spontaneous type 2 theta but it can be elicited with eserine, an anticholinesterase, and by reticular formation electrical stimulation and later abolished with atropine and scopolamine (Vanderwolf et al., 1975). Control injection of atropine methyl nitrate, which does not cross the blood-brain barrier, had no effect on type 2 theta (Kramis et al., 1975; Whishaw, Bland, Robinson & Vanderwolf, 1976).

Whishaw, Robinson and Schallert (1976) have argued that intraventricular administration of anticholinergics is inefficient in blocking type 2 theta. However, Dajas, Gaztelu, Zavalla, Macadar and Garcia-Austt (1983) reported that intraventricular injections of nicotinic ligands produced theta of three to five hertz (Hz, cycles per second) which was abolished by a systemic injection of atropine. They concluded that nicotinic ligands depressed a tonic inhibition thus allowing facilitation of muscarinic excitation of theta, subsequently blocked by a muscarinic antagonist.

The action of systemically administered drugs cannot easily be localized. Elicitation or blockade of hippocampal

theta may result from the drug action on the brainstem, the septum or the hippocampal formation (Stumpf, 1965). Thus, Brazhnik and Vinogradova (1986) have argued that hippocampal theta is solely the result of the septal cellular activity. They studied the effect of anticholinergic drugs on the septal bursting pacemaker and theta correlated cells. Anticholinergic drugs affected the overall power of theta bursts in the whole theta frequency band and no frequency shift was observed. They suggested that only secondary cholinoceptive neurons were inhibited thus reducing the number of rhythmic firing cells. However, true pacemaker cells were still active but would respond only to strong stimulation from the brainstem, producing hippocampal theta of high frequency.

An alternative argument for the selective blockade of atropine sensitive theta is to suppose two different afferent systems for the two types of theta. Some authors have suggested that type 1 theta may receive a non-cholinergic afferent (Whishaw, Bland, Robinson, & Vanderwolf, 1976; Sinclair, Seto & Bland, 1982; Bland et al., 1984). There exists anatomical evidence for a non-cholinergic projection from the medial septal diagonal band nuclei to the hippocampal formation (Lynch et al., 1977).

The entorhinal cortex has also been suggested as a possible source of type 1 theta. Following entorhinal lesions, Vanderwolf and Leung (1983) reported that only atropine sensitive theta remained and supposed that the entorhinal input may mediate type 1 theta. They also noted that the remaining atropine sensitive theta sometimes appeared during movement. This observation together with Dudar, Whishaw and Szerb's (1979) report of a high ACh release rate in the hippocampus during movement are evidence in support of the notion that both types of theta are coactive.

Theta has been recorded in the hippocampus of the rabbit, rat, guinea pig, gerbil, cat, and dog (e.g. rabbit: Green & Arduini, 1954; rat: Vanderwolf, 1969; guinea pig; Sainsbury, 1970, gerbil: Whishaw, 1972, cat: Green & Arduini, 1954; dog; Arnolds, Lopes Da Silva, Aitink, & Kamp, 1979). In primates theta studies are few (Green & Arduini, 1954; Arezzo, Tenke, & Vaughan, 1987). Robinson's (1980) review of species differences among lower mammals led him to conclude that there probably were not major species differences in the neural system underlying atropine-resistant, movement-related theta but that there were definitely species differences in the neural system responsible for producing atropine-sensitive, immobility-

related theta. These differences may very well be related to differences in the behavioral strategies various species have adapted to their particular environment, in agreement with Winson (1972).

In 1973, Ranck published the results of an extensive study on hippocampal cells' firing repertoires and behavioral correlates in the rat. Recording from the dorsal hippocampus and dentate area, he categorized cells as either theta or complex spike cells. According to their firing repertoire, theta cells always fired single action potentials while complex spike cells could sometimes exhibit a complex spike in addition to single spikes. A complex spike is a series of two to seven individual spikes in which the amplitude of individual spikes changes during the series, usually decreasing. The rate of firing was always faster for theta cells than for complex spike cells. During both paradoxical sleep and wakefulness, the two cell types usually fired in phase with the locally recorded theta. Only theta cells showed an increased firing rate along with greater frequency of slow wave theta. Theta cells always fired more rapidly during theta (occuring either during movement or tonic and phasic paradoxical sleep) than during LIA. Finally, the behavioral correlate of rapid firing in

theta cells was identical to the behavioral correlates of the theta rhythm, while the behavioral correlates of complex spike cells were unrelated to those of the theta rhythm.

O'Keefe and Dostrovsky (1971) described 'movement' cells which were clearly the same as Ranck's theta cells. O'Keefe's (1976) place units with complex spikes had a low resting firing rate and higher firing rate during automatic behaviors than during movement whereas displace units with simple spikes were identical to Ranck's theta cells, increasing their firing rate with hippocampal theta and losing their rhythmicity during LIA.

Bland et al. (1980) demonstrated in urethane anesthetized rabbits that electrophysiologically identified pyramidal cells firing occasional complex spikes increased their discharge rate and rhythmicity during theta. They also reported that basket cells did not show theta modulation of their discharges as much as CA1 pyramidal cells or dentate granule cells. Suzuki and Smith (1985), in an attempt to harmonize Ranck's and Bland et al.'s findings, have reported that granule cells can be distinguished from both complex spike cells and theta cells, and that a small number of complex spike cells increase discharges during theta. Granule cells were found to fire rhythmically during

theta just like theta cells but unlike theta cells they exhibited long silent periods during alert immobility.

Feder and Ranck (1973) studied theta cells and concurrent slow wave theta in the rat in order to determine whether or not a clearly voluntary, gross motor pattern would be accompanied by theta once the motor pattern became stereotyped and automatic through intensive operant training. They concluded that the unit and EEG activity during bar pressing, when well learned, were not of the theta mode. However they reported that during the approach to the reward, both unit and the EEG were in the theta mode, a contradictory and unexplained finding.

Bland, Seto and Rowntree (1983) reported a linear increase in multiunit discharge rates with increasing frequency of theta during both theta behavior categories, type 1 and type 2 theta, in the rabbit. The augmented discharges could have been the result of a small number of cells' increased response or the result of more recruited cells. The firing rate was always greater during type 1 behaviors than type 2 behaviors even at equivalent frequencies.

Theta cells were renamed theta-on cells with the discovery of the reciprocal theta-off cells in urethane

anesthetized rats (Colom, Ford & Bland, 1987). Theta-on cells increased their rhythmic discharge rate with increasing theta frequency. Theta-off cells were always silent during theta frequencies of 5 Hz or more and began firing rhythmically, increasing their firing rate as theta frequency decreased. They fired non-rhythmically and at high rates during LIA. Colom and Bland (1987) have reported the existence of theta-on and theta-off cells with a tonic firing pattern in urethane anesthetized rats. Theta-on and theta-off cells described in Colom et al. (1987) were termed phasic to contrast them with the tonic cells. Tonic theta-on cells fired non-rhythmically at a constant rate during theta and had a higher discharge rate during theta than during LIA. Tonic theta-off cells never fired during theta and fired at low rates, non-rhythmically, during LIA.

Bland et al. (1984) performed extracellular recordings of CA1 and dentate cells in the freely moving rabbit and reported that eserine elicited firing rhythmicity synchronized with theta. Both rhythmic cellular discharges and the theta rhythm elicited with sensory stimulation were abolished with atropine sulfate, replaced by the slow wave activity and discharge patterns occurring during LIA. During type 1 theta behaviors, neither the slow wave nor the

cell rhythmicity were affected. However, a reduction in the cell firing rate was reported. This indicated that the blockade of type 2 theta had some effect on type 1 theta. Finally, atropine did not affect the cell discharge rate or the EEG pattern during LIA behaviors.

In running rats, atropine injections decreased the firing rate of theta cells (Buzsaki, Leung & Vanderwolf, 1983). Bland and Bland (1986) observed a similar reduction of unit activity following medial septum lesions in freely moving rabbits. Medial septal lesions produced a reduction of hippocampal cell discharge rate and rhythmicity in the type 1 theta condition while no difference was observed during the type 2 condition.

In summary, the behavioral studies of hippocampal theta cells have shown that motor and sensory correlates exist and that only sensory related slow wave theta and accompanying cell discharges are sensitive to cholinergic manipulations. Conceptually, both types of theta activity are believed to be reflections of integrated neuronal processes within the hippocampus (Bland, 1986). Theta cells are closely related to slow wave theta, their rhythmic discharges are phase-locked to a constant portion of the wave and their discharge rate varies in parallel with the slow wave frequency.

Reports of decreased firing rates following atropine treatment of moving rabbits and rats strongly suggest that the cholinergic input is somehow subtracted from the motor input.

The present research was designed to extend earlier findings and to systematically quantify the discharge rate of theta cells during movement, immobility with sensory stimulation and immobility in awake rabbits before and after the administration of atropine sulfate. Precise research hypotheses are defined in the methods section along with the statistical analysis.

METHODS

Subjects

Dutch belted rabbits (Oryctolagus cuniculus) of either sex, weighing between 1.7 and 4.1 kilograms (supplied by the Animal Care services of the University of Calgary) were used in this study. They were housed individually in stainless steel cages and allowed free access to food (16% rabbit pellets, United Feeds) and water. The circadian cycle was adjusted so that these nocturnal animals received 12 hours of light during the night and were left in the dark during the day at which time the experiments took place.

Surgical Procedure

The data were obtained from 12 rabbits. Each adult rabbit was anesthetized for surgical procedures using sodium pentobarbital (30 mg/kg) delivered through the marginal ear vein. Xylocaine or marcaine was injected subcutaneously in the cheeks and on the top of the head to provide local analgesia. The rabbit was placed in the stereotaxic apparatus and the plane between bregma and lambda was

levelled to horizontal. The body temperature was maintained at 38 degrees centigrade using a Harvard Instrument temperature servosystem. The heart rate was continually monitored via a Grass EKG tachograph equipped with an alarm system set at four and eight beats per second.

An uninsulated tungsten wire was placed in the cortex, anterior to bregma, to serve as an indifferent electrode. A tungsten microelectrode (0.3 megohms) insulated with Kynar and the tip electrolytically etched (1 micron) was used as a reference electrode. It was implanted in the dentate gyrus through a 0.5 mm hole drilled in the skull over the right hippocampus (posterior bregma 5.0 mm; lateral midline 5.0 mm; ventral to dural surface 4.8 mm). At the same posterior and lateral coordinates, a 3.0 mm trephine hole was drilled in the contralateral side of the skull. A number 20 nylon nut was then placed over the hole to accomodate the movable microdrive system (Bland, Sinclair, Jorgenson and Keen, 1980). In some rabbits, two wells were installed one above each hippocampus at the same coordinates and the reference electrode was placed on the edge of the right well. The ground electrode consisted of a Winchester subminiature connector soldered to a jewellers screw and placed in the skull close to the well. The electrodes were then attached

to a female nine-pin amphenol plug and the entire assembly fixed to the skull with No. 18-8 pan head screws and dental acrylic. Following surgery the animals were allowed a minimum one week recovery period before testing procedures began.

Apparatus

Electrical signals from the brain were passed through an instrumentation amplifier circuit (TI 412) with gain set at 10 (developed by U of C technical services) and located in the male amphenol plug assembly. They were led through a nine-pin electromechanical commutator and into a Grass model P511 wide-band AC preamplifier wide open. The signals from the reference electrode were then attenuated and displayed using a Grass model 7B polygraph and a 5100 series Tektronic storage oscilloscope. The polygraph amplifiers were generally set at one Hz and 35 Hz for the half amplitude low and high filter settings, respectively. The signals from the movable microelectrode were, in addition to this, lead off from the P511 preamplifier to a Kronhite filter and then to the oscilloscope. Filter settings for the unit activity were narrow band (-3 dB at high pass end (300 Hz) and the

low pass end (3 KHz)). All signals were stored on a TEAC XR-30 FM tape recorder for subsequent computer analysis.

Experimental Procedure

Units were isolated with electrolytically sharpened and Kynar insulated tungsten microelectrodes (one micron tip, 1-10 megohms at 100 Hz) carried by the microdrive. The microdrive was placed in the well at each testing session. As many as 10 tracks were made in each rabbit. Once electrical contact was established at the dural surface five minutes were allowed to pass in order for the cerebral tissue to stabilize. Then the electrode was slowly lowered. Phase relations of theta between the dentate reference electrode and the movable electrode provided information of electrode tip location during the experiment (Bland and Whishaw, 1976).

Behavioral testing was carried out in a large Faraday cage (123 x 65 x 64 cm), the front and top of which was clear glass. A movement-sensing device installed on the floor of the cage provided an output displayed concurrently with brain activity on the polygraph paper. Both the oscilloscope and an audio channel allowed unitary activity

to be selected and isolated. Once isolated units were judged to be stable testing began. Behavior was coded on an FM tape recorder by pressing two buttons which produced one volt, DC square waves of opposite polarity. For 20 minutes mobility and immobility were coded by an observer looking only at the animal. During this period, pure tones were presented through dual speakers mounted on either side of the cage and prerecorded on a Sony tape deck. Tactile stimuli were also given by hand strokes while the rabbit was immobile. Behaviors were grouped into three categories: (1) Type 1 theta behaviors included walking, hopping, postural shifts, rearing, head movements and manipulatory movements of the paws. (2) Type 2 theta behaviors were defined as alert immobility during the presentation of sensory stimuli such as pure tones and stroking. (3) Type 2 LIA behaviors consisted of automatic motor patterns such as alert immobility, licking and chewing. Following baseline recording, the rabbit was injected with atropine sulfate (50 mg/kg, ip) and approximately 10 minutes were allowed to pass until the theta activity during type 2 theta behaviors was abolished. Behavior coding as described above was repeated during an equivalent time span.

At the end of the study, the animals were euthanized

with an overdose of sodium pentobarbital, perfused with saline and 10% neutral formalin, and the brains removed. Microelectrode tip and track locations were reconstructed from unstained coronal brain sections, prepared by freezing the brains with dry ice (CO_2) and sectioning them at 80 microns.

Data Analysis

Data from the three behavioral categories were analyzed by a PDP 11-73 computing system (Digital Equipment Corp.) in the following manner: at the completion of an experiment a paper chart protocol of all signals (with the exception of the unit activity) was created by running the signals from the FM tape into a Grass polygraph. Auditory signals were led through a Schmidt trigger and displayed as a square wave on the polygraph paper. The footage indicator of the tape recorder was coded onto the paper chart by means of a manual signal marker. This protocol was then used to determine the segments of the tape that would be analyzed by the computer for the three behavioral categories as indicated by the behavioral codes.

For all conditions, a minimum sample of 30 seconds was

analyzed. Slow wave signals were sampled at 1000 Hz and converted electronically to a digital signal, and in the case of theta waves, the positive peaks marked. Unitary discharges were first led into an electronic window discriminator (WP-instrument) and then entered into the computer as digital events. Eight data plots were put out on a Houston Instruments x-y plotter: (1) raw data sample (one to two seconds) of slow wave activity with simultaneous unit discharges; (2) histogram of theta frequency; (3) interspike interval histogram; (4) normalized theta waves with standard error 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; (8) autocorrelation of the spike train.

Statistical Analysis

Because of the difficulty in maintaining a clear recording of a single neuron in freely moving animals, it was expected that approximately ten cells would constitute the sample of a repeated measures design. Both the small sample and the interest in studying every cell intensively

suggested that some hypotheses would be most appropriately tested with non-parametric or single subject (the subject being a neuron in this case) statistical methods. Non-parametric tests such as randomization tests are not based on assumptions of large sized samples. Thus, they do not require the assumption of random sampling from a normal population or any population in particular. In other analyses, sufficient data were collected to warrant the use of parametric tests.

The hypothesized positive correlation between the firing frequency of each theta cell and the simultaneous slow wave theta frequency was measured by the Pearson product-moment correlation coefficient (r), based on 30 one-second paired observations. The correlation coefficients were tested according to a randomization test. The process of randomization consists of 10,000 random pairings of the 30 cell discharge measurements with the 30 slow wave theta measurements and the calculation of a correlation coefficient for each pairing. The probability value of an obtained coefficient is determined by the proportion of the 10,000 r's which are as large as the coefficient obtained in the experiment. For example, in a given behavioral condition, a coefficient of .66 associated with a

probability value (p) of .01 is obtained by randomization. This means that no more than one percent of 10,000 random permutations of the data yielded r's as large as .66 and it can be concluded that there exists a very small probability that the two variables are independent of each other. The correlation test used in this research was constructed by Edgington (1980, chapter 8), the description of the computer program (number 8.2) for this test is given on page 205 (Edgington, 1980).

The design allowed comparisons of pre and post atropine recordings of unit firing and accompanying theta waves in three behavioral categories. The analysis of these data was guided by the following hypotheses: 1) That the discharge rate of theta cells and the frequency of the theta rhythm would vary according to the animal's behavior. 2) That during type 1 theta behaviors, the mean discharge rate would be greater than during type 2 theta and LIA categories, and there would be no difference between the latter two. 3) That the mean theta frequency would be higher in the type 1 theta condition than in the type 2 theta condition. 4) That at equivalent theta frequencies, the mean firing rate would be greater during type 1 theta versus type 2 theta behaviors. 5) That theta cells' discharge rate would be

positively related to the concomitant slow wave theta frequency. 6) That administration of atropine sulfate would abolish type 2 theta and the rhythmicity of theta cells during type 2 behaviors. 7) That atropine would reduce the firing rate only during type 1 theta behaviors without affecting the rhythmicity of the discharges.

Parametric procedures were applied according to the descriptions of Pedhazur (1982) and Glass and Hopkins (1984). The latter reference was also consulted for significance tabled values. Any difference between mean firing rates of theta cells in three behavioral categories was tested according to Newman-Keul's all possible contrasts method. The difference between the theta frequency in two behavioral categories was tested by an analysis of variance. Analysis of variance was also used to test the difference between the mean discharge rate of theta cells in two behavioral categories for equivalent theta frequencies. The variance among mean discharge rates before and after atropine in three behavioral categories was analyzed by multiple regression. Then, analysis of variance was used in order to compare pre and post mean firing rates for each behavioral category and the test's significance was determined by randomization (Edgington, 1980 chapter 5,

program 5.1 on page 113). Any difference between theta frequency pre and post atropine was tested with a t-test by randomization (Edgington, 1980 chapter 4, program 4.5 on page 84). Finally, the same t-test by randomization was applied to data of a particular cell for which the atropine treatment did not abolish accompanying type 2 theta. Both discharge rate and theta frequency were analyzed.

RESULTS

An effort was made to isolate single neurons before recordings were taken. After each experiment, good isolation was verified in agreement with Ranck's (1973) criteria: all amplitudes were similar to each other and a fixed window discriminator setting could be effectively used from beginning to end of the tape, and shapes of action potentials as observed on a fast sweep were the same. Even these criteria did not assure that only single neurons were recorded.

Results are based on data from 14 neurons. For two of these neurons short data segments were available and the mean discharge rates were calculated but no measure of slow wave theta was performed. Thus the two cells were not included in single cell analyses. Each neuron was recorded from a different rabbit except in two animals where two cells were isolated, on separate occasions. Only theta cells were selected, that is rhythmically firing neurons, encountered by the movable electrode in a vertical path through the dorsal hippocampus. Pharmacological data were obtained from eight of 14 cells, the remaining six were no longer in the recording site following the drug injection.

Histological verification confirmed the placement of the reference electrode in or above the granular layer of the upper blade of the dentate gyrus. Many tracks were reconstructed and found to be perpendicular to the hippocampal layers penetrating as deep as the lower blade of the dentate gyrus.

Because of the small sample size, it was important to include as many cells as possible in the analysis. The first concern was to establish that no difference existed between the six cells for which only baseline information was available and the eight cells which were recorded from for long enough time to allow post-drug data to be collected. Both mean discharge rates and mean theta frequency were compared by regression analysis of the baseline data. Discharge rates were obtained for all three behavioral categories but theta frequency was calculated only for type 1 theta and type 2 theta behavioral categories because only these records consist of slow wave theta.

The first analysis was performed by regressing groups and behavioral categories on the mean discharge rate of 14 cells with repeated measures on the last variable. The resulting source table (see Table 1) indicates that grouping the data did not significantly add to the explained variance

Table 1

Analysis of variance source table of mean neuronal discharge for two groups (n_1=8, n_2=6) in three behavioral categories

SOURCE	SS	DF	MS	F
Group	708.70	1	708.70	0.34
Subjects within groups	25014.34	12	2084.53	
Category	6821.99	2	3411.00	11.96*
Group X Category	313.48	2	156.74	0.55
Residual	6846.09	24	285.25	

* F (2,24) = 3.40, p<.05

nor did the interaction term of group and behavioral category. Only within group variance and behavioral categories significantly accounted for the mean discharge rate variance. Similarly, the regression analysis of mean theta frequency for two groups of cells in two behavioral categories with repeated measures on the last variable showed no significant difference between the groups (see Table 2). It was concluded that neurons from the two groups did not differ significantly from each other and that all could be included in further analysis.

Theta cells

The interest in studying cells related to the hippocampal theta rhythm was focused on cellular rhythmic firing phase-locked to theta waves and positively related to theta frequency.

The degree of discharge phase-locking was estimated by the cross-correlation between unit firing and phase of the normalized theta wave derived from the reference recording in the dentate area. By superimposing hundreds of discharges recorded in 30 seconds samples on the 360 degrees continuum of a theta cycle, a maximum phase value during

Table 2

Analysis of variance source table of mean theta frequency for two groups ($n_1=n_2=6$) in two behavioral categories

SOURCE	SS	DF	MS	F
Group	0.67	1	0.67	1.86
Subjects within groups	3.62	10	0.36	
Category	4.10	1	4.10	136.67*
Group X Category	0.00	1	0.00	0.02
Residual	0.58	22	0.03	

* F (1,22) = 4.30, $p<.05$

which most discharges occurred and a correlation coefficient were obtained. For 14 cells analyzed in this manner, correlation coefficients ranged from 0.74 to 0.97. Cell location was grossly estimated from the depth of the moving electrode and from the local theta phase relation to the reference theta. Discharges from cells found in the CA1 region were correlated with the dentate theta and a correction factor of 180 degrees was imposed on the resulting maximum phase value to yield a value referring to the local waves. For eight cells located in the CA1 region the mean phase-lag was 256 degrees with a standard deviation (s.d.) of 70 degrees and 291 degrees (s.d. = 80) during type 1 and type 2 theta behaviors, respectively. The mean phase-lag for four CA1 cells during post-type 1 theta behaviors was 236 degrees (s.d. = 90). Six dentate cells had a mean phase-lag of 153 degrees (s.d. = 36) and 171 degrees (s.d. = 40) in the type 1 and type 2 theta categories, respectively. Four of these cells had a mean phase-lag of 160 degrees (s.d. = 45) in the post-type 1 theta category. It appeared that cells believed to be located in the CA1 field discharged preferably on the positive phase of the local theta (peak negativity set at 180 degrees) whereas cells from the dentate area fired on the negative phase of the

local theta.

In order to test the hypothesis of a positive relationship between theta cells' discharge rate and accompanying theta frequency, a randomization test on correlation coefficients (r) was carried out. Every coefficient was based on a sample of 30 one-second segments of slow wave theta and cellular discharges during type 1 and type 2 theta behaviors pre-atropine for each of 12 neurons as well as during type 1 theta behaviors post-atropine for six of the 12 neurons. The one-tail randomization test was applied to each r associated with 28 degrees of freedom. Correlation coefficients and statistical significance, mean discharge rate and mean theta frequency for two categories are reported in Table 3. Six cells showed a significant (significance set at .05) positive correlation in both categories for the baseline state. Four cells did not exhibit this relation in either of the pre categories and two cells' discharges were positively correlated to theta frequency only during type 1 theta behaviors not during type 2 theta behaviors. Among the six cells studied under the effect of atropine, three retained their positive relationship, and two remained non-significantly positively related. Unexpectedly, one cell switched firing pattern

Table 3

Mean firing rate per second (D), mean theta frequency (F) and correlation coefficients before (n_1=12) and after atropine (n_2=6) in two behavioral categories

	baseline						post-atropine		
	type 1 theta			type 2 theta			type 1 theta		
n	D	F	r	D	F	r	D	F	r
1	156	7.77	.67***	80	6.99	.86***	138	7.00	.54***
2	75	7.77	.60***	54	7.05	.58***	45	7.43	.60***
3	65	7.87	.33*	31	7.26	.33*	45	7.12	.59***
4	49	8.14	.08	22	7.18	-.31	52	8.09	.38*
5	41	7.59	-.06	24	6.40	.23	18	7.18	.13
6	29	7.15	.14	20	6.64	.20	28	7.07	-.08
7	94	7.46	.59***	50	5.77	.41**			
8	79	7.94	.61***	65	7.21	.14			
9	59	6.81	.37**	32	6.04	.62***			
10	36	6.90	.42**	21	6.30	.47**			
11	53	7.47	.58***	46	6.71	-.33			
12	32	7.90	.24	11	7.30	-.06			

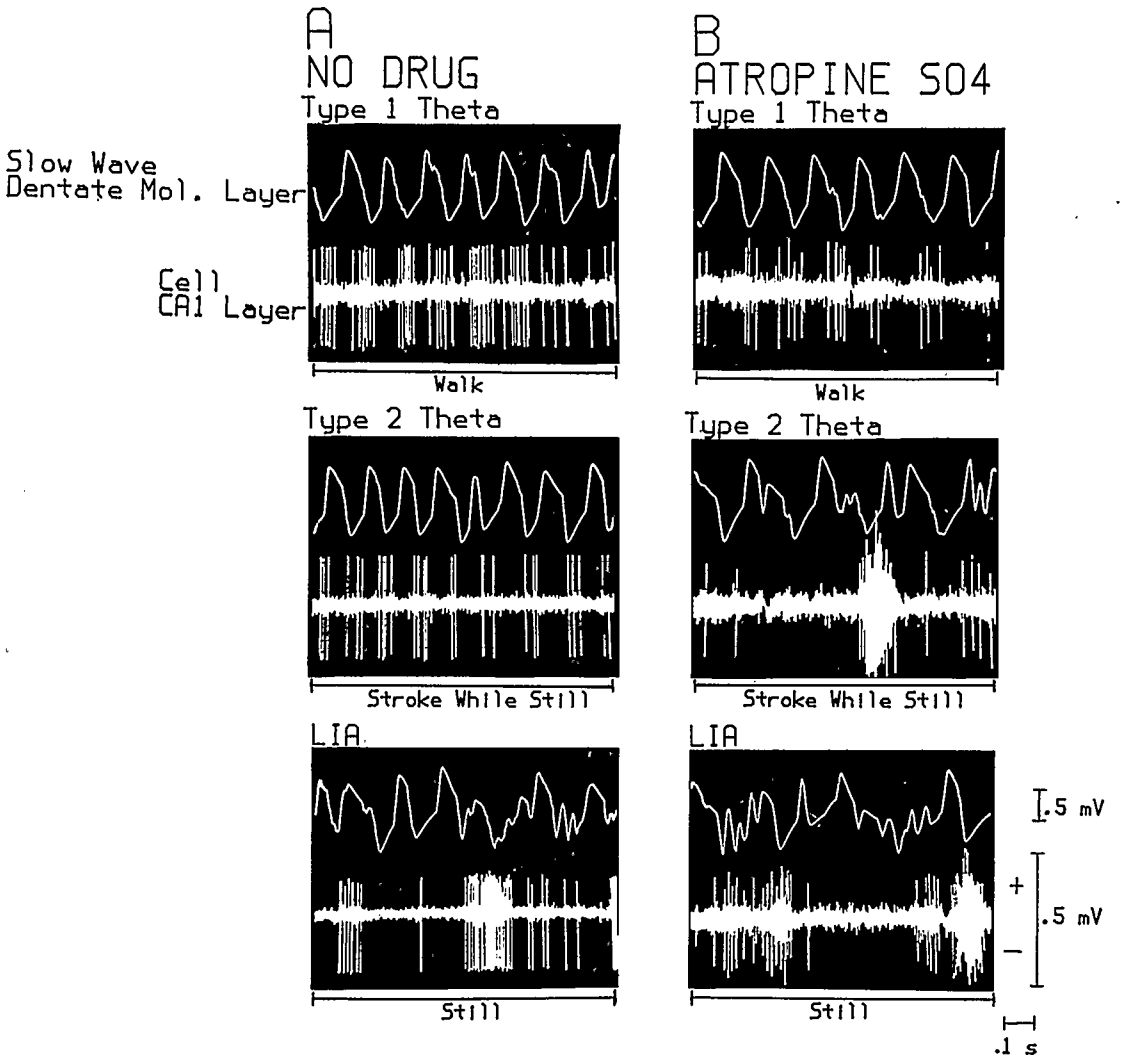
one-tail test
* sig set at .05
** sig set at .01
*** sig set at .001

after the drug and discharge frequency became positively related to the on-going theta frequency. In this particular case, the drug did not abolish the theta rhythm accompanying type 2 theta behaviors and there was a significant positive relationship for both theta categories.

Behavioral categories

Significant differences in the firing rate of theta cells and in the slow wave theta frequency range have both supported the distinction between type 1 and type 2 theta behaviors. It has been shown that during type 1 movement-related behaviors theta cells' firing rate and concurrent theta frequencies were greater than during type 2 sensory and immobility related behaviors. Even for the same theta frequency, discharge rates were greater in the type 1 theta category in comparison to type 2 theta category. Furthermore, no significant difference was found between discharge rates during type 2 theta and LIA behaviors. Analog records of EEG and theta cell discharges during the three behavioral categories pre and post-atropine are shown in Figure 2. By comparing type 1 and type 2 theta categories in the no drug state (equivalent theta

Figure 2. Analog records of hippocampal EEG and theta cell discharges in three behavioral categories before and after the administration of atropine.


A
NO DRUG
Type 1 Theta
Slow Wave
Dentate Mol. Layer
Cell
CA1 Layer
Walk
Type 2 Theta
Stroke While Still
LIA
Still
B
ATROPINE SO4
Type 1 Theta
Walk
Type 2 Theta
Stroke While Still
LIA
Still
.5 mV
+
.5 mV
−
.1 s

frequencies are shown) it appears that theta cells fire less during sensory-related than during movement-related behaviors.

An analysis of variance of the mean theta frequency for 12 neurons with repeated measures on type 1 and type 2 theta categories yielded significant results (see Table 4). The mean theta frequency was greatest during type 1 theta behaviors (7.56 Hz versus 6.74 Hz).

Baseline mean discharge rates for 14 neurons were compared according to three a priori hypotheses by Newman-Keuls' method for all possible contrasts and a per-contrast type-I error rate of 1%. The three behavioral comparisons were type 1 theta versus type 2 theta, type 1 theta versus LIA and type 2 theta versus LIA. Indeed, comparisons yielded significant differences between the mean discharge rate in type 1 theta category (66.66, s.d = 36.04) and that in either type 2 theta (37.88, s.d. = 19.70) or LIA (46.47, s.d. = 29.03) categories. The difference between type 2 theta and LIA categories was found to be non-significant at the .01 alpha level. The studentized range statistic for the contrast comparing type 1 theta and type 2 theta was q = 6.48, critical q (14,3) = 4.89. The second contrast between type 1 theta and type 2 LIA gave a q = 4.55, critical q

Table 4

Analysis of variance source table of mean theta frequency for 12 neurons in two behavioral categories

SOURCE	SS	DF	MS	F
Category	4.03	1	4.03	134.33*
Within subjects	4.64	11	0.42	
Residual	0.30	11	0.03	

* F (1,11) = 4.84, $p<.05$

(14,2) = 4.21. The third contrast opposing type 2 theta and LIA categories was q = 1.93, critical q (14,2) = 4.21, thus a non-significant difference.

In order to compare the firing rate of theta cells in the two theta behavioral categories during equivalent theta frequencies, the overlap between the respective ranges was determined. For each of 12 neurons a minimum of 4-5 seconds of type 1 and type 2 theta and a maximum of 21-22 seconds of type 1 and type 2 theta were selected. The mean sample size of type 1 and type 2 theta was 15 seconds (s.d. = 6). Equivalent theta frequencies ranged from 6.00 Hz for the two categories to 7.95 Hz for type 1 and 7.91 Hz for type 2 categories (grand mean of 7.16 and 7.09 respectively with a s.d. of .42 for both). The mean discharge rate of each cell for two categories with equivalent theta frequencies was computed and an analysis of variance was carried out. The grand mean for type 1 theta behaviors was 57 discharges per second (s.d. = 30), that for type 2 theta was 41 (s.d. = 26). The source table (see Table 5) provides evidence that theta cells fired at a significantly higher rate during movement related theta than they did during sensory processing related theta even for equivalent slow wave frequencies.

Table 5

Analysis of variance source table of mean discharge rate of 12 neurons in two behavioral categories with equivalent theta frequencies

SOURCE	SS	DF	MS	F
Category	1637.46	1	1637.46	29.51*
Within subjects	17110.23	11	1555.48	
Residual	610.38	11	55.49	

* F (1,11) = 4.84, p<.05

Anticholinergic manipulation

As predicted, the administration of atropine sulfate abolished theta during sensory processing related behaviors in seven cells. In the case of one neuron, administration of atropine did not abolish type 2 theta slow wave. Because the cholinergic blockade was unsuccessful for this eighth cell, it was not analyzed with the others but rather as a single case. Data from seven cells were subjected to a pre-post comparison by regression analysis on the mean discharge rates before and after the drug treatment in three behavioral categories with repeated measures on the two variables. Table 6 contains the regression source table. Both pharmacological state and behavioral category accounted significantly for the firing rate variance and the interaction term did not meet the 5% significance level. An analysis of variance by randomization with repeated measures was performed on the mean discharge rate of seven neurons in the type 1 theta behavioral category to verify that the pharmacological effect was specific to this behavioral category. The resulting probability value ($p = .0156$) confirmed the hypothesis of a reduction in the firing rate

Table 6

Results of regressing pharmacological state and behavioral category on the mean discharge rate of seven neurons

SOURCE	SS	DF	MS	F
Within subjects	27372.50	6	4562.08	
State	3089.15	1	3089.15	9.80*
Category	6969.83	2	3484.92	11.06**
State X Category	591.10	2	295.55	0.94
Residual	9457.01	30	315.23	

* F (1,30) = 4.17, $p<.05$

** F (2,30) = 3.32, $p<.05$

during type 1 theta category post-atropine. Table 7 shows the mean firing rate and standard deviation of these cells pre-post and the percent reduction that resulted. The same analysis was performed on data from the type 2 theta behavioral category where the difference between pre and post was found to be statistically non-significant (p = .1406). Finally, the difference was tested for the pre-post type 2 LIA behavioral category, the average discharge rate was significantly reduced after the drug treatment (p = .0156). Figure 3 shows the effect of the anticholinergic blockade on the mean discharge rate of all seven neurons in three behavioral categories.

The effect of atropine on type 1 theta frequency was also analyzed. A t-test by randomization was used on 30 measures of type 1 theta frequency pre and post atropine for each of five neurons. For four cells, there was a significant pre-post reduction (significant p values ranged from .0001 to .0113). In reference to Figure 2 (pp 74-75), the depressing effect of atropine on theta frequency and cells' firing rate during movement-related behaviors is visible in the upper two panels. After the atropine treatment, theta was no longer elicited by sensory stimulation and the cell's rhythmic firing was abolished.

Table 7

Pre-post atropine mean, grand mean firing rate and percent reduction of seven neurons during type 1 theta behaviors

Pre	Post	% Reduction
29.13 (7.32)*	27.77 (9.03)	5
41.40 (13.48)	18.17 (7.76)	56
45.53 (8.22)	25.15 (6.91)	45
65.00 (24.32)	44.87 (15.50)	31
74.90 (12.78)	53.40 (21.33)	29
118.90 (29.23)	95.77 (41.47)	19
156.00 (46.33)	137.87 (44.13)	12
75.84	57.87	

* Standard deviation enclosed in brackets

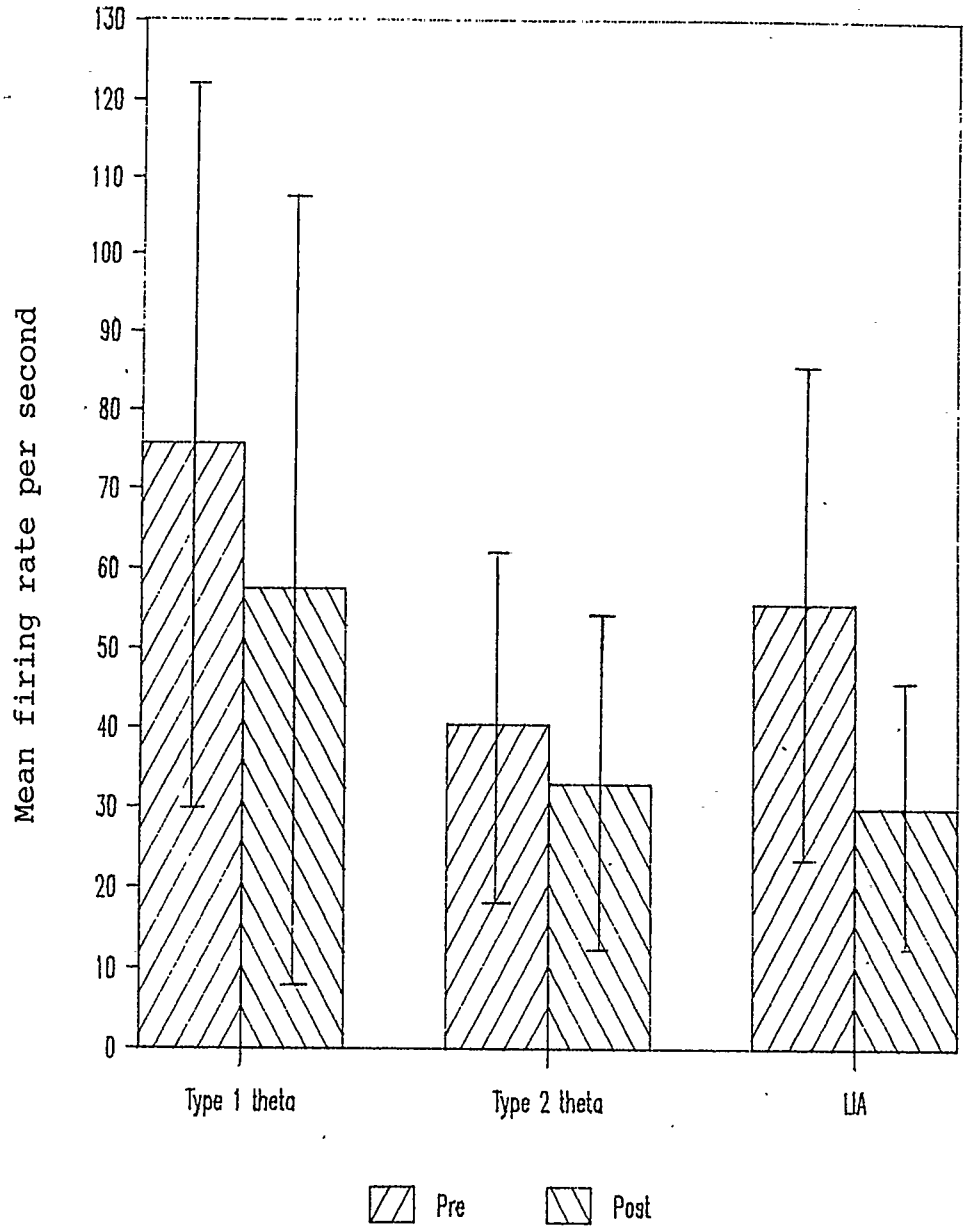


Figure 3. Average firing rate with standard deviation of seven theta cells in three behavioral categories pre and post-atropine.

In the LIA category, the EEG appeared least affected by atropine but the cell's firing rate decreased significantly.

The cell for which atropine treatment did not abolish accompanying type 2 theta was analyzed singly for any difference in the discharge rate pre-post in three behavioral categories and any difference in theta frequency pre-post in two behavioral categories. Each independent t-test was calculated on samples of 30 seconds. A two-tail t-test on type 1 theta frequency showed no significant difference between pre and post means (p = .6786) and neither did the same test applied to type 2 mean theta frequency (p = .5749). Mean discharge rate for three behavioral categories was tested for any difference between pre and post states and the only significant result to emerge was for the immobility and LIA category where the discharge rate was greater post-atropine (p = .0171). In summary, analysis of this neuron yielded no significant difference of theta frequency and cell firing rate in two theta categories between baseline and post states. Only LIA mean firing rate was significantly different after atropine treatment.

DISCUSSION

The present research supports the hypothesis of a cholinoceptive sensory input contributing to the generation of type 2 hippocampal theta rhythm. The administration of atropine sulfate to freely moving rabbits had two expected effects. It blocked the slow wave theta rhythm and accompanying cellular rhythmicity during type 2 sensory related behaviors and reduced the firing rate of theta cells during type 1 theta behaviors, a condition during which both sensory and movement systems are normally coactive. Atropine also reduced theta frequency during type 1 behaviors, a result which could have been predicted by the sensorimotor integration functional model. In addition, the study of theta cells has confirmed previous findings and extended our knowledge of their properties. This class of neurons is related to the theta rhythm in such a way that a change in theta frequency is concomitantly manifest in their firing rate. Both cellular and field activities differ in the three behavioral categories considered. During movement, the cells' firing rate and the frequency of theta waves are at their highest; during sensory processing without movement, theta frequency is lowest and cellular

discharges are as numerous as during immobility without sensory stimulation; this third category is correlated with irregular waveforms in the hippocampus which are termed LIA.

Cholinergic projections to the hippocampal formation have been shown to originate in the medial septal and diagonal band nuclei of the septum (Lewis & Shute, 1967; Nyakas et al., 1987). Septo-hippocampal connections have been associated with the characteristic hippocampal EEG pattern called theta rhythm. The septal involvement in hippocampal theta is crucial because ablation of the medial septum completely disrupts theta (Green & Arduini, 1954). However, the mechanism by which the septal input results in the rhythm is unknown. The cholinergic sensitivity of theta was demonstrated in the awake animal. In the immobile rat and rabbit, systemic injection of eserine produces long trains of theta and the rabbit can spontaneously produce theta during immobility. In both instances, theta is abolished by the administration of atropine sulfate (Vanderwolf et al., 1975). Extracellular recordings in the septum and the hippocampal formation have revealed a class of neurons which fire rhythmically, phase-locked to theta waves and increasing their number of discharges

simultaneously with increasing theta frequency. These neurons have been called theta cells (e.g. Petsche et al., 1962; Ranck, 1973; Rose, Diamond & Lynch, 1983). In the awake rabbit, hippocampal theta is present with movement (type 1 theta behaviors) and sensory processing during immobility (type 2 theta behaviors) (Kramis et al., 1975). Systemic injection of atropine sulfate abolishes slow wave theta only during type 2 theta behaviors (Kramis et al., 1975; Vanderwolf, 1975). Several findings suggest that both types of theta are coactive during movement. A sensorimotor integration model of the hippocampus has been proposed by Bland (1985; 1986) which stipulates that the atropine-sensitive component of the theta rhythm, when removed by pharmacological means, is associated with a reduction of synaptic depolarization of theta cells and hence a reduction in their discharge rates.

During spontaneous behavior within an observation box, the rabbit hippocampal EEG consists characteristically of theta waves of 4-9 cycles/second. If the animal moves, the mean theta range is 7-8 Hz and if it remains immobile while being subjected to auditory or tactile stimulation, the mean theta range is 6-7 Hz. The data show that theta frequency, on average, is significantly higher during movement than

during immobility with sensory stimulation. This statement confirms previous observations (e.g. Kramis et al., 1975).

With extracellular recording of hippocampal cells, one can find cells that rhythmically fire in phase with theta waves. Fourteen theta cells were isolated throughout the CA1 and dentate regions of both hippocampi. Computer analysis of the relation between discharges and the phase of the normalized theta wave produced correlation coefficients between .7 to .9 for a given portion of the theta cycle. In this experiment, only cells from the dentate area preferably discharged on the negative phase of local theta waves. Cells recorded from the CA1 region fired on the positive phase of the local theta. In a recent literature review, Bland (1986) observed that phase-locking reports were quite variable among species and drugs used. Other studies on freely moving rabbits showed that discharges occured on the negative phase of the local theta (Bland et al., 1983; 1984; Sinclair et al., 1982). The phase-locking preference of cells from CA1 in the present study conformed to the findings of Buzsaki et al. (1983) in freely moving rats and of Colom and Bland (1987) for both recording areas in urethane anesthetized rats. Selection bias in this investigation of theta cells may account for the obtained

results.

Another characteristic of theta cells which has been observed by many authors is that their discharge pattern is closely related to the frequency of accompanying theta waves (e.g. Ranck, 1973). Even though individual cells exhibit a particular firing range, almost all cells recorded from showed a positive relationship to theta frequency during type 1 and type 2 behaviors as well as during type 1 behaviors post-atropine. This finding is in agreement with the linear regression analysis performed by Bland et al. (1983). The relationship suggests that theta cells code the level of activity, in terms of frequency, of the theta rhythm.

Like theta frequency, theta cells' firing frequency varies according to the animal's behavior. The firing rate has often been said to be highest with the occurrence of hippocampal theta and lowest during LIA (Feder & Ranck, 1973; Ranck, 1973; Bland et al., 1980). Further, Bland et al. (1983) reported that there were more firings during type 1 theta than during type 2 theta. Recordings taken in this experiment indicated that the mean firing rate of cells was higher during type 1 behaviors than during type 2 and LIA behaviors. If the firing rate was positively related to

theta frequency and theta frequency was higher during type 1 versus type 2 behaviors, it logically follows that the firing rate would be highest during type 1 versus type 2 behaviors. However, the data analysis showed that for selected equivalent theta frequencies cells discharge on average more often during type 1 behaviors than during type 2 behaviors, in agreement with Sinclair et al. (1982) and Bland et al. (1983). The difference in firing rate is evidence in support of the differentiation between the two theta behavior categories. No statistically significant difference exists between the mean firing rate during type 2 theta and LIA behaviors. This suggests that theta cells remain active during LIA.

The aim of the anticholinergic manipulation was to abolish type 2 theta and quantify the effect it had on the firing rate of theta cells during the three behavioral categories. The cholinergic blockade of slow wave theta, as previously reported by Vanderwolf (1975), Kramis et al. (1975) and Bland et al. (1984), was successful during recordings of seven out of eight neurons. The exceptional case of one neuron is difficult to interpret. Although the usual dose of atropine (50 mg/kg, ip) was administered, slow wave theta was not abolished during sensory related

behaviors. Further, the cell's discharges became positively correlated to theta frequency during both type 1 and type 2 theta behaviors whereas the relationship was not significant for baseline data. The mean firing rate significantly increased after the drug treatment during LIA behaviors only. No other changes were noted. The most obvious explanation may reside in the data recording itself. Indeed, close examination of the tape revealed that extracellular spikes lost amplitude in the post-atropine testing condition and there seemed to be less filling in by other cells which would result in clearer rhythmicity and better correlation with theta waves. Other possible causes for the unsuccessful atropine treatment may have a metabolic origin. For example, the erroneous injection of atropine in the digestive organs rather than in the peritoneal cavity would cause the drug to be metabolized before it could reach the brain via the blood stream. The presence of degradative enzymes specific to atropine in this species would have a similar effect. Still, it would not be expected that the cell's firing pattern would change following an unsuccessful drug manipulation. It remains a possibility that this neuron was different from the other seven theta cells.

Thus post-atropine data was analyzed for seven theta cell recordings. Atropine decreased the mean theta frequency during type 1 theta behaviors. In view of the cholinergic sensitivity of the theta rhythm and theta cells firing rate, (and the positive relationship between the two), it could be argued that the blockade of the sensory input would reduce theta frequency as well as discharge frequency. The latter was reported by Bland et al. (1983) whereas the former might have gone undetected because no quantitative analysis was carried out.

The cholinergic blockade is reflected in the mean firing rate of theta cells which was significantly reduced during movement-related theta but unchanged during sensory-related behaviors, except for the loss of rhythmicity in the firing pattern, in agreement with Bland et al. (1984). Reduction of the firing frequency ranged from 5 to 56 percent for the seven neurons. Similarly, Bland and Bland (1986) have shown that lesion of the medial septum reduced cellular discharges during type 1 behaviors but not during type 2 behaviors of freely behaving rabbits. Theta cells also decreased their firing rate during LIA behaviors following atropine injection, an unreported effect. It has become evident from the literature that the hippocampal EEG

contains other important activity patterns which differ from the theta rhythm. Behavioral correlates of these other field potentials are poorly understood but there still is an interest in their origin and meaning. The effect of atropine on the cellular discharges during LIA behaviors can only suggest that irregular activity may have a cholinergic component originating in the only known source of ACh to the hippocampus, the septum. Spectral analysis of the immobile rat EEG following the administration of atropine shows that there is an increase of irregular slow activity and a decrease of fast activity (Leung, 1985). How the drug may affect these two wave patterns and decrease the number of theta cell discharges is unknown.

The morphological identification of theta cells could not be attempted in this study. Many authors have speculated on their own observations, basing their conclusion mainly on cell dispersion and small number throughout the hippocampal formation, and suggested that theta cells are interneurons (Feder & Ranck, 1973; Buzsaki et al., 1983 and others). There does not seem to be agreement on the physiological criteria for the identification of hippocampal cells. The best way appears to be the use of intracellular dye techniques.

This research was designed to ascertain some assumptions and observations that have been integrated into a sensorimotor functional model of the hippocampus by Bland (1986). The principal findings that have led to this conceptualization are as follows. Hippocampal theta is correlated to two behavioral categories: movement with continuous sensory processing and immobility with sensory processing. Slow wave theta recorded during movement is typically of higher frequency than theta recorded during immobility and sensory processing and only sensory related theta is sensitive to cholinergic manipulations. Similarly, theta cells fire at a higher rate during movement than during immobility with sensory processing and their rhythmicity is disrupted by anticholinergic drugs during sensory processing only. Further, both sensory and motor activities are supposed to be coactive and their coactivation is reflected in the higher theta frequency and cell firing frequency during spontaneous movement as well as in the reduced firing rate post-atropine during movement. The coexistence of two distinct types of theta and theta cell responses to cholinergic manipulations are the bases for the assumption of at least two separate, motor and sensory, inputs which are integrated in the hippocampus.

The functional link between type 1 and type 2 theta, as formulated by Bland, may be that the sensory processing system provides a priming input to the hippocampal motor system. From the facts listed above it follows that type 2 theta is a neuronal response to sensory stimulation and that the firing rate of theta cells in this category may reflect the intensity of the sensory input. This in turn may provide a priming stimulus for the preparation of movement or change of ongoing movement. If it could be shown that the theta cells' firing during sensory processing preceeding movement was directly related to a following movement, the model would prove to be quite realistic.

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