FIRING OF NEURON PAIRS IN THE HIPPOCAMPO-SEPTAL AXIS OF THE RAT: CELL TYPES AND THEIR INTERACTIONS DURING THETA AND NON-THETA STATES

By

JOSEF ZAIDE, M.A.

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AUTHOR: Josef Zaide, B.A. Concordia University

M.A. McGill University

SUPERVISOR: Dr. R.J. Racine

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ABSTRACT

The behavioral and neuronal functions of the hippocampus, a major component of the brain's limbic cortex, are not well understood. One major clue to its role in behavior is that its gross electrical activity (EEG) shows striking correlations with behavior. Special significance has been attached to the hippocampal theta rhythm, but, despite extensive research concerning the precise dimensions of behavior correlated with theta, it is still not clear if the presence of theta indicates that the hippocampus is actively involved in the control of behavior. In an attempt to describe some of the neuronal interactions that occur during theta, the activity of pairs of neurons in the hippocampus and septum was recorded in rats paralyzed with succinylcholine chloride.

In Section One it was seen that hippocampal EEG in paralyzed rats was not substantially different from the normal. Long trains of clear theta as well as irregular EEG were seen in the immobilized rat. In Section Two cells in hippocampo-septal sites were categorized. One of the neurons of the pair was usually in dorsal CA1; the other was located in the lateral septum (LSN) in a region shown to receive the terminals of the hippocampal projection cell (P cell). Typical cell types were seen in CA1: rhythmically bursting "theta" cells (B cells) and complex-spike cells. The A cell was probably the CA1 projection cell (pyramidal cell). Cells in the LSN (C cells) were characterized by a lack of bursting. The majority were slowly firing cells. In

Section Three the activity of different cell types during different EEG rhythms was described. Firing patterns of A and B cells were similar to those described in other work: A cells tended to decline in rate during theta, while B cells increased in rate providing an initial indication that during theta hippocampal transmission to target zones is attenuated. The C cells did not generally show substantial changes in activity during EEG changes, although in some C cells a weak theta rhythm was detected.

In the last section (Section Four) an attempt was made to detect correlations between hippocampal and septal spike trains which could be indicative of a transsynaptic influence of P cells and to determine if such correlations changed as a function of EEG state. The cross-correlation of "naturally" occurring firing is not a standard tool in the analysis of complex mammalian forebrain circuits and so, this study was also an exploration of whether such an approach was practical in the analysis of complex circuits.

In the cross-correlograms of an appreciable number of cell pairs it was possible to detect changes in target cell firing which appeared to be due to a direct influence of P cell populations. Most of these changes consisted of increases in rates of septal neurons; for a smaller number of pairs, the occurrence of a hippocampal event was associated with a decrease in target cell firing. Most changes in septal cell firing occurred during irregular EEG patterns. During theta, the firing of P cells was relatively ineffective in biasing the state of the septal cell. These results were interpreted in terms of a possible tendency for a greater number of P cells to fire more synchronously during irregular EEG. It was also found, in agreement

with other studies, that the activity of P cells was relatively suppressed during theta, again suggesting that theta represents a state of attenuated output.

On the basis of the present findings, it can be tentatively conclude that, during theta, it is unlikely that the hippocampus is actively involved in behavioral expression; it is more likely that hippocampal output function is exerted during irregular EEG and associated behaviors, during which time it acts partially to excite septal cells.

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ABBREVIATIONS

AR	Autorhythmicity
AUTO	Autocorrelogram
CC	Cross-correlogram
CV	Coefficient of variation
DBB	Diagonal band of Broca, vertical limb .
EEG	Electroencephalogram
E-I	Excitation-inhibition
HIA	Anterior continuation of the hippocampus
g. cell	Granule cell
ISI	Inter-spike interval
LIA	Large-amplitude irregular activity
LSN	Lateral septal nucleus
MSN	Medial septal nucleus
OM	Operating mode
p. cell	Pyramidal cell
PDS	Paradoxical sleep
POF	Post-commissural fornix
PRF	Pre-commissural fornix
PST	Post-stimulus time histogram
s.	Stratum (as in s. moleculare)
SIA	Small-amplitude irregular activity
SWS	Slow-wave sleep
TIH	Time-interval histogram

GENERAL INTRODUCTION

> Physiological psychology is an act of faith; it is the illogical assertion that to proceed with faulty assumptions is better than to do nothing at all and that, as knowledge is acquired, the imponderable problems will disappear. (Goddard, 1980)

I. Background and rationale.

For many people, the most intriguing and easily identified component of the human brain is its highly convoluted outer layer, the neocortex. Beneath the neocortex, encircling the upper knob of the brain stem, lies another, older type of cortex which, although less known, is equally fascinating. Like neocortex it is layered, but its layering scheme is simpler; while six or seven layers are recognized in new cortex, the older cortex consists of three basic layers. The older cortex has been given a variety of names such as allocortex or paleocortex. Because paleocortical tissue forms a border or limbus about the brain stem, it has been called the limbic lobe. To the first anatomists one portion of the limbic lobe, in certain views, looked like a sea horse. The Latin name hippocampus was assigned to this structure, which, in later work, was identified as the oldest of all cortical tissue, archicortex. It is with the functions of the hippocampus that this thesis is concerned.

Because in human and other primate brains there has been such a disproportionately large expansion of neocortical tissue, the hippocampus has been pushed towards the bottom of the brain where it lies

alongside temporal neocortex (near the ear). In the rat, the experimental subject of this study, a relatively small portion of the hippocampus is found at the bottom of the brain. The rat hippocampus continues to sweep upwards, curving like a slender banana, towards the upper, midline regions of the brain. A view of the rat brain with neocortical tissue peeled away so as to expose the hippocampus can be seen in Figure 1A. As indicated in the figure, the rat hippocampus occupies a substantial volume of the brain; its relative size is greater than that of man.

The rudimentary internal structure of the hippocampus has attracted a great many researchers interested in understanding basic properties of neural tissue or in constructing models which could account for complex cortical phenomena. While these approaches have been partially successful, they have not contributed substantially to an understanding of overall hippocampal function in behavior. As Purpura (1959) has pointed out, the simplicity of hippocampal structure is deceptive:

> We are concerned about how easily one can be fooled about "simplicity of structure". Certainly structural homogeneity means very little in terms of diversification of intrinsic synaptic organization. (p. 185)

After about eighty years of intensive effort (measured from the work of Ramón y Cajal at the turn of the century), there is no consensus concerning hippocampal function either at a physiological or behavioral level. The presence of a number of comprehensive reviews makes a detailed review of the literature superfluous here (see CIBA Foundation Symposium 58, 1978; Isaacson & Pribram, 1975; O'Keefe &

Nadel, 1978). Briefly, the literature of the past two decades has implicated the hippocampus in animals in such processes as arousal, motivation, attention, habituation, memory, learning, voluntary behavior, response inhibition, response to non-reward, spatial orientation, and so on. In man there have been two dominant views, one in which the hippocampus is perceived as part of a system responsible for emotional experience and behavior (MacLean, 1949; Papez, 1937) and one in which the hippocampus is implicated in the formation of memory (Penfield & Milner, 1958; Scoville & Milner, 1957).

A discussion of the reasons behind the divergence of viewpoints would constitute a sizable treatise on conceptual, methodological, and technical problems of physiological psychology. Some of these issues, as they relate to hippocampal function, have been considered by Nadel and O'Keefe (1974) and O'Keefe and Nadel (1978). A major source of the difficulty may be related to the isolation of the hippocampus from primary sensory-motor areas. Anatomically, there are no direct outputs to motor areas nor do primary sensory systems find their way directly into the hippocampus. The information relayed to the hippocampus is of a complex, multi-modal nature. Electrical stimulation of the hippocampus does not elicit clear cut motor responses and it is difficult to decide if those effects that are elicited are due to functions controlled by hippocampus or to the propagation of abnormal seizure activity to distal sites which are involved in the behavior. Destruction of hippocampal tissue produces a myriad of effects; there is little agreement concerning the nature of the behavioral deficit following a hippocampal lesion. Finally, the behavioral functions of

the major input and output areas of the hippocampus are also complex and largely unknown. The hippocampus, then, has not typically been related in any simple way to sensory or motor processes. Instead, it is seen as a complex integrator of pre-processed information or as a modulator of processes controlled at other levels of the nervous system. The only view which had linked hippocampal function to first-order sensory-motor processes was that of Ferrier (1876; discussed in Brodal, 1947) which regarded the hippocampus as the olfactory cortical receptive site, a view which became untenable after the publication of Brodal's (1947) work.

O'Keefe and Nadel (1978), in the first book-length attempt to integrate data from a variety of approaches into a theory of hippocampal function, have proposed, mainly on the basis of observations of single-cell activity, that the hippocampus is a necessary component of neural circuitry responsible for a translation of the environment into a spatial or cognitive map. It is still a speculative view, not sufficiently substantiated to receive wide support. Vinogradova (1978) has criticized it on several grounds, her most pointed objection being that experimental findings are directly related to the experimenter's expectations:

> These are very interesting data, but it is curious how we find in the brain what we are looking for! For example, in my earlier experiments I was specifically interested in <u>time</u> perception in the hippocampus and I have beautiful extrapolation in hippocampal units. . . . So I suspect that space and time and in fact <u>all</u> dimensions of the world are going through the hippocampus! (p.197)

Present perspective. The present study takes as its starting point what is probably the most generally agreed-upon phenomenon in the literature concerned with hippocampal behavioral functions: in the rat a specific class of behaviors are invariably accompanied by a readily discriminated pattern of hippocampal electrical activity termed theta rhythm. Other brain areas also give rise to electrical patterns which vary as a function of the behavioral state, but none has shown the striking patterns which are characteristic of hippocampal EEG during waking behaviors.

Despite the ease with which correlations between hippocampal EEG and behavior are detected, they have not led to an unequivocal inference about hippocampal function in behavior. Two problems have contributed to the difficulty. First, during behavior a large number of processes, some observable, others not, co-vary; it has been difficult to establish which specific dimension of behavior relates to the theta rhythm. Second, it is uncertain if theta represents an active hippocampal state.

It is with the latter problem that this study is concerned and a number of interrelated questions are pursued here: Does the occurrence of theta during certain behaviors reflect an active involvement of the hippocampus in the control of those behaviors or do other EEG patterns perhaps indicate a greater degree of hippocampal involvement? What is the nature of hippocampal function during theta and non-theta states?

For many years questions such as these have been posed by many researchers, particularly during conference discussion sessions (see

CIBA Symposium, 1978, pp. 321-324 or Purpura, 1959, p. 177). Their significance to an understanding of hippocampal function in behavior might best be discerned against a brief historical backdrop.

In one of the first observations of theta, Green and Arduini (1954) noted that in the excited, mobile rabbit or after presentation of various sensory stimuli to the paralysed rabbit, theta rhythm, a slowly oscillating, nearly sinusoidal waveform, dominated the hippocampal EEG, while a small amplitude, high frequency "desynchronized" pattern appeared in the neocortical EEG. That a specific, easily identifiable component of hippocampal EEG was correlated with a relatively circumscribed behavioral state (arousal) motivated a large number of similarly oriented studies in a variety of species. Unfortunately, these studies did not result in a unified view of EEG-behavior relationships (see Black, 1975). Vanderwolf's (1969, 1971) descriptions of hippocampal theta and its behavioral correlates in the rat have gained the widest support. Two other major classes of hippocampal EEG, each with its own set of behavioral correlates, were also described by Vanderwolf (1971). These patterns are discussed in more detail in Section One.

The essential point here, and it is a point that cuts across species differences in EEG-behavior correlates (see Winson, 1972), is this: regardless of the nature of the EEG-behavior correlation observed, it was assumed by many workers, without direct evidence, that one or another of the principal types of hippocampal EEG patterns represented its functional state. In many cases no attempt was made to specify how activity in the hippocampus during some EEG pattern allowed

the hippocampus to participate in the control of the behavior or process in question. Arguments were invoked to support one or another view, but most were without empirical foundation.

In the earliest views (Green & Arduini, 1954) it was proposed that because theta waves were correlated with behavioral arousal (and cortical desynchronization), and since theta could be induced by activation of the brainstem reticular core, it represented an active state of the hippocampus. Behavioral arousal, however, does not necessarily require active participation of all neural areas, nor does it specify in itself which neural areas should be active.

For Grastyán (1959) the presence of theta in the cat indicated that the hippocampus was in an "inhibited functional state". Grastyán (1959, p.120) noted that electrical stimulation of the hippocampus inhibited various types of movements in animals such as orienting movements or conditioned responses, suggesting that the hippocampus acted to inhibit movements. He also observed that during desynchronization of the hippocampal EEG the cat tended to be in a state of aroused immobility while during theta it performed orienting movements. These and other incidental observations indicated to Grastyán that the hippocampus was functional during its desynchronized state, at which time it acted to suppress a neural system involved in production of orienting movement. During theta, the hippocampus was inactive or suppressed, allowing for the orienting system to be released from hippocampal inhibition and orienting movements to appear.

The results of other stimulation studies have not been consistent. Also, the behavioral effects, in many cases, may have been due

to spread of afterdischarge. Furthermore, the parallels between electrically-induced behaviors and natural behavior occurring during a specific EEG pattern are questionable. For these, and other, reasons it is difficult to establish on the basis of stimulation evidence whether the correlation between hippocampal EEG desynchrony and "immobility" implies that the hippocampus is active during the immobility.

If it were possible to reliably elicit an unambiguous behavioral pattern with non-seizure producing hippocampal stimulation, our understanding of hippocampal function might be appreciably advanced. It might also provide evidence that whatever EEG pattern appeared during that behavior pattern was indicative of an active hippocampus. Nevertheless, the evidence would be indirect; it would still be necessary to show that the hippocampus was more active during this pattern than other EEG patterns. As Grastyan (1959, p. 143) observed, "the final solution of this problem can be obtained only from experiments with microelectrodes".

The traditional belief that regularly oscillating slow waves indicated an inactive state was also influential in forming the view that during theta the hippocampus was inactive. Douglas (1967), in a major review article, expressed this bias:

> It challenges belief that a brain structure could generate very high-amplitude sine-like waves and yet be functioning in any discrete fashion. . . .(p. 420)

For Adey (1966), however, theta in cats was a definite sign of information processing in the hippocampus. In humans, Penfield and Milner (1958) had written about the reliance of memory formation on intact hippocampi. Adey's observations of EEG during learning proce-

dures in cats suggested that theta was essential to the formation and recall of memory traces. These results and conclusions were directly opposed to those of Grastyan and an attempt at reconciling the two positions was made by Bennett (see Bennett, 1975). Despite Bennett's original leanings towards Adey's views, his findings in the cat supported those of Grastyan. That is, as learning progressed, theta did not persist, as claimed by Adey, but was replaced by irregular EEG activity. Bennett, also tried to determine whether theta was necessary for those behaviors it accompanied by administering a theta-blocking drug during a learning task. He found that the learning and performance of acquired movements that were normally accompanied by theta were not disrupted, suggesting that hippocampal events giving rise to theta were not essential to the task.

The opinion that theta rhythm signalled a non-functional state of the hippocampus became more widespread as the large volume of data generated by the animal lesion research began to be integrated. The basic finding was that animals without a hippocampus were not impaired in tasks which in intact animals were accompanied by theta rhythm. Douglas (1967) put it this way:

> It is of some interest that both (Grastyan et al, 1959; Adey et al, 1960) found prominent theta rhythms during the learning or performance of tasks which the lesion literature indicates do not require hippocampal functioning: . . . If one assumes that theta indicates that the hippocampus is functioning. . . . then one would be forced to predict that removal of the hippocampus should result in a deficit on these problems. (p. 421-422)

Vanderwolf (1969), and others, found that theta in the rat accompanied "voluntary" movements (broad-ranging exploratory movements) and studies of Black (see Black, 1975) demonstrated that it was probab-

ly with movement itself and not some other process that occurred concurrently with movement that theta was correlated. The lesion research made it clear, however, that voluntary movements were not disrupted by hippocampal damage; if anything, a large segment of the literature suggested that the animal had difficulties in suppressing such movements. Many researchers adhered to the hypothesis that the hippocampus in rats was involved in some sort of response inhibition (Kimble, 1968; McLeary, 1966). The most parsimonious integration of the data suggested that during theta and voluntary movement the hippocampus was inactive. Vanderwolf (1971) opposed both conclusions. With respect to the former he suggested that the disinhibition of behavior could be due to infringement of the damage on the dentate region since he had noted that stimulation of the dentate area suppressed voluntary movement, while hippocampal stimulation had no effect. Others, however, have found that mild hippocampal stimulation does produce suppression (Grastyán, 1959, e.g.) and the findings of suppression with dentate stimulation have not been replicable (Kramis, 1972, cited by Bennett, 1975 and Vanderwolf, 1976 personal communication). Vanderwolf also felt that it was unlikely that the hippocampus was inactive during theta because of observations that hippocampal units fired in phase with theta. But, as will be noted later, inferences about the state of "activity " of a neural zone based on single cell firing, are highly dependent on the type of cell which is active.

A unique attempt to reconcile the lesion studies with the EEG data is that of Altman et al (1973). These workers have concluded from the lesion literature that hippocampal tissue does in fact participate

in a response "braking" function. Unlike other workers, they did not go on to make the inference that the hippocampus was suppressed during movement (and theta). Instead, they proposed that, during movement initiation and maintenance, there was a concurrent activation of a septo-hippocampal braking system which was necessary to ensure that "motor action can be delicately controlled". When the aroused animal was ready to engage in voluntary motor activity the septo-hippocampal system was triggered into action and hippocampal theta was seen. Theta represented "the alerting of the braking system without engagement" and persisted throughout movement because, in their view, movement involved repeated preparations to stop.

In Altman et al's view, theta is apparently an intermediate level of hippocampal function. If the animal must stop its voluntary movement, the hippocampus becomes maximally functional and generates its most activated pattern, small amplitude irregular activity. On the other hand, if the animal is quiescent; if it is not prepared to make a voluntary act and is not in the process of moving, the braking system is not required and large amplitude activity appears in the hippocampus as it achieves its least functional state.

The foregoing analysis is by no means exhaustive or representative of positions taken with respect to the role of theta. The examples provided do serve to illustrate the major symptoms of the problem that this thesis is concerned with: on the basis of the correlation of hippocampal EEG and behavior any of a number of conclusions concerning

the behavioral role of the hippocampus are possible. While findings of lesion, stimulation, and other more analytic EEG studies (see Black, 1975) place constraints on the type of theory derived, the evidence they furnish, as noted above, remains indirect.

The present study, by recording the activity of single cells in the hippocampo-septal circuit, attempts to specify some of the neuronal interactions that form the substrate of hippocampal action during theta and non-theta states. Such descriptions potentially could clarify the nature of hippocampal involvement in those behaviors with which its EEG patterns have been correlated.

Single unit activity in the hippocampus has been studied by a large number of research groups, but their observations do not readily apply to the questions asked here. It is critical, in an analysis of the relative state of activity in the hippocampus (or any neural zone) during a particular brain state, to identify the type of neuron giving rise to the unit record; many studies have not done so. It is well known that most neural regions contain interneurons (local circuit, short-axon elements) which serve to modify the activity of the long-axon projection neurons in the region. Some interneurons will act to suppress activity of the projection cells. In the ventral horn of the spinal cord, for example, the local circuit Renshaw cell exerts inhibition on the motoneuron which projects to the muscle fibre. In the hippocampus there is also a class of interneurons (the basket cells, probably) which inhibit the activity of the hippocampal projection cell. When recording single unit activity, mistaking one type of element for the other could lead to opposing views of the function of the area in question.

Implicit in this approach is the assumption that it is the activity of projection cells that forms the measure critical to interpreting whether an area is actively involved in, or "functional" during, behavior. This assumption is held by many researchers; as will be seen, those workers that do attempt to identify elements recorded in the hippocampus, equate hippocampal function with the behavioral correlates of the activity of what they believe to be the projection cell. This assumption is also implicit in the present research. In some views function is described in terms of the transformation performed by the network, given certain inputs, by virtue of its internal circuitry. In any case, it appears logical to assume that execution of function can be measured most conveniently in terms of activity of projection elements, since part of the circuitry constituting the system is composed of elements that extend to other systems.

It is also assumed here that an important factor in defining the functional state of a neural zone is whether activity in projection elements is capable of affecting the target zone or of being decoded by systems innervated by the output units. Thus, in this study, activity of output elements of the hippocampus was monitored simultaneously with the activity of septal target cells. This activity was subjected to a cross-correlational analysis which provided a measure of hippocampal influence on septal cells under different EEG conditions.

The recording of pairs of units would have provided maximal information in behaving rats. Pilot studies, using chronic microelectrode recording techniques, indicated, however, that it would be difficult to isolate a reasonable number of cell pairs with usable signal-

to-noise ratios in the freely moving rat. For this reason microelectrode probes were done in rats immobilized with succinylcholine chloride, a neuromuscular blocking agent.

The approach used here was not intended to provide a specific test of any one hypothesis of hippocampal functioning in behavior. It was partially an attempt to apply a methodology not often used in the analysis of mammalian nervous system, the cross-correlation of pairs of units, in order to evaluate some assumptions about the information-processing state of the hippocampus during certain broad classes of behavior (or EEG pattern).

In the remaining portions of the Introduction some fundamental anatomical and electrophysiological characteristics of the hippocampo-septal axis are described and further elaboration of the scope and rationale of this study are provided.

II. Terminology and description of areas.

HIPPOCAMPUS

The term hippocampus has been used in a number of ways. Most often it includes three areas: the subiculum proper, Ammon's horn (cornu Ammonis), and the dentate region (fascia dentata in rodent or dentate gyrus in primate). Occasionally, hippocampus has been used to refer to Ammon's horn alone. The research here is concerned primarily with Ammon's horn (which will be called horn); the term hippocampus will be used to refer to a combination of all three areas.

Internally the hippocampus is organized along both functional and structural lines. Functionally, the electrophysiological evidence

indicates that the transsynaptic relay of information between hippocampal divisions is confined to a lamella, a thin section or slice of hippocampus running transversely to the longitudinal axis (Andersen et al, 1971; Rawlins & Green, 1977). The approximate orientation of such a slice is seen in Figure 1A in anterior and posterior hippocampus. The lamellar principle of information flow is analogous to columnar organization in neocortex; it hypothetically allows for adjacent segments of hippocampus to carry out different functions with contrast between those functions enhanced by a possible lateral inhibition.

If a slice of hippocampus is removed and magnified, its structural divisions can be discerned. Such a slice, depicted schematically in Figure 2A, reveals two types of structural organization: one consists of the major subdivisions and fields of the hippocampus; a second is the layering schema. The most obvious division is that between fascia dentata and the horn.

Hippocampal subdivisions.

Fascia dentata. The dentate area, demarcated from the horn by the hippocampal fissure, is characterized by the alignment of granule cells (g. cells) in a neat V-formation. The g. cell, the dominant cell type, has only an apical dendritic complex arising from the cell body; it possesses at least two axonal branches, one of which is conveyed in the mossy fibre system to adjacent horn and a second which innervates local circuit interneurons. The portion of the dentate closest to the fissure has been termed the buried blade; that portion adjacent to thalamus, the exposed blade (Andersen, cited by O'Keefe & Nadel, 1978).

The peripheral dendrites of the g. cell are found in the outer rim of the dentate, near the fissure, and make up the stratum moleculare. The tightly packed g. cell bodies and the dense basket cell terminal plexus in which they are enmeshed, form the stratum granulosum. The exiting g. cell axons and a variety of other elements such as the basket cell soma and the pyramidal cells of the horn, comprise the stratum polymorphe.

Ammon's horn. In remaining portions of the hippocampus the pyramidal cell (p. cell) is dominant. Lorente de Nó (1934), noting that in different parts of the hippocampus p. cells were morphologically different and were arranged differently, divided the horn into its principal cell fields, CA1-4. Further subdivisions were made by him, but have not been widely used and are excluded from discussion here.

As indicated in Figure 2A,B, the p. cell bodies of CA1-3, restricted to a single layer, form a semi-circle the lower limb of which falls partially between the blades of the dentate. Pyramidal cells are found in the polymorphe layer of the dentate (hilar region) as well, where they constitute area CA4, but they differ in several respects from those of CA1-3. They are scattered, no longer contained in a thin sheet; morphologically they are modified, and according to Swanson and Cowan (1977), the principal axon does not go out beyond the hippocampal formation.

The smallest, most densely packed p. cells are found in area CA1, clustered in several rows. A number of CA1 p. cell axonal branches were described by Lorente de Nó; in some figures the p. cell axon is seen bifurcating with one branch directed rostrally toward septum, a

second caudally toward subiculum. Recent studies to be discussed shortly have indicated the presence of CA1 terminal material in both septum and subiculum, but it cannot be determined from these studies if one cell projects to both, or if different populations of cells project to different areas. It is also likely that another axon collateral terminates locally in the vicinity of the basket cell.

Areas CA2 and CA3 are characterized by giant pyramidal cells, arranged irregularly in several rows. A single CA3 p. cell has at least two axons: a main, relatively thick axon, destined for the septal area and a collateral axon going to area CA1 in the Schaffer collateral system. Since CA3 projects to other areas within the hippocampal formation (subiculum, entorhinal cortex, contralateral hippocampus, the same as well as different CA3 segments), a single CA3 cell could have as many as six or seven axon branches. Whether a single cell projects to all destinations or different groups project to different areas is unknown. Not all CA3 p. cells have a Schaffer collateral; those nearest CA2 are least likely to project to CA1 and most likely to give rise to a longitudinal association system.

The CA3 and CA1 p. cell are distinguished by a number of morphological features other than size. For example, the apical dendritic shaft of the CA1 p. cell bears a large number of side branches; both soma and the proximal part of the shaft do not have thorns. In CA3 the apical shaft does not give off side branches, but does have thorns and tends to divide into a number of vertical branches.

Blackstad (1956), following Ramón y Cajal, called the horn sector adjacent to subiculum, corresponding to CA1, the upper region or

regio superior; the lower region encompassing CA2, CA3 was called regio inferior. Blackstad could not distinguish CA2 from CA3 on the basis of staining characteristics or interconnections. Whether field CA2 should be considered a separate field is uncertain. Swanson and Cowan (1977) could not make the distinction on the basis of efference. On the other hand, Chronister et al (1974) claimed it was a small, but clearly delimited area. In Lorente de No's study the major distinction between CA2 and CA3 was that the CA2 apical shaft was devoid of thorns.

Subiculum. The term subiculum refers to the area bounded by the CA1 and presubicular border. The boundary between CA1 and subiculum is defined partially by the fact that the Schaffer collaterals come to a halt at the beginning of the subiculum. The subicular p. cells are loosely arranged, deviating from the stratification in CA1.

Hippocampal layering.

Although the horn, like the dentate area, consists of three basic strata, it has been apportioned into seven different layers, primarily on the basis of the structural characteristics of the p. cell (see Fig 2A).

1. The alveus is the first layer encountered in a probe starting at the hippocampal surface. It consists primarily of p. cell axons although incoming axons also run in the alveus. 2. Stratum oriens consists of the basal dendrites of the p. cell. 3. Stratum pyramidale contains the p. cell bodies with the basket cell terminal plexus. 4. The main shaft of the p. cell apical dendrite is found in stratum radiatum. 5. Stratum lacunosum is seen mainly in CA1 since it consists largely of the collaterals of the CA3 p. cell destined for the CA1 cell. 6. The

most superficial layer, stratum moleculare, contains the p. cell apical dendritic plexus. 7. Stratum lucidum is found only in CA3 and consists mainly of g. cell axons travelling to CA3.

The different strata, besides containing different p. cell (or g. cell) components, are also characterized by a number of other neuronal elements such as fibres and terminals associated with extrinsic and intrinsic efferent systems. They also contain different cell types. Lorente de Nó (1934) distinguished between 13 different cell types in Ammon's horn alone on the basis of dendritic (input) and axonal (output) characteristics and went on to note that his descriptions were based upon a preliminary analysis. Three different types of pyramidal cells were observed in the pyramidal layer and two different types of basket cells were found in stratum oriens. Other cell types were distributed in oriens, radiatum, and lacunosum-moleculare. In the hilar region alone Amaral (1978) distinguished between 21 different cell types.

Aside from the p. and g. cells, the most important type of cell is the basket cell, a short-axon internuncial found in s. polymorphe of the dentate, in s. lucidum/moleculare of field CA3, and in s. oriens of CA1. According to one estimate (Andersen et al 1963), in CA1 there is about one internuncial for every 200 to 500 p. cells; probably they are sparse in other areas as well. Nevertheless, these cells are supposed to play an important role in hippocampal physiology because of their ramifying termination on the p. and g. cells.

It appears that the only cell types projecting outside of their respective regions are the granule cell and the pyramidal cell, al-

though a recent report by Chronister, and DeFrance (1979) suggests that cells projecting out of the hippocampus may be found in layers other than the pyramidal layer. That g. and p. cells are dominant numerically and project out of their respective areas justifies the emphasis placed on their analysis; the basket cell, intimately involved in regulating p. and g. cell activity also merits attention. In Lorente de Nó's (1934) view:

> The best and least commital method of describing Ammonshorn is to consider it as composed of a layer of pyramidal cells, which are the origin of the efferent pathways and a series of cells with short axis cylinder, subordinated to the pyramidal cells. (p.122)

It is clear, however, at least according to a reductionist view, that a complete account of hippocampal function must eventually take into consideration the large assortment of other cell types found in the hippocampus.

SEPTUM

As pointed out above, the hippocampus, a part of the limbic lobe, is cortical tissue. The septal area, on the other hand, is a major subcortical component of the limbic system. It is situated just anterior to the hippocampus beneath the corpus callosum, extending anteriorly to frontal cortex. Ventrally, it is bound by the olfactory tubercule and preoptic area; laterally it is limited by the lateral ventricles. Like other subcortical areas the septum is divided into a number of nuclei, distinguished by conventional criteria such as development, cytoarchitecture, connections, chemistry, etc.

Recent work by Swanson (Swanson, 1978; Swanson & Cowan, 1976, 1979) has suggested that rat septum can be divided into four principal areas: lateral, medial, ventral, and posterior. A lateral-medial distinction has long been recognized (see Fox, 1940). The medial septal nucleus (MSN) and the nucleus of the diagonal band (DBB-vertical limb) make up the medial portion. According to Swanson (1978) the distinctions between the MSN and DBB are arbitrary. A mixture of very large and small cells are found in both and connections are similar in both. The term medial septal nucleus will be used to apply to both regions.

In the cat and opposum (Fox, 1940) the MSN consists of a small-celled anterior portion and a large-celled posterior portion. The small-celled portion according to Fox is closely related to the anterior continuation of the hippocampus (HIA), another midline septal nucleus found at the anterior border of the septum and which contains small, pyramidal-type cells. The large-celled posterior portion is allied to the DBB. Figure 2C shows a coronal section through the septum at the level of the HIA.

The lateral septal nucleus (LSN) is the largest of septal nuclei and, in the cat, appears anteriorly adjacent to HIA, just dorsolateral to it, before the MSN has appeared (Fox, 1940). On the basis of cell density and size as well as connections, it has been divided by Swanson into three parts: dorsal, intermediate and ventral. Most of the lateral septum contains a medium sized cell; the dorsal division contains the largest cells, most loosely arranged, while ventral septum is composed of smaller, more tightly packed cells. A section through the LSN is depicted in Figure 2D.

The posterior septal nuclei are two in number: the septofimbrial nucleus, which is a caudal continuation of the LSN (Fox, 1940-cat), and which contains cells larger than LSN cells and more scattered (Swanson, rat). The other is the triangular septal nucleus which contains smaller, more densely packed cells.

The ventral sector of the septum is comprised of the bed nucleus of the stria terminalis and of the anterior commissure and is a heterogenous area.

III. Hippocampal outflow to septum: Anatomical organization.

Attempts to unravel the organization of the hippocampal efferent system have frustrated the most determined of neuroanatomists. Lorente de Nò (1934), somewhat despondently, stated:

> The main efferent path of the Ammon's horn is the fimbria, a bundle so extremely complicated that all my attempts to analyse it completely have failed in the same way as the old authors failed. (p. 143)

Some of the reasons for the difficulties are hinted at in Figure 1A. The hippocampus, a large neuronal mass, curves in several directions as it ascends over the thalamus from its ventro-temporal position to its dorso-septal location; naturally, the disposition of the p. cell fields changes accordingly along with the trajectory of the fibres they give rise to. These fibres, ensheathing the mass of the hippocampus, gather rostrally at the septal bottleneck, converging on and perforating the smaller, narrow septal volume, making a topographic analysis difficult to achieve. Also, in order to reach the septal targets, axons from one portion of the hippocampus must travel across the other hippocampal

zones. Thus, septal sites of terminal degeneration seen after a lesion of a particular hippocampal area will likely consist both of targets of the zone lesioned as well as other zones giving rise to the damaged fibres of passage.

The application of recently developed neuroanatomical techniques (see Lasek, 1975), particularly autoradiography, has greatly reduced the "fibres of passage" problem and has led to several important revisions in conceptions of hippocampal outflow. A discussion of these revisions follows a brief elaboration of the terms applied to the major fibre systems carrying hippocampal output.

Depending on its position in the subiculum or Ammon's horn, the p. cell will use one of three routes to arrive at the rostral pole of the hippocampus - the alveus, fimbria, and dorsal fornix (see Fig. 1). The fimbria is a thick bundle of fibres, running along the lateral edge of the hippocampus, while the dorsal fornix, a thinner bundle, runs along the medial or subicular edge. Alvear fibres, encasing the hippocampus on its deep and superficial surfaces, run between the fimbria and dorsal fornix, many merging with these latter systems in their ascent towards the septal pole. Alvear and fimbral fibres gradually lose their identity as they enter the septum, contributing fibres to the ventral hippocampal commissure and the body of the fornix. The dorsal fornix also gives off fibres to the main fornix body, but, according to several sources, portions of it continue rostrally along a midline trajectory as a discrete fibre bundle (Blackstad, 1956; König & Klippel, 1963; Raisman et al, 1966). There are indications in older studies that the dorsal fornix or portions

thereof may arise in non-hippocampal areas (Blackstad, 1956; Kõnig & Klippel, 1963; Ramón y Cajal, 1955). It is clear, however, from more recent work that a large portion of the dorsal fornix contains pyramidal cell axons (Siegel et al, 1974; Swanson, 1978).

The body of the fornix continues rostrally dividing into two major components: 1. the columns of the fornix which descend as compact bundles just posteriorly to the anterior commissure and are often termed the post-commissural fornix (POF) and 2. the pre-commissural fornix (PRF) which is simply a rostral extension of the fornix body, descending in diffuse fashion rostral to the anterior commissure. The disposition of the POF and PRF is shown schematically in the sagittal section of Figure 1B.

The work of Raisman et al (1966) initiated contemporary research efforts to ascertain precisely which pyramidal cells connected with which target areas via which of the aforementioned fibre systems. Using conventional lesion-degeneration techniques, these workers found that dorsal-anterior CA1 projected primarily to anterior thalamus and mamillary bodies through dorsal fornix and the POF. A slight ipsilateral projection to the midline portion of the medial septal nucleus (as well as triangular and septo-fimbrial nucleus) was also seen. Axons of p. cells in posterior CA1 took a more convenient route through the dorsal fimbria to enter both pre- and post-commissural circuits. The pre-commissural terminations were mainly in ipsilateral septum, localized to an area in medial and lateral septum just lateral to the sparse terminal zone of the anterior CA1 projections. Axons of more posterior CA3, 4 cells were carried in the ventral fimbria and distrib-

uted bilaterally mainly to more lateral portions of the septal nuclei not occupied by CA1 terminals (see Fig. 3A).

While the Raisman et al work was incomplete with respect to outflow from more dorsal CA3, 4 as well as the tempero-ventral hippocampus, the basic view of hippocampal efference to septum proposed by these workers was that CA1 projected unilaterally to more medial septum while CA3, 4 projected bilaterally to more lateral septal areas.

The lesion-degeneration study of Siegel et al (1974) in rat, gerbil, rabbit, and cat was concerned solely with the topographical nature of hippocampal outflow to septum. In general, their findings agreed with those of Raisman et al. As Figure 3B shows, they found that dorsal hippocampus projected to medial septum and ventral hippocampus to lateral septum in all species. However, while Raisman et al's findings indicated a segregation of septal terminals according to the CA field of origin, Siegel et al.'s results suggested that the terminations were independent of CA field.

Recent studies using autoradiographic and horseradish peroxidase tracing techniques have not only refined the older descriptions of the topography of hippocampal impingement on septal neurons, but have also presented a quite different view of the general nature of hippocampal efference, in the rat at least. Whether these findings will hold in other species is an empirical question. Swanson (1978, p.45) has already reported that preliminary findings in monkey indicate differences from the rat.

Probably the most striking observation disclosed by the newer methods (Meibach & Siegel, 1977; Swanson & Cowan, 1975, 1976, 1977; see

also the lesion work of Chronister et al, 1976) was that the primary, if not the sole origin of the POF circuit was in cells of subicular (subiculum, presubiculum, parasubiculum) areas; cells in Ammon's horn ($CA1-^{3}$) projected exclusively through the PRF. These findings constituted the greatest upset to the belief that the origin of the POF was in Ammon's horn proper, particularly in CA1. The reports of Raisman et al (1966), and earlier workers, of POF degeneration after lesions of Ammon's horn cells or fibre systems are easily explained by assuming that all these workers severed fibres passing from the subicular area rostrally to the POF circuit.

A second unexpected finding was that the $CA1-^{3}$ p. cell axons carried in the PRF terminated mainly in the lateral septal nucleus. It could not be determined whether distribution of radioactivity in the septofimbrial and triangular septal nuclei was terminal or fibrous (Swanson & Cowan, 1977). In both Swanson's and Meibach's work the medial septal nucleus was devoid of radioactive labelling, an observation directly opposed to those of Raisman et al and Siegel et al. The most plausible explanation of the discrepancy is again in terms of the disruption of fibres of passage. In this case, however, it is unlikely that the source of the passing fibres was subicular, since amino acid injections in the subicular area, while leading to radioactivity in the lateral septal area, did not produce radioactive labelling in MSN. It is possible that the MSN degeneration produced by the antero-dorsal lesions of Raisman et al and the Siegel group was due to disruption of dorsal fornix fibres originating in extra-hippocampal areas, perhaps the cingulate cortex.

A third major reconceptualization of hippocampal efference, derived principally from Swanson and Cowan's (1977) work, concerns the relative importance of the caudally-directed output of fields CA1-3 to subicular, perirhinal and entorhinal cortices. While the work of Hjorth-Simonsen (1971, 1973) and Andersen et al (1973) has resulted in a growing awareness of the presence of non-fornical, caudally-directed efference from Ammon's horn, Swanson and Cowan's (1977) findings emphasized the importance of this projection. In discussing their results, Swanson and Cowan (1977) pointed out that while approximately 450,000 cells are found in the pyramidal layer of CA1-3 in the rat, only 50,000 to 100,000 fibres enter the PRF. Given that some of the PRF fibres are subicular in origin, and that CA1-3 does not enter the POF, the possibility that axons of horn cells are mainly caudally directed is not an unlikely one.

Details of hippocampal (CA1-3, subiculum) outflow to the lateral septal nucleus, as described by the autoradiographic studies of Meibach and Siegel (1977) and Swanson and Cowan (1977) will now be considered (see Fig. 3C,D). Both groups of workers agree that zones of termination of CA1,2 and 3 in the septum are ordered partially according to the dorsal-ventral (septo-temporal) position of the p. cell. Dorsal p. cells, regardless of their CA field, project to dorsal septum, while cells in temporal hippocampus project to ventral septum. Swanson's work also provides direct evidence of a medial-lateral organization, at least as far as anterior hippocampus is concerned: medial lying CA1 or CA3 cells of dorsal hippocampus will project to more medial septum, while lateral CA1 and CA3 cells will terminate in

more lateral septum (dorsal septum in both cases). On the surface Meibach and Siegel's findings are not in accord with this principle. They showed that both dorsal CA1 and CA3 terminals were confined to dorsomedial septum. Diagrams in Meibach & Siegel indicated, however, that dorsal CA1 and CA3 infusions were restricted to more medial portions.

A medial-lateral organization principle may also apply to dorsal versus ventral hippocampal output. As Fig. 3C indicates, not only do progressively more postero-temporal hippocampal areas project to more ventral septum, but they also tend to terminate more laterally. A septo-temporal and medial-lateral topographic organization of hippocampal outflow to septum is not surprising in view of the fact that the hippocampus, in curving ventrally over the thalamus, is also progressively displaced more laterally.

The two research groups also agreed that: 1. each level of the hippocampus projects to septum through a specific portion of the fimbria-fornix complex. CA1 utilizes both dorsal fornix and fimbria to enter the septum; CA3 is conveyed in fimbria. 2. CA1 projections are strictly ipsilateral, while CA3 output is approximately homotopically bilateral. 3. All parts of the subiculum, besides projecting to the POF, enter the PRF to terminate in the ipsilateral LSN. Dorsal subiculum, like dorsal CA1-3, projects to dorsal septum; ventral subiculum projects to ventral septum.

A major area of disagreement between the two research groups concerned the relative density and extent of subicular, CA1, and CA3 termination. In Meibach & Siegel's view, the dominant source of PRF

termination in the LSN was the subiculum. The ipsilateral termination of CA1 was relatively light, with the bilateral termination of CA3 lighter still. Also, the termination of Ammon's horn in septum was seen to be confined to the posterior half, while subicular terminals were distributed throughout the rostro-caudal extent of the septum.

Swanson & Cowan agreed that subicular termination in septum was extensive; however, their findings showed that CA1 termination was also substantial and extended rostrally to the level of the anterior continuation of the hippocampus. And, while Swanson's findings indicated that CA3 terminals did not extend as far rostrally as those of CA1, the distribution of CA3 in more caudal septum was fairly heavy, covering the entire lateral septal complex. Although Swanson & Cowan suggested that, for a given level of hippocampus, CA1 and CA3 terminals were similarly distributed in septum, their figures indicated that CA3 distribution was more extensive than that of CA1.

In summary, it appears that some aspects of the distribution of hippocampus to septum are dependent on the cell field of origin and not on the particular location of that cell field in space. The greater rostro-caudal extent of CA1 termination as well as the bilateral termination of CA3 compared to CA1's ipsilateral termination are examples. On the other hand, the topographic arrangement of the p. cell terminals within the lateral septum appears to depend on the location of the p. cell within the hippocampus and not on the CA area they fall in. Additionally, Swanson & Cowan found that CA2 projection was similar to that of CA3 while CA4 did not project to areas outside the hippocampus.

The results of lesion studies as well as transport studies, and as will be noted later, of electrophysiological studies do not support Andersen et al's contention that CA1 projects exclusively to subiculum. While the rostral contribution of CA1 may be small relative to its caudal outflow (Swanson, 1978, p.46), the recent tracing studies indicate that it is clearly present. It should be again recalled that the more recent studies have reported data based only on the rat. Andersen's observations were made in rabbit. Species differences are clearly possible.

IV. Hippocampal outflow to septum: Electrophysiological aspects.

Several groups of researchers have recently demonstrated that the principal effect of electrical stimulation of hippocampus on cells in LSN is an early short-latency excitation followed by an inhibition of variable duration (E-I response - DeFrance et al, 1971, 1973a,b,c; Edinger et al, 1973; Gutnick & Feldman, 1977; McLennan & Miller, 1974). The following discussion first considers the excitatory component, then the inhibitory component.

Excitation.

DeFrance et al (1971, 1973b), stimulating in various hippocampal cell fields and fibre systems (dorsal fornix, anterior fimbria, anterior and posterior CA1, and anterior CA3-4), concluded that the early negative-going extracellular field response they recorded from the LSN of rats and later cats reflected excitation of septal target cells. Intracellular recordings revealed graded depolarizations (EPSP's) and action potentials at latencies corresponding to the extracellular negativity.

Depending on the stimulation locus, different zones within the LSN were activated, the locations of which closely corresponded to many of the neuroanatomical findings cited in the previous section. For example, stimulation of dorsal fornix and anterior CA1 yielded an E-I pattern confined to dorsal portions of the medial part of the LSN, maximizing about 250 um from the midline, while stimulation of posterior CA1 evoked responses throughout dorsal LSN, maximizing more laterally and ventrally. Stimulation of anterior fimbria (ipsi- and contralateral) as well as anterior CA3-4 also produced potentials maximizing more laterally (about 750 uM from the midline), although contralateral effects failed to extend as far rostrally as ipsilateral effects. With contralateral CA3 stimulation, responses were found mainly in dorsolateral LSN. These latter findings do not agree with Meibach and Siegel's view that both anterior CA1 and CA3 project to dorso-medial LSN.

The latencies to the peak of the initial extracellular negativity for all areas stimulated ranged from about 4 to 7 msec, and were related to proximity of stimulating and recording sites. (DeFrance estimated velocities of 1-3 m/sec in fimbria-fornix fibres; distances from sites stimulated to recording sites ranged from about 3 to 6 mm.) The application of a number of standard electrophysiological procedures (resistance to anoxia, response recovery after paired pulses, correlation of response latencies with conduction velocities and distances) helped establish the monosynaptic basis of these responses.

Long-latency responses were also detected by DeFrance et al (1973b latencies of 15-20 msec). These responses, evoked with stimula-

tion of anterior fimbria, were confined to more postero-ventral levels of the LSN.

In the work of McLennan & Miller (1974) medial and lateral septal responses to stimulation of fimbria and fornix were examined in the rat. In contrast to DeFrance's findings these workers claimed that no responses were evoked in the LSN following stimulation of dorsal fornix. They did report, however, that with fornical stimulation, a short-latency negative extracellular field potential was evoked at maximum amplitude in "a restricted zone of the medial septum". Since this response was nearly identical to the dorsomedial response described by DeFrance et al (1973b) after stimulation of the dorsal fornix, it is conceivable that the area defined as MSN by McLennan & Miller included overlying dorsomedial LSN. Given that DeFrance's studies were performed in rats with hippocampo-septal areas exposed, while McLennan & Miller's rats were intact, it is likely that DeFrance's estimates of the microelectrode tip position were more accurate.

In agreement with DeFrance's work, McLennan & Miller observed a short-latency discharge (4-7 msec latency) in the dorsal half of LSN, following stimulation of anterior fimbria and maximizing about 0.75 mm from the midline. The latter workers also stimulated posterior fimbria and besides the early response in dorsal LSN, observed a long-latency response of 12-16 msec in ventral LSN which appears to correspond to the long-latency response seen by DeFrance. With frequency following procedures, McLennan & Miller determined that the early, dorsally situated, response was probably monosynaptic, while the later, ventral response was polysynaptic.

Edinger at al (1973) investigated extracellular unit responses in medial and lateral septum of the cat to stimulation of dorsal and ventral hippocampus. Responses to dorsal stimulation, irrespective of CA field, were distributed in more dorso-medial septum , while ventral stimulation produced responses localized to more ventro-lateral septum. In a general way these data conformed to the anatomical facts. That cells in the medial septal nucleus responded at all is in apparent disagreement with the newer studies, but not with the lesion data. DeFrance et al (1978) also reported excitation of cells in the MSN of cats following fimbria and fornix stimulation. There are three possible explanations of the discrepancy between the newer anatomical findings and the physiological data. Firstly, there may in fact be a sparse hippocampal projection to the medial septal nucleus which is not detected by autoradiography. Second, the dendrites of cells in MSN may extend into the LSN (DeFrance et al, 1978; Swanson, 1978), and thirdly, in the cat there may be projections to the MSN.

The latencies to initial excitation of medial and lateral septal units in Edinger et al (1973) fell into two classes. Most units were activated at 10-30 msec latencies; for a much smaller number of units with no discernible topographic distribution, latencies ranged between 30-60 msec. Frequency following techniques suggested to Edinger et al that the short-latency responses were monosynaptic.

Inhibition.

The following discussion reviews evidence which suggests that within the lateral septum there is a widespread group of inhibitory elements which might be activated by two sources: collaterals of

primary inputs (hippocampal) to the septum and recurrent collaterals of septal cells.

In DeFrance et al's (1972, 1973b) investigations the conclusion was reached that the extracellular positivity following the initial negative deflection was probably due to synaptic inhibitory processes (IPSP's). Intracellularly, these potentials were accompanied by graded hyperpolarizations, which continued to appear even in the absence of prior action potentials. Increasing the membrane potential by chloride diffusion resulted in a reduction and eventual reversal of the stimulus-evoked hyperpolarization. During the hyperpolarization, spontaneous discharges were suppressed, again without necessarily being preceded by a stimulus-evoked discharge, supporting the view that the inhibition was externally and synaptically imposed and not due solely to refractory processes intrinsic to the neuron.

The evocation of suppression without preceding discharge also suggested that the inhibition was more spatially diffuse than excitation, a suggestion supported by the finding that with low intensities of stimulation the IPSP could occasionally be evoked without preceding EPSP's; presumably the recording electrode was situated outside the excitatory focus in such cases. The latency of the IPSP in such cases was about 4.5 msec longer than EPSP latency, indicating a polysynaptically produced inhibition mediated by interneurons. McLennan and Miller (1974), recording from spontaneously active septal cells, reported, however, that stimulus-produced inhibition of units was never seen without their prior excitation and suggested that the suppression of unit activity was due to recurrent collateral inhibition. The differ-

ence between the two studies may lie in stimulation intensity (number of hippocampal elements activated).

More direct evidence in support of a recurrent collateral inhibitory network in LSN comes from McLennan & Miller's findings that stimulation of MSN produced a short-latency antidromic activation of LSN cells followed by suppression of spontaneous activity. Similarly, DeFrance found that stimulation of MSN antidromically suppressed responding of LSN neurons to fimbrial stimulation.

That inhibition is mediated by a population of inhibitory cells different from cells initially activated is supported by observations of DeFrance et al (1972) and McLennan and Miller (1974) that in extracellular recordings a smaller amplitude, unitary discharge would sometimes rapidly follow (by 1-3 msec) the initial large amplitude discharge produced by hippocampal stimulation. In McLennan and Miller's work, these events were seen as ripples or a brief burst which followed initial excitation by 1-2 msec. They never occurred spontaneously and were observed throughout the LSN but were most often seen in more medial sites. DeFrance et al (1972) also noted the occasional, burst-like property of these discharges and found that the responses of the early discharge to pulse pairs differed from those of the smaller amplitude discharges. Also, their analogues could not be recorded intracellularly.

The duration of the post-excitatory inhibition was variable. In DeFrance the intracellularly recorded hyperpolarization lasted from 40 to 120 msec regardless of fibre pathways activated; concomitant suppression of discharges lasted about 50 msec. In Edinger et al,

suppression of extracellularly recorded spikes persisted for 50-500 msec, while in the McLennan & Miller study it ranged from 50-700 msec. The latter researchers also noted that the duration of inhibition was longer when spontaneous discharge rates were low. Both McLennan and Miller (1974) and DeFrance et al (1973b) observed a rebound excitation following the inhibition.

Edinger et al (1973) reported that in about 100 cells located throughout the septum of their cats, hippocampal stimulation produced a pure suppression of discharge activity lasting anywhere from 50-500 msec (a duration equal to that of the post-excitatory inhibition seen in other septal cells). While these cells did not display the well-defined topographical organization seen in cells responding with initial excitation, there was a slight tendency for cells inhibited by dorsal hippocampal stimulation to lie more laterally than cells excited by dorsal stimulation. Conversely, cells suppressed by ventral stimulation tended to fall more medially than cells activated by ventral stimulation. An important corollary observation was that those cells in medial septum activated by dorsal stimulation were inhibited by ventral stimulation and vice-versa. Also, while some cells were suppressed exclusively by dorsal stimulation and others by ventral stimulation, about 30% of cells were inhibited by both. Very little convergence of excitatory influence from different hippocampal levels had been observed. These data are consistent with DeFrance's notion of a widespread interneuronal inhibitory network activated both by recurrent collaterals of cells receiving more spatially circumscribed primary afferent drive as well as by afferent collaterals.

Summary.

The preceding sections of the Introduction have been aimed at furnishing a structural and functional "wiring diagram" of the p. cell projections to the septum. Such a schema provides the basis for an analysis of how naturally occuring changes in firing of p. cells will be interpreted by the septal cell and which cells will do the decoding.

It is important to note that the hippocampo-septal system is not an open-loop system: cells in the lateral septal nucleus project to the medial septal area which in turn projects back to the hippocampus. Also, activity in a CA1 p. cell, for example, may influence septal cells through direct projections or indirectly through a relay in the subiculum. It is completely unknown whether the CA1 cell that projects to the subiculum also extends collaterally to the septum or if different p. cell populations project to different areas.

The next part of the Introduction evaluates a body of evidence which bears directly on the understanding of hippocampal function during its various EEG rhythms: the nature of hippocampal unit activity during EEG.

V. Hippocampal unit activity during EEG rhythms.

In many of the initial studies of hippocampal electrophysiology the hippocampal cortex was perceived as a relatively simple neuronal system which could be utilized in the construction of explanatory models of more general electrophysiological phenomena. The microelectrode work of Renshaw et al (1940) was perhaps the earliest application

of this strategy. Green and his co-workers also followed this approach trying to elucidate the cellular basis of the theta rhythm, hoping it would clarify the bases of more complex neocortical events such as the EEG and evoked potentials. The work of Petsche and Stumpf, and the inquiries of Kandel, Spencer and Fujita were also guided by broader questions of neuronal function. Much of this work was published between 1954 and 1964 (see Passouant, 1962) and critically reviewed by Green (1964).

This work is of interest from the present standpoint because, insofar as it attempted to specify how unit events gave rise to theta, it provided a body of evidence bearing directly on the nature of hippocampal action during theta. Other EEG patterns were not considered in these studies; the emphasis was on theta. Most of the work was done in acutely prepared rabbits. In most cases it was assumed that pyramidal cells were at the electrode tip.

A principal finding of these studies was that the theta wave, as seen extracellularly by a large or micro-electrode, was <u>generated</u> by changes in the transmembrane potential of the p. cell (Green & Petsche, 1961; Green et al, 1960, 1961). The findings of Fujita & Sato (1964; see also Fujita, 1975; Fujita & Iwasa, 1977) furnished critical support for the evolving concept that the membrane changes during theta were associated primarily with post-synaptic potentials (excitatory and inhibitory). With intracellular recordings from identified pyramidal cells these workers observed rhythmic oscillations of the transmembrane potential occurring in phase with extracellularly recorded theta. As with neocortical EEG phenomena it appeared that action currents played

a minimal role in theta generation. Thus, although the p. cell was directly responsible for the generation of theta, the presence of theta, by itself, was not predictive of the probability of firing in p. cells.

The studies of Green were the first to furnish an empirical description of p. cell discharges during theta. Green and Machne (1955) reported rhythmic discharges of single cells in hippocampus during theta while Euler & Green (1960) observed that hippocampal bursts (like those seen in cerebellum) often consisted of spikes of different amplitudes, usually descending. This bursting discharge was termed by them the inactivation response since it was thought that the gradual blocking of spike production was due to sodium inactivation. Later the burst was called a complex spike by Ranck (1973).

The complex spike was hypothesized by Green (see Green, 1964) to be intimately involved in theta production, partially because it occasionally recurred in rhythmic fashion and could be elicited by events similar to those triggering theta waves. This position did not contradict the view that theta was composed of synaptic potentials, because Green thought that the large wave of depolarization supporting the complex burst was due to a summation of smaller EPSP "wavelets". A number of observations challenged the soundness of this view. Fujita and Sato (1964) noted that during intracellular theta, p. cells often did not discharge in complex fashion. The extracellular work of Green himself, discussed below, also demonstrated that theta was not invariably linked to complex spiking. Kandel and Spencer (1961), in their detailed intracellular analysis of cat p. cell discharges, presented

strong evidence that complex bursts were due to summating depolarizing after-potentials, events which appeared to be linked to factors intrinsic to the p. cell membrane. This view was bolstered by Fujita's (1975; Fujita & Iwasa, 1977) demonstration that the complex spike was an all-or-nothing response and could be produced by intracellular injections of depolarizing current. Thus, while the complex spike could be triggered by synaptic potentials, once activated its evolution was independent of input. Again it appeared that there was no immediate connection between p. cell spikes and theta.

The studies of Green et al (1960) showed that the activity of hippocampal cells during theta was quite variable. When theta was evoked by midbrain electrical stimulation, there was a tendency for inhibition of firing. The inhibition was sometimes followed by a rebound excitation, a phenomenon also observed by other research groups (Andersen & Eccles, 1962; Fujita & Sato, 1964; Kandel & Spencer, 1961). When theta was evoked by skin shocks, only intense shocks suppressed discharges. Theta induced by amphetamine or eserine was generally accompanied by a rhythmic bursting of cells. In one case, however, rates increased, while in the other a decrease was seen. Green et al (1960) reported that in most situations putative pyramidal cells did not fire rhythmically during theta, although the spikes occurred at the same phase of the theta wave. And, as noted by Green & Petsche (1961), the same cell might fire during one portion of theta and not another. More recent descriptions of hippocampal unit activity have revealed that besides phase relations, there are other regularities in the relation of unit firing and hippocampal theta. Before discussing

these studies, research dealing with the network contributions to theta will be briefly reviewed in an attempt to predict the kinds of neural events that are necessary to trigger rhythmic post-synaptic potentials in the p. cell. An understanding of these events may lead to predictions of probability of p. cell discharge during theta.

Probably the most extensively worked out explanation of rhythmic EEG activity in general is the autorhythmicity (AR) model of Andersen and Sears (1964). In this model a local circuit composed of two cell types is required: a projection cell and an inhibitory interneuron. The projection cell, via a recurrent collateral terminates on a basket cell while the inhibitory element terminates on a number of projection cells. This basic circuit is probably present in the hippocampus. Strong, long-lasting suppression of p. cells of a recurrent nature has been observed by several researchers (Andersen et al, 1964a; Kandel et al, 1961) and Andersen et al's (1964 a,b) findings have suggested that the recurrent inhibition is mediated by an interneuron, probably the basket cell. A second major requirement of the AR model is that the projection cell show a post-inhibitory rebound. As already pointed out, p. cells show this characteristic.

In the AR model, rhythmic activity is initiated when extrinsic input activates projection cells and consists of the self-sustaining oscillation between inhibition (IPSP's) and rebound excitation (EPSP's) and discharge in those cells. A necessary consequence and cause of this rhythmic oscillation is a rhythmic and synchronous (because of interneuron divergence) firing of projection cells. These outcomes do not seem to hold for hippocampal neurons. It has been noted that while

p. cells may discharge rhythmically during theta, they are as likely not to do so. The question of synchronicity of cell discharge during theta will be dealt with at a later point.

Another difference, and a critical one, between the facts of hippocampal theta and the AR model is that theta is not autorhythmic. In the AR model a single excitatory afferent volley to the projection cell is sufficient to initiate rhythmicity. While the hippocampus contains the circuitry necessary for self-sustaining oscillatory behavior, it requires constant priming or pacing from cells in the medial septal area. It is a well known fact that lesions of the MSN eliminate theta and that rhythmic discharges in the septum are in phase with theta (Petsche et al, 1962). It has consequently been supposed that rhythmic activity of septal cells drives hippocampal p. cells into regular discharge (Stumpf, 1965a). That septal activity will activate p. cells is an assumption made by a number of early and contemporary workers (see Izquierdo, 1975 and references therein). Much of the evidence indicates however, that septal termination in the hippocampus serves to suppress p. cell activity.

Intracellular studies have shown that p. cells undergo prolonged inhibition following fornical stimulation (Andersen et al, 1964a; Kandel et al, 1961). The results of stimulation in the deafferented fornix preparation indicate that the inhibition is mediated by recurrent collaterals, but does not rule out the possibility of feed-forward inhibition in the intact preparation. Andersen et al (1964a) have claimed to record intracellularly from a basket cell and have observed its excitation following fornical stimulation. Finch and Babb

(1977) have recorded extracellular responses from a single putative basket cell following fornix stimulation and have reported a high-frequency burst response, the rapid portion of which lasted for a time approximately equal to the IPSP duration in p. cells. Interestingly, the duration of the burst decreased with repeated stimulation. Franzini et al (1975) have shown that lesions of the MSN result in increases in discharge rates of CA1 pyramidal cells during theta, and Miller and Groves (1977) have found a significant reduction in numbers of CA1 pyramidal cells inhibited by peripheral stimulation following septal lesions. The electrophysiological findings are in agreement with the anatomical data which indicate termination of medial septal afferents in regions containing the interneuron population (Mosko et al, 1973; Raisman et al, 1966). Lynch, Rose, & Gall (1978) with a transsynaptic tracing technique have located a labelled cell corresponding to an interneuron in the dentate area of the rat following injection in the medial septum. There has been some controversy over whether MSN cells project at all to area CA1, and there is other evidence which suggests that some septal input may be excitatory. Although this work will not be reviewed here, it can be said that MSN input, regardless of its precise termination, serves to inhibit p. cell activity.

If those medial septal cells which discharge rhythmically during theta do in fact project to the hippocampal interneuronal population, at least partially, it would be expected that basket cells should discharge rhythmically during theta, closely coupled to the theta wave. The studies of Ranck (1973; Wolfson et al, 1979) have

indicated that this, indeed, might be the case. While contemporary and early researchers have recorded cells bursting rhythmically during theta, they have not attempted to identify the cell type giving rise to the bursts, assuming their origin in p. cells. Ranck's work is unique in its thorough attempt to distinguish between hippocampal cell types. It also represents a significant break from previous studies in its recording of single cells from rats free to move.

In observing about 370 cells in dorsal hippocampus in moving rats, Ranck noted they could be classified into two basic types. The discharge patterns of one type of cell, termed a "theta" cell, were closely related to slow wave theta and those behaviors which have been correlated with theta. During theta and only during theta this cell type increased its firing rate, often discharging in rhythmic bursts, more or less synchronously with theta. The other cell type, the "complex-spike" cell, was defined principally on the basis of the fact that at some time during the recording session (usually during slow wave sleep) the spike was complex. Theta cell discharges never showed a complex form. The two types were different in a number of other respects outlined in Table 1. Together the electrophysiological differences pointed to different anatomical cell types. That theta cells were generally found in regions thought to contain the interneuron population, while complex-spike cells were most often encountered in the pyramidal cell body layer suggested that theta cells were basket cells and complex-spike cells were pyramidal cells. Many of Ranck's distinctions have been confirmed by O'Keefe (1976).

Of particular interest here is Ranck's observation that discharge rates in the two cell types were approximately inversely related: theta cell spike frequencies were highest during slow wave theta while complex spike activity was suppressed over much of the theta period. When complex spikes did occur during theta, it was for brief periods and only under special circumstances, defined by the particular behavioral correlate of the cell. In the work of O'Keefe and Nadel (1978) most complex spikes occurred at rates "considerably less than one per second" during a theta-related behavior. High rates (10 Hz) of p. cell firing were seen during theta, but only if the rat was in a particular "spatial" relation to the environment. During the brief periods of complex-spike cell firing during theta, the spikes were also phase-locked to the theta wave, but it appeared that firing of complex-spike cells was apt to be less regular than that of the theta cell. One possible inference, which requires experimental test, is that phase relations of complex-spike cells with the theta wave are due to local circuit action, while theta cell phase relations result from the impingement of external circuits,

In Ranck's work, sustained discharging of complex-spike cells was more likely to be observed during slow-wave sleep. During paradoxical sleep (during which theta is present), Ranck found that complex spike probability was quite low. Out of 25 cells monitored during SWS and PDS only three occurred at higher rates during PDS.

The work of Ranck and O'Keefe also suggested that p. cell discharges are not synchronous during theta. Both researchers found that when the rat was active (presumably during theta), the firing of

different cells was correlated with a different behavior or place field. It has also been noted, in several studies, that when more than one cell is at the microelectrode tip (as is most often the case) the firing of the different cells will occur during different segments of the theta train (Hill, 1978, Figs. 3,6; O'Keefe & Nadel, 1978, Fig.23). On the other hand, p. cell firing appears to be synchronized during LIA (O'Keefe & Nadel, 1978). The work of Noda et al (1969), in which cross-correlograms were used to assess the degree of relationship between hippocampal units recorded at the same tip, provided direct evidence that hippocampal units are not synchronized during arousal or REM sleep; during these states the cross-correlogram was flat. During SWS and intermediate sleep, peaks in the cross-correlogram about zero suggested synchronicity of discharge.

Summary.

The outcomes of those unit studies which distinguish between hippocampal cell types as well as some studies which do not, indicate that during much of slow-wave theta pyramidal cells are relatively inactive. During select portions of theta, however, there is firing in units corresponding to the hippocampal pyramidal cell. These findings lead to the tentative conclusion that theta during normal waking behaviors is not a unitary state, at least in terms of the discharging of underlying neuronal elements. Neuronal interactions within the hippocampus during theta are complex, with different cells doing different things at the same moment in time. Notions of hippocampal function which perceive the hippocampus as active or inhibited during any particular state appear to be oversimplified.

The remaining portions of the thesis are taken up with a further analysis of the activity of single cells in the hippocampo-septal axis. After a description of the general methods used to obtain and process the data, the observations are divided into a number of sections:

Section One: What is the nature of hippocampal EEG in the paralysed rat? Does it differ substantially from that seen in the normal?

Section Two: What electrophysiological cell types are found in the hippocampo-septal circuit?

Section Three: What types of activity changes are seen in hippocampo-septal cells during EEG changes?

Section Four: How does the hippocampus influence septal activity? Does its influence change as a function of EEG state?

GENERAL METHOD

Experimental strategy.

One of the aims of this thesis was to describe hippocampal output, particularly under natural conditions. A number of approaches were tried during pilot experiments. The first was to electrically stimulate the hippocampus and record extracellular responses in various target areas. This was done in four rats anaesthetized with urethane and later in 26 rats immobilized with succinylcholine chloride.

In addition, hippocampal EEG was monitored during normal behavior in 13 pilot animals. This provided a baseline range of responses against which to evaluate the changes in hippocampal EEG with paralysis. Changes in septal unit activity during changing hippocampal EEG in these rats were also noted after paralysis. The results of these pilot experiments are not presented here since the experiments were repeated in the main study. It was noted that, in many cases, gross hippocampal EEG underwent a number of changes with paralysis. To help evaluate whether the paralysed rat's theta was similar to that of the normal rat, atropine sulphate was administered to 4 pilot animals during normal and paralysed states and effects on theta assessed. The reasons for doing this are given in Section One.

The results of this thesis are based primarily on 16 additional animals in which the strategy was to record pairs of units in the hippocampo-septal circuit of one hemisphere, while monitoring EEG with

a gross electrode situated in the hippocampus of the other hemisphere. The experimental procedures have been divided into five phases: selection of animals, surgery, acute experimental procedures, histological procedures, and data analysis.

Experimental animals.

Sixteen experimentally naive, male Long Evans rats from the McMaster Psychology Department colony were housed either singly or in groups. These rats, approximately 3 to 4 months old, weighed between 268 to 382 grams (mean weight of 325 g) at the time of surgery. Other than daily handling, no special treatments were applied prior to surgery or between surgery and the acute experimental day. Lights went on at 6 AM and off at 8 PM and the rats had continuous access to Purina lab chow and water.

Surgery.

Rats were anaesthetized with intraperitoneally administered doses of sodium pentobarbital (Somnotol, MTC Lab., Hamilton, 65 mg/kg) and, when necessary, with supplementary doses of chloral hydrate (standard dose of 60 mg). They were then placed in a Kopf rat stereotaxic instrument with the incisor bar set to match the requirements of the Pellegrino and Cushman (1967) rat brain atlas (5 mm above the interaural line). In each animal 2 or 3 holes, about two millimeters in diameter, were drilled in the skull overlying the brain regions to be probed with the microelectrodes. In all rats, the holes were over the right hemisphere. Dura was left intact during surgery and the

holes were filled with bone wax. Nylon nuts were cemented over the holes with dental acrylic. Following surgery nylon screws were turned into these nuts to protect the openings.

Once preparation of the microelectrode access holes was complete, a bipolar twisted wire electrode was lowered into the left hemisphere of each rat using conventional stereotaxic procedures. The electrode was made of two strands of 255 uM nichrome wire insulated with thin enamel except for the tip cross-section. The tips were separated by the layers of insulation and in all rats they were aimed at the pyramidal cell layer of dorsal CA1. The electrode was cut so that one tip was slightly higher than the other, but the orientation of the tips with respect to the pyramidal cell layer was not standardized.

A jeweler's screw soldered to a miniature connecting pin was turned into the skull just posterior to lambda to function as the ground electrode while a second screw, placed in the skull overlying the frontal area, acted as reference for the microelectrode. One or two other small screws were inserted into the skull to serve as additional anchors for the dental cement which fixed the entire assembly in place. Before the acrylic hardened, two shallow anchor holes were formed on both the right and left lateral surfaces of the cement by a metal assembly mounted on each ear bar. During the acute recording phase the rat was firmly held by positioning the assemblies to fit in the indentations so formed. This eliminated the need for ear and nose bars and held the animal's head at the proper stereotaxic angle. Towards the end of surgery penicillin was injected intramuscularly (40,000 I.U.).

Acute experimental procedures.

One to two weeks after surgery the rat was placed in a copper-shielded room where testing was carried out over a period of time averaging 12 hours. Testing was initiated in the morning during the rat's light cycle and always extended into the dark cycle.

Baseline EEG recording. Prior to the injection of the paralysing drug five to ten minutes of EEG was recorded from the CA1 macroelectrode with the animal free to move in a large wooden test box. The EEG signals, carried by shielded Microdot cables, were written out on EEG chart paper with an ink-writing oscillograph (Grass-Model 7AE) and also recorded FM on magnetic tape (Ampex). The low and high frequency filters on the polygraph were set to attenuate frequencies less than 0.3 Hz and greater than 3 kHz. Monitoring EEG during behavior permitted evaluation of the normalcy of rhythms recorded at the hippocampal electrode. Also, since EEG was recorded during paralysis, the baseline EEG provided a reference by which to judge the state of the paralysed animal.

Immobilization, artificial respiration. The rat was injected intraperitoneally with succinylcholine chloride (Anectine, Wellcome Burroughs, 4 mg/kg) and, as soon as the initial muscle-activating effects of the drug subsided, the immobile rat was respirated with a constant-volume respirator (Harvard Model 680).

The method of linking the animal to the respirator was adapted from Roberts (1973). A short plastic cylinder with a thin tautly-stretched rubber diaphragm at one end was connected to the air delivery tubing of the respirator with a one-hole rubber stopper inserted into

the other end. The rat's snout was placed into a small hole in the diaphragm. With the respirator delivering a set volume of 7 to 10 ml of room air at 70 strokes per minute, it was possible to maintain the rat for as long as 24 hours. The respirator was placed in a grounded metal screen cage to minimize electrical interference with electrophysiological signals.

Once respiration appeared stable, the rat was placed in a stereotaxic instrument and its head was automatically fixed at the required stereotaxic angle by inserting the ear bar mounted assemblies into the indentations in the headcap. Anectine was infused intramuscularly throughout testing at a nominal rate of 4 mg/kg/hr (Harvard infusion pump). Heart rate was monitored by two needle electrodes inserted beneath the skin along the chest area. Core temperature was monitored with a rectal thermometer. An infrared heat lamp was used to keep core temperature at normal levels.

Shortly after immobilization, heart rate was typically about 360 beats per minute and showed "spontaneous" as well as environmentally sensitive irregularities. After about one or two hours heart rate stabilized at 380 to 480 bpm. Few irregularities were seen after the initial period of instability; when they did occur they could be compensated for by adjusting air volume at the respirator, by closing the rat's jaws to prevent leakage at that point, or by adjusting the position of the snout in the rubber diaphragm to compensate for leakage at that juncture. All measures used (heart rate, temperature, electrophysiological activity) indicated that these preparations were reasonably stable throughout the testing period. Abnormalities in these

measures produced either a delay in testing until recovery, or termination of the experiment.

Pain-associated trauma to the rat was minimal: since the rat's head was held by the cement cap and microelectrode holes were predrilled, there were no wounds or pressure points. Artificial respiration was accomplished with a nose cylinder, rather than tracheal intubation methods. The major source of trauma was probably derived from paralysis itself. Generally, recording did not start until one to three hours after immobilization, at which time indices of the rat's condition had stabilized and some adaptation to the paralysed state had presumably occurred.

Electrophysiological recording procedures and equipment. Monitoring of hippocampal EEG was continued during immobilization and maintained throughout testing except for those times when the macroelectrode was used to electrically stimulate the hippocampus. Recording of EEG was similar to that used during baseline EEG recording.

To prepare for extracellular single cell recording, the protective nylon screws were removed and bone wax cleared away from the hole. Dura was removed and one glass micropipette was centred over the hippocampal access hole while a second pipette was placed over the septal opening. The micropipettes, with tip diameters 2-3 uM, were filled with near-saturated sodium chloride solution and each was held by a micromanipulator (Narishige).

Silver (silver-chlorided) wires conducted changes in electrical potential at the pipette tips to two high-impedance pre-amplifiers (Grass P15). The changes in potential were referred to the anterior

skull screw; grounding was achieved by connecting the posterior screw to the P15 ground. The brain signals at the microelectrodes were further amplified by the oscillograph amplifiers (Grass 7P5B wide-band AC EEG pre-amplifier). Filters at both stages of amplification were set to attenuate frequencies below 0.3 Hz and above 3 kHz for the septal microelectrode. The oscillograph 60 Hz notch filter was used as well. Subsequent to the second stage of amplification slow potential changes (field potentials) were separated from fast potential changes (action potentials) at the hippocampal tip by low and high pass filters. The low-pass filter allowed frequencies below 50 Hz to pass without attenuation; the half-amplitude cut-off frequencies for the high-pass filter were 500 Hz and 3 kHz.

The microelectrode signals were monitored on a dual beam oscilloscope (Tektronix 502) and sample portions were recorded on magnetic tape with a seven-channel recorder (Ampex PR500). The two channels of fast activity were recorded on direct channels while the slow activity was recorded on FM channels. Thus, four channels of electrophysiological information were recorded concurrently:

1. EEG from the hippocampal macroelectrode.
2. Slow activity from the hippocampal microelectrode.
3. Fast activity from the hippocampal microelectrode.
4. Fast activity from the septal microelectrode.

The high frequency channels were also connected to loudspeakers via gating units to permit auditory monitoring of action potentials.

Data collection: fast activity. Data collection began by establishing a dorsal-ventral microelectrode zero level. The pipette tips were

aligned visually with the skull surface and then lowered until visual and auditory monitoring of the signals indicated passage of the tips from air to cerebrospinal fluid. The tips were then lowered an additional .5 mm to the zero level, a point presumably in close proximity to the brain surface.

Units at the hippocampal electrode were sampled first. The electrode was lowered in small increments until the typical popping sound associated with neural discharges was heard at the loudspeaker. Movement of the electrode was continued until the signal to noise (S/N) ratio was maximal. If the S/N ratio was adequate and the discharge pattern was stable and did not appear to be injury-induced, a search for units at the septal level was initiated. When two stable units with reasonable S/N ratios were registered concurrently, their activity was recorded on magnetic tape and EEG chart paper. Occasionally, only one channel of spike activity was sampled. Because of difficulties inherent in finding and recording two clear trains of spikes simultaneously, in most cases, particularly in hippocampal probes, the record consisted of several spikes of different amplitudes. If the largest spike was distinct from others in the record it was accepted for analysis. Units that were small, but could be clearly distinguished from baseline noise levels and were the only units evident, were also processed.

The duration of a sample "run" varied from several minutes to over an hour; no statistical analyses were performed during data collection. Two general types of observations were noted, however: one was of the general appearance of the activity at the pipettes. A

second was the electrophysiological "depth profile." That is, as the electrodes were lowered, notes were made of the correlation between the depth reading on the micrometer dial and the nature of fast activity registered by the microtips at that depth. Cell-poor areas or fibre-rich areas were electrically silent relative to cell-dense areas. Spontaneously quiet, cell-rich areas could be identified because the pipette might provoke a characteristic high frequency irritative or injury discharge in otherwise silent cells. Also, a given spontaneously active area might have a unique signature in the record. The medial septal nucleus, for example, was readily identified by the presence of rhythmically bursting cells.

Starting with the original zero level, then, and revising on the basis of the electrophysiological readout, it was possible to ascertain when the microtips were passing through the corpus callosum, when they were approaching the hippocampal pyramidal cell layer, and so on. Changes in hippocampal slow activity at the microelctrode as a function of depth were also noted, permitting the construction of a theta depth profile. These procedures provided an important adjunct to histological procedures used in the identification of brain areas giving rise to a particular unit record.

When the depth profile and micrometer readings indicated that hippocampal units were being sampled, the hippocampal pipette was left at that depth for a number of runs. Activity at its tip was paired with different target units on each run. The hippocampal microtip position was altered if the S/N ratio declined or if a reasonable number of target pairings had been recorded. To obtain different

hippocampal-septal pairings either pipette could be moved up or down. In some animals, to successfully record spontaneous activity at the microelectrode, required more than one penetration. The final penetration usually provided most of the recorded activity.

Several factors contributed to selectivity in the data sampling procedures. One factor, inherent in microelectrode recording, was that only larger elements with stronger signal capacity were registered. Also, only cells spontaneously active were recorded. Only on the rare occasion was an attempt made to find optimal conditions of firing for cells normally silent. Another source of bias was spike frequency. Of those elements spontaneously active, it was probably the more rapid ones, more easily detected aurally, that were selected. Only a small proportion of elements outside of the septo-hippocampal complex were sampled. Usually, these were elements which showed systematic changes in activity as a function of environmental changes. A majority of units encountered in the septo-hippocampal complex with reasonable S/N ratios were recorded for later analysis.

Data collection: slow activity. Samples of slow activity from both hippocampal macro- and microelectrodes were recorded throughout the experiment both on FM tape and on EEG chart paper. For each sample run an attempt was made to include "spontaneous" transitions from one type of hippocampal EEG pattern to another. To produce regularization of EEG activity the rat was exposed to various types of sensory influences such as tail pinching, stroking of vibrissa, or blowing. A punctate, forceful blowing of air on the rat was particularly effective in eliciting long bouts of clear theta activity. The time of occurrence

of stimulus presentation was marked on magnetic tape by verbal signal.

Several other procedures were implemented to alter electrical activity at the macro- or microelectrodes.

a. Electrical stimulation - cells under the CA1 macroelectrode tip were activated and changes in ongoing activity at the contralateral microtips were recorded. These data helped to determine the location of the microelectrodes and also provided information about dorsal CA1 influence on septal cells under conditions of artificial activation.

b. Injection of atropine sulphate - in two animals atropine sulphate (25 mg/kg) was administered intraperitoneally at the conclusion of testing and effects on hippocampal EEG at various time intervals following injection were assessed. In a separate pilot study four additional animals with macroelectrodes implanted in hippocampus were paralyzed and the effects of atropine sulphate (25 mg/kg) on theta were studied.

Histological procedures.

At the end of testing the rat was deeply anaesthetized with an overdose of sodium pentobarbital. The microelectrodes, with tips at the most ventral recording sites, were cemented in place. The rat was removed from the respirator and perfused through the heart with normal saline followed by 10% formalin. The brain was extracted from the skull and stored in 10% buffered formalin for at least 48 hours after which it was frozen and the region containing the electrode track was sliced coronally into 30-50 μm sections. Brain slices were mounted on slides and stained with thionin.

Data processing.

All data analyses were based on information recorded on magnetic tape (off-line) and were done after the acute experiment. Calculation of statistics and reduction of data to histogram form were performed by a PDP-8/A or 8/E digital computer (Digital Equipment Corporation).

Processing of spike trains. Prior to computer processing, both channels of unit activity were filtered to attenuate slow fluctuations in baseline and passed through "gating" units (Schmitt Trigger circuits - Fersch) which generated brief, constant amplitude rectangular pulses in response to "gated" events at the input. The raw record of Ammon's horn activity typically consisted of spikes of several amplitudes. The largest spike, usually complex, was relatively easy to distinguish from smaller spikes. The smaller events, which often varied in amplitude, were difficult to distinguish from each other particularly because fast activity was usually superimposed on relatively large amplitude EEG activity which was difficult to reduce without concomitant severe attenuation of fast activity. One of the gating units contained a pulse height discrimination circuit and, where feasible, it was used to allow for separate analysis of large and small pulses in the hippocampal record. Gating level for complex spikes was set to include as many of the diminishing amplitude spikes in the burst as possible without overlapping with smaller spikes. Large and small units were discriminated to allow for later evaluation of the cross-correlation of the two events. It was noted that small spikes in the record often behaved

like "theta" cells of Ranck (1973) while larger spikes were similar to the complex-spike discharge of Ranck. This observation is expected if one assumes that when the microtip is in the basal end of s. pyramidale it is registering the activity of nearby internuncials. To perform a cross-correlation of the two events could potentially provide a strong test of the hypothesis that basket cells inhibit p. cells. The occasional overlap in amplitude between the small spikes and the smallest of the complex-spike cell spikes prevented a clear interpretation of this data and the results are not included here. But, the behavior of the small cells was considered in the classification of cell types (Section Two) and their patterns of firing during EEG were also noted (Section Three).

The septal record usually consisted of one dominant height which was relatively easily discriminated from other spikes. Only the largest of the target spikes was processed during a computer run. The standardized output pulses of the gating units served as input to the PDP-8. These pulses were registered at two Schmitt triggers located on a PDP-8 peripheral device.

Undoubtedly the most critical and time-consuming aspect of data analysis involved those procedures undertaken to ensure that only action potentials of the particular cell being studied were sensed by the computer. To accomplish this the outputs of the Schmitt triggers at the PDP-8 peripheral unit were displayed simultaneously with and compared to the raw record on a dual-trace storage oscilloscope both prior to and during the processing run. The PDP-8 Schmitt outputs were also relayed to loudspeakers to permit auditory as well as visual comparisons of raw and gated activity.

In certain runs there was too great an overlap in spike amplitudes to permit gating of individual units; other runs showed spontaneous changes in spike amplitude suggesting electrode movement; while others showed sudden uncharacteristic losses of cell activity not attributable to experimental treatments. All these cases were rejected for analysis; only those displaying stable electronically discriminable spikes were processed.

The starting and stopping points of computer analysis of a particular spike train pair depended on the appearance of hippocampal EEG. In some sample runs clear distinctions between regular activity and a large amplitude irregular activity could be seen. In the majority of runs the EEG was more uniform and spontaneous transitions from one pattern to another were difficult to note. Procedures for processing of spike data differed in these two cases. In both situations, however, two separate computer analyses were performed for each sample run.

In those sample runs where EEG patterns could be distinguished, the analyses provided a comparison between unit activity during irregular and regular EEG. Processing for each of these patterns began with its appearance on the oscilloscope screen and was terminated automatically if a total of 1021 pulses for both channels was counted during the occurrence of that pattern. If the pattern of interest was interrupted by a change in EEG before a collection of 1021 spikes occurred, the program was temporarily halted and was restarted with the reappearance of the relevant EEG pattern. In some cases, if the overall duration of a particular pattern was brief and/or if firing rates were low in both

channels, the analysis of spike activity during a particular EEG pattern consisted of less than 1021 spikes. Since this method of analysis relied heavily on the validity of the assumption that for different segments of a given EEG pattern the relationship between firing of neurons was constant, this assumption was put to the test for several cell pairs by comparing histograms for adjacent as well as non-adjacent segments of a given EEG pattern. In all cases tested, the histogram features for different segments remained the same.

On occasions when it was difficult to discriminate the various EEG patterns by eye, a comparison was made between unit activity during "spontaneous" EEG and EEG affected by sensory stimulation. For these sample runs processing was initiated at the beginning of the run and continued until the delivery of a sensory stimulus to the animal was indicated. Analysis began at the time of stimulation and extended for variable periods beyond the cessation of the arousing stimulation. In many cases analysis included delivery of a number of different stimuli.

Computer reduction of spike trains. The programming used to sense and reduce the two channels of pulses arriving at the PDP-8 Schmitt triggers was written in this laboratory. At its core was a routine that created a list of "words" identifying the source of events (channel one or two) and the time interval elapsed since the preceding event. Measurement of inter-event times by the computer clock was carried out at a single resolution of 0.8 msec.

Based on the information in the computer's word list two types of data summaries were generated: 1. A statistical summary was printed

at the teletype and 2. One of several different histograms was displayed on the oscilloscope. The counts in the histogram bins were stored on flexible diskette and later redisplayed for photography or plotting on an X-Y plotter.

The statistical summary consisted of number of spikes counted (n), mean interspike interval (m), standard deviation of the ISI's (s), and the coefficient of variation (c = s/m) for each channel. The coefficient of variation was useful in the assessment of relative variability of distributions. A small coefficient suggested that the distribution was composed of fairly regularly occurring non-bursting events. A large coefficient was indicative of the presence of very short intervals (bursting) mixed in an irregular or regular way with long silences (ISI's).

Two types of histogram were generated for each spike channel. The first histogram, a conventional time interval histogram (TIH), was a bar graph plot of the frequency distribution of intervals between each spike and the immediately following spike (first-order intervals) up to a maximum interval of 816 msec.

A second histogram was the autocorrelogram (AUTO). It required for its construction that the interval between each spike to every subsequent spike be calculated (zero order through nth order) up to a maximum interval of 816 msec. Because all orders were considered, its form reflected whether certain intervals tended to repeat themselves regularly as a function of time after spike occurrence. While the TIH estimated the probability of occurrence of the next spike as a function of time following the reference spike, the AUTO provided an estimate of

the probability of occurrence of all spikes subsequent to the reference spike (up to a maximum interval).

A third histogram, the crosscorrelogram (CC), expressed the relation of firing between the two channels. It plotted the frequency distribution of intervals from each spike in one channel to every subsequent and preceding spike in the second channel up to a maximum of 408 msec. Thus, the crosscorrelogram (CC) provided an estimate of the probability of encountering a spike in train B as a function of some time increment occurring both subsequently (positive time) and prior (negative time) to a spike in train A. It should be recognized that negative time from train A to B is equivalent to positive time from B to A. Thus, the CC potentially can characterize the influence of A spikes on B and of B spikes on A. Conventionally, in the CC the origin is at the centre of the abscissa, while positive and negative times from A to B extend to the right and left of zero respectively. Thus, the right half of the CC estimates the probability of discharges in B as a function of time after an event in A.

Since ordinate values represent the number of cross-intervals of a particular duration (or number of spikes occurring at a particular cross-interval), a flat CC indicates that discharges in one channel do not tend to follow those of the other at some preferred interval(s) or that regardless of the time elapsed since a spike in one channel, the probability of the occurrence of spikes in the second channel is the same. The discharges in the two channels in that case would be unrelated. However, if the CC is not flat, then the possibility that firing in one channel affects the firing in the other must be considered.

There appear to be two fundamental types of physiological events which can induce a correlation between two spike trains (Moore et al, 1970). The cells involved may be connected either mono- or polysynaptically, with one producing excitation or inhibition on the other. Alternatively, the two neurons may share a common input such as a presynaptic neuronal element or other physiological inputs including hormonal, blood CO_2, blood pressure, temperature, as well as electrical field effects (ephaptic, etc.). Both these factors will give rise to what Moore et al have termed primary effects. They occur close to the origin of the CC and in most cases can be distinguished from each other by the fact that if two neurons share inputs, the CC associated with their simultaneous activity, will have a relatively broad peak which straddles the origin (see Kristan, 1970). Since common input tends to produce simultaneous discharges in the two cells recorded from, firing in A tends to be followed by firing in B and vice-versa, producing peaks on either side of zero. On the other hand, trans-synaptic effects are reflected in the presence of CC features which do not straddle the origin, but are seen relatively close to it, to the left or right depending on which element produces the reference event. However, it is also possible that features which do not straddle the origin may also arise from common inputs. This is unlikely for those cases which originate in non-neural factors or field effects, but it is a good possibility in the case of neuronal input. If area A projects to areas B and C, the branch from A to B may be longer, or it may have an interneuron interposed, or it may be more or less myelinated, or of different diameter. All these factors will result in different arrival times of impulses to B and C from A.

Besides those CC features which are attributable to primary effects, a second, ubiquitous source of peaks and troughs lies in what has been termed secondary effects by Moore et al. These are the contributions attributable mainly to the individual discharge characteristics of hippocampal and septal neurons (periodicity, e.g.) and they are typically seen further away from the origin. In many instances these individual patterns are repeated in the CC's and it is partially for this reason that AUTO's have been included in the analysis; they serve as indispensable aids to the interpretation of CC features.

SECTION ONE. Analysis of EEG.

This section is intended to serve two main functions. First, it attempts to assess changes in normal hippocampal EEG that follow as a result of paralysis. Large differences between paralysed and freely-moving rats would place serious constraints on extrapolations to the normal rat. Second, since comparisons of discharge patterns were made during irregular and regular EEG, more detailed descriptions of these EEG patterns in paralyzed rats are furnished here.

Hippocampal EEG: Patterns in normal rats. In the normal, awake rat three fundamental types of EEG patterns can be discerned when gross or microelectrodes are situated optimally with respect to the generators of hippocampal rhythms. According to Vanderwolf (1969, 1971) each pattern is associated with a different class of behaviors. Since his descriptions have received the widest support (Black, 1975, O'Keefe & Nadel, 1978; Ranck, 1973, Winson, 1974), they will be used as a standard here. Examples of these patterns, recorded in two pilot animals (12, 13), are presented in Figure 5.

Two of the EEG patterns have an irregular appearance to the naked eye. One of these (SIA or small amplitude irregular activity) appears as a flattening of EEG, typically lasts for one or two seconds, and appears to be associated mainly with sudden cessation of ongoing movement (Vanderwolf, 1971).

A second type of irregular activity is of larger amplitude than SIA and accordingly it has been termed large amplitude irregular

activity (LIA). LIA consists of a sequence of slow irregularly spaced waves of different forms and amplitudes with occasional fast (50-100 msec) EEG spikes intermixed. LIA appears either when the awake rat is motionless or when the rat is performing stereotyped behaviors such as chewing, licking, grooming, defecating, urinating, etc. A type of large irregular activity, not described extensively in the literature, is also present during non-waking states. A few reports (O'Keefe & Nadel, 1978; Whishaw & Vanderwolf, 1973; Winson, 1974, 1976a) as well as observations made here (see Fig. 5), suggest that as the rat progresses from alert (upright) immobility to drowsy immobility and to slow-wave sleep, the amplitude of LIA increases and a greater number of large amplitude spikes and bouts of spindling are seen in the hippocampal EEG. O'Keefe & Nadel (1978) have noted that EEG spikes during LIA are most frequent during slow wave sleep and quiet sitting, less frequent during eating and drinking, and least likely to occur during grooming. Black (1975) reported that when a rat is immobile during the occurrence of a signal predicting shock, there are fewer LIA spikes than when the animal is immobile during rest periods, suggesting that LIA spikes increase as level of arousal decreases. In agreement with this suggestion is the finding that LIA spikes associated with slow-wave sleep are suppressed when the animal becomes aroused, either spontaneously or because of environmental disturbance (O'Keefe & Nadel, 1978 and Fig. 5). All these impressions have been substantiated in a quantitative study by Hartse et al (1975) who recorded hippocampal EEG spikes from the ventral hippocampus of the cat. Spikes were rarely seen during active waking, becoming more frequent with decreased arousal.

A third dominant form of hippocampal EEG pattern, consisting of a rhythmical series of slow waves, appears to be unique to the hippocampus. These waves have been called rhythmical slow activity (RSA; Stumpf, 1965b; Vanderwolf, 1971) and theta waves in earlier literature (Jung & Kornmüller, 1938; Green & Arduini, 1954). Frequency of theta varies both within and between species; in the rat it has been reported to extend between 6 and 10 Hz. It accompanies non-automatic behaviors such as running and exploratory movements and is also present during active or paradoxical sleep.

Some researchers have considered that the theta continuum is composed of discrete frequency bands which are related to different classes of behavior (Gray, 1971) while others have assumed that different frequencies are related to different "intensities" of a common class of behaviors. The latter has been the working assumption of Vanderwolf and co-workers for a number of years. The common class of behaviors associated with theta have been termed "voluntary" or Type 1 behaviors. The low frequency end of the theta spectrum has been associated with behavioral states occuring just prior to voluntary movement (movement "intention") as well as smaller amplitude voluntary movements. Higher frequencies have been associated with the initiation and performance of large movements (Vanderwolf, 1969,1971; Whishaw & Vanderwolf, 1973). This scheme is changing. More recent studies (Kramis et al, 1975; Vanderwolf, 1975) have suggested to these researchers that the low and high frequency ends of theta may not be the outcomes of changes in the activity of one neural system, but the consequences of activity in two different systems. One of these

systems is thought to be responsible for the generation of a slow theta (4-7 Hz), the behavioral correlates of which have not yet been clearly established in the rat. The other system results in a faster theta of 7-10 Hz, correlated with large-amplitude voluntary behaviors. Vanderwolf's findings are of particular relevance because of supplementary findings that slower and faster theta rhythms appear to be disrupted by different pharmacological agents, suggesting the operation of two neural systems. Brief bouts of slow theta, occurring during immobility just prior to an active avoidance response, were not seen subsequent to atropine sulphate, a cholinergic blocker, while fast theta, if it was clearly present in the record, survived atropine treatment. Similarly, the relatively long bouts of slow theta seen in still, but alerted rabbits were also abolished by atropine while fast theta during movement was spared. On the other hand, in both rabbit and rat under deep ether or urethane anaesthesia only slow theta of about 5 Hz was seen. This slow theta was subsequently abolished by atropine (Vanderwolf, 1975; Kramis et al, 1975).

Observations of an atropine-sensitive theta in the behaving rabbit are relatively unambiguous; long trains of pure, large amplitude theta during immobility have often been reported (Winson, 1972, e.g.) and Kramis et al (1975) demonstrated severe deterioration of this theta after atropine. The outcomes of atropine administration in normal rats are more difficult to interpret, mainly because slow theta, in the rat, is usually ephemeral and of relatively small amplitude; to the naked eye it is rarely as clear as fast theta (see Vanderwolf, 1975, and Fig. 5). Since Vanderwolf (1975) has shown that even fast theta disappears

after atropine if it is not clearly recorded, it is conceivable that the selective elimination of slow theta in the behaving rat is due to its lesser clarity.

Hippocampal EEG: Depth profile analyses. Until recently it was thought that theta recorded throughout the hippocampus was a consequence of a single neuronal generator - the pyramidal cell in CA1. Early mapping studies of theta amplitude and phase in curarized rabbits had demonstrated that as a microelectrode descended through dorsal hippocampus, theta amplitude reached one peak in stratum pyramidale, tapered to a null point in stratum radiatum and then grew to a second peak in the vicinity of the distal portion of the pyramidal cell apical dendrite. The ventral peak was about twice as large as the dorsal one and the two were 180 degrees out of phase with the change in phase occurring abruptly at the null point (Green et al, 1960). Vanderwolf's early studies in normal rats supported these findings: the best theta was recorded in CA1 with electrode tips bracketing s. pyramidale. Electrode tips in the dentate area consistently registered fast activity or theta mixed with fast activity. Similar differences between CA1 and dentate EEG were seen when a moving electrode penetrated the hippocampus of urethane-anaesthetized rats, which presumably emitted what would now be labelled atropine-sensitive theta (Vanderwolf, 1971; Whishaw & Vanderwolf, 1973).

Winson (1974, 1976a, b) has repeated Green et al's depth mapping studies in freely-moving rabbits and rats using movable microelectrodes. In the rabbit depth profiles were similar to those of Green et al with one significant exception: the large ventral peak was

localized to the area of s. moleculare of the buried blade of the dentate area. This profile (called "rabbit" profile here) was identical for all three conditions which gave rise to theta: movement (fast theta), active sleep (slower theta) and alerted immobility (slow theta). A similar profile was plotted by Bland et al (1975) in the urethane-anaesthetized rabbit (slow theta); maximal ventral amplitude was reported to arise in s. moleculare or s. granulosum. These researchers also found that CA1 theta could be completely abolished by various treatments (cooling, surgical) without a loss of dentate theta, while loss of dentate theta resulted in the disappearance of CA1 theta.

Depth profiles in the freely-moving rat differed from the rabbit profile in a number of ways: there was no null point, the 180 degree phase shift was a gradual one, and the maximal dentate peak was ventral to that seen in rabbit- closer to s. granulosum (Winson, 1974). In addition, Winson found that waveforms and amplitude modulations for dorsal and ventral thetas were different. Taken together these data suggested that in both rat and rabbit there were two coupled theta generators, one in the dentate area (the granule cell) and one in CA1 (the pyramidal cell). Both generators were capable of showing either slow or fast theta depending on the animal's state. The frequencies of theta at dorsal and ventral maxima were always the same.

Effects of muscle paralysis on hippocampal EEG. A number of studies have indicated that hippocampal EEG patterns are essentially unchanged during paralysis. Irregular activity is seen if the animal is undisturbed and theta patterns occur spontaneously and during sensory arousal. Transitions between EEG patterns also appear to be normal,

but it is possible that relative proportions of the patterns are changed. Whishaw et al (1976) suggested that arousal patterns, measured by neocortical and hippocampal EEG, are dominant in the paralyzed rat. The frequency of theta is, however, consistently low, ranging between 4 and 7 Hz (Black & Young, 1972; Klemm, 1972; Routtenberg, 1970; Whishaw et al, 1976; Winson, 1974, 1976a). Body temperature, and blood levels of carbon dioxide and oxygen were found to be normal in the paralyzed rats in some of these studies, so it is unlikely that changes in theta were due to unphysiological consequences of paralysis.

More detailed study of effects of gallamine and curare, two paralytic agents, on hippocampal EEG have been done by Whishaw et al (1976). During gallamine paralysis spontaneous theta frequency ranged from 5 to 8 Hz; with curare mean theta frequency was 5.4 Hz (range was not reported so it is difficult to say if frequencies above 7 Hz were present at all). Injection of atropine sulphate in gallamine rats eliminated all theta frequencies below 7 Hz (spontaneous theta as well as theta associated with hypothalamic electrical stimulation). Effects of atropine on spontaneous theta in curarized rats were not reported. The authors did state, however, that theta of 7.6 Hz associated with hypothalamic stimulation in curarized animals was present after injection of atropine in doses of 20 mg/kg. In brief, both slow and, less frequently, fast theta were seen in paralysed rats with faster frequencies (at least those evoked by hypothalamic stimulation) surviving atropine administration.

Besides the tendency of paralysis to reduce theta frequencies,

it also has a significant effect on the theta depth profile. Winson (1976a) has found that the profile in normal rats undergoes a number of changes after curarization: 1. the gradual phase shift in s. radiatum is replaced by a null point and an abrupt phase shift, 2. the ventral peak is displaced dorsally so it is closer to the fissure and 3. the dorsal and ventral thetas become more similar in waveform and amplitude modulation. In other words, the paralysed rat's profile looks more like that of the rabbit.

Winson (1976a) has attempted to determine whether differences in theta profile between normal and paralysed rats were due to direct central effects of curare or to indirect consequences of paralysis. The microelectrode in the curarized rat was moved to the theta null level in s. radiatum and fixed in place. Curarization was discontinued and the time course of movement recovery and reappearance of theta were compared. If the change in profile was a secondary consequence of paralysis, then with movement recovery, theta should show simultaneous recovery. This was not the case. Complete recovery of theta at the null point followed complete movement recovery by about 30 minutes, suggesting that curare was altering the normal profile at least partially through direct central action.

Although hippocampal EEG patterns are relatively normal in paralyzed animals, the possibility of direct central action of curare (or other neuromuscular blocking agents) as well as the presence of a slower theta, also seen in deeply anaesthetized animals, emphasizes the question of the normalcy of information processing in paralysed animals. Although not intending to do so, Black and Young (1972) have

provided what is probably the clearest answer to the question of whether slower theta in paralyzed animals is an indication of disturbances in information processing. During baseline training, these authors established that dogs showed more theta during cue-evoked movement than during cue-evoked immobility. Parallel results were obtained during curare paralysis: the stimulus that had triggered movement in baseline tests was accompanied by more theta than the stimulus associated with immobility. As in other studies, theta during paralysis was reduced in rate. These results indicated that processing of inputs during paralysis was not unlike processing in the normal state and also suggested that the slow theta during paralysis was a function of the same system responsible for the faster thetas during movement. Atropine effects were not assessed.

The present study:

1. Effects of succinylcholine chloride (Anectine) on theta. During paralysis recordings of hippocampal EEG were obtained via two electrodes: the moving hippocampal microelectrode, which sampled activity in different strata of the right hippocampus and the fixed macroelectrode, the tips of which were intended to sample EEG activity in left dorsal hippocampus. The following evaluation of changes in theta with paralysis is based primarily on EEG recorded by the gross electrode.

Clear signs of the gross electrode tip were seen in the histological material of 15 of the 16 rats. Ten of the 15 tips were in the dentate region; five were in CA1. The tip locations are summarized in the two coronal sections of Figure 4, which also notes approximate

amplitudes and frequencies of theta at each site, both before and after paralysis.

EEG prior to Anectine administration, recorded during periods of vigorous exploratory movements by the rat, showed clear theta in sites dorsal to the fissure (Fig. 6). In sites near the dorsal granule cell body layer, a larger amplitude theta was seen, the clarity of which was contingent on the proximity of the tip to the granule cell bodies; tips closest to s. granulosum showed high frequency activity mixed with theta. EEG in rats with tips in the dentate hilus consisted mainly of very high frequency and large amplitude components which in some rats prevented visualization of theta. However, appropriate filtering revealed that theta was present, but masked by high frequency components (Fig. 7).

Paralysis had some obvious effects on theta activity. Reductions in amplitude (modal reduction of 100 μV) as well as in frequency (modal reduction of 2.5 Hz) were common (Fig. 4). The extent of alteration in theta depended both on time elapsed since paralysis induction and the area recorded from. In most rats the largest changes were seen during the first hour of paralysis, with rapid recovery of more normal EEG thereafter. The most extreme change in theta was seen in Rat 20 which showed the largest amplitude of theta before paralysis (about 1.5 mV). After paralysis theta amplitude was reduced by about one mV (Fig. 6). Over the course of the experiment no recovery of theta amplitude was seen at the gross electrode. As Figure 6 shows, sleep associated LIA appeared to be intact at the gross electrode and an obvious theta was recorded by the microelectrode in CA1.

In rats with high frequency components in the EEG, there was a general tendency for those components to drop out for some time after paralysis induction, so that in several cases, theta during paralysis, although slow, was more easily discerned than before paralysis. In time, high frequency components were reinstated, but not to baseline levels. This phenomenon was particularly clear in three rats (28, 32, 33), all with tips in CA4 (Fig. 7).

2. Theta depth profiles. Observed amplitude profiles (null points and phase shifts were not investigated) corresponded closely to those reported in the literature. Clear theta was often seen in neocortex. As the microtip approached the vicinity of the pyramidal layer (where single unit discharges were maximal) theta amplitude was reduced and theta difficult to discern. Further descent into radiatum resulted in an increase in clarity and amplitude of theta in an area devoid of spontaneous discharges. Clean, maximal-amplitude theta was detected at a level corresponding to the fissure, about 200 uM dorsal to the first electrophysiological indications of a concentrated cell area (granule cell layer of the buried dentate blade). As the electrode approached the granule cell bodies, theta became less clear, obscured by high frequency activity which dominated the record right through the hilus. Amplitude decreases and a reappearance of theta were associated with depths corresponding to s. moleculare of the exposed blade.

3. Effects of atropine sulphate on theta during paralysis. In two of the paralysed rats of the main study (26, 33) as well as in four pilot animals (1-4) the effects of atropine sulphate (25 mg/kg) on theta were assessed. A dose of 25 mg/kg of atropine was selected for a number of

reasons. First, Vanderwolf (1975) has found that doses ranging from 25 mg/kg to 150 mg/kg (which are typically considered large doses) all produced similar neocortical EEG-behavior relations. Second, while Vanderwolf (1975) reported no fatalities with doses of 150 mg/kg, in this study two pilot rats administered 50 mg/kg of atropine during paralysis died approximately one hour after atropine administration. No deaths occurred in the 6 rats given 25 mg/kg doses during paralysis. Third, atropine in 25 mg/kg doses produced typical changes in hippocampal EEG. Large amplitude spike and slow waves began to dominate the EEG some 5-10 minutes after injection of atropine; peak effects appeared to be reached at about a 20-30 minute delay.

The effects of atropine on theta are summarized in Figure 8, which shows theta in 4 rats (1, 4, 33, 3) during normal movement (Normal), subsequent to sensory stimulation during paralysis (Paralysed) and subsequent to sensory stimulation during atropine (Para + Atropine). There are a number of points made in this figure:

1. The slow theta in paralysed rats is not abolished by atropine. This is seen in the tracings for rats 1 (top) and 3. In rat 1 frequency of theta during movement (column 1) is 7.5 Hz dropping to about 5.5 to 6.5 Hz in response to puffs of air during paralysis. This frequency falls in Vanderwolf's slow range of theta frequencies. Tracing a) of column 3 shows that 30 minutes after atropine (4 hr. after paralysis onset) a puff of air on the rat is effective in eliciting a short-lived bout of theta of 6.5 Hz.

In rat 3 pre-paralysis rates of about 7 Hz during movement are maintained during paralysis (column 2, a). Cooling the rat by 2 °C

reduces theta frequency to about 6 Hz (column 2, b). As seen in tracings a, b, and c of column 3, blowing on the rat at 30 minutes, 45 minutes and 70 minutes respectively after atropine produces theta of 6 to 6.5 Hz. The maintenance of slow theta rates in response to stimulation was seen in rat 26 as well, not shown in the figure.

2. As seen in the tracings for rats 4 and 33, in which fast theta rates are seen during paralysis, the effect of atropine on these fast theta rates are similar to the effects on slow rates. As with slow theta, bouts of fast theta in the paralysed, atropinized rat are brief.

Taken together, the EEG tracings for different rats indicate that atropine in paralysed rats does not distinguish between slower and faster theta frequencies. This is borne out in within-rat data as well. In rat 3 for example, when core temperature is allowed to rise to normal levels (tracing d - 38.2 ^{o}C) fast theta is seen with external stimulation. In rat 1, theta frequencies are seen to increase with recovery from paralysis (tracing b-d). Atropine effects on the slow frequencies in a) are similar to effects in b) and c). Tracing d shows theta with the rat mobile, 150 minutes after atropine. Tracing 1a, at the bottom of the figure, shows atropine effects in the unparalysed, normal rat 30 min after injection (25 mg/kg). The black lines indicate periods of theta during movement.

4. Irregular EEG patterns in the paralyzed rat. Descriptions of EEG which follow are based on activity recorded at both macro- and micro-electrodes. If theta was not clear at the gross electrode (Rat 20, e.g., Fig. 6), the micropipette was used to monitor changes in EEG.

In most rats, EEG during non-theta segments appeared to be similar to the LIA accompanying immobility or automatic behaviors in normal rats. LIA spikes were seen, but seemed to occur less frequently than during LIA in normal rats. In a minority of rats spontaneous occurrences of sleep-associated LIA were seen (very large amplitude irregular waves mixed with fast spikes and spindles; Figs. 6, 7; Rats 20, 28) usually late in the experiment.

In Figure 6 the post-Anectine EEG activity for rats 25 and 30, prior to sensory stimulation, provides examples of irregular EEG. Note that some of this activity contains slow regularities; the occasional high-frequency spike is seen. Subsequent to sensory arousal clear theta is present. In Rat 25 it is relatively brief, reverting to pre-arousal EEG after about 6 secs; in Rat 30 it is long, persisting beyond the 14 sec segment in the figure. In most instances, since spontaneous regularities were brief and difficult to detect, comparisons of unit activity patterns were made between such spontaneous and sensory-induced EEG patterns. Since sampling times of unit activity often extended beyond the cessation of sensory-induced theta, there was overlap of EEG states within both EEG categories. What was considered irregular EEG contained transient slower regularities, while regular activity contained irregular patterns. The duration of irregular activity during theta states was reduced by introducing a number of different stimuli. Thus, despite what appeared to be an overlap of states within any one condition, it was expected that periods of arousal contained more theta than spontaneous periods. A computer-based autocorrelogram analysis of EEG for a majority of sample runs confirmed this expectation.

Discussion

The different gross EEG amplitudes and patterns observed at different electrode sites prior to paralysis, as well as differences in EEG as a function of microelectrode depth during paralysis, agree well with results of previous studies: largest amplitudes have been reported in the dentate area (Winson, 1974, 1976a, b, e.g.) and fast activity has been associated with the CA4-hilus region (Whishaw et al , 1976). The methods used here were not intended to determine whether Anectine was affecting theta depth profiles. Nevertheless, the changes in Rat 20's gross EEG following paralysis hint at possible Anectine effects on the ventral theta peak, similar to effects of curare reported by Winson (1976a). The location of Rat 20's electrode tip as well as the fact that pre-Anectine theta amplitude was largest in this rat suggest an optimal orientation of the gross electrode with respect to the ventral generator.

If Anectine does alter the rat depth profile, it might be doing so through direct CNS effects. Winson's (1976a) work suggests that the effects of curare on rat depth profiles are mediated centrally. An unexpected finding, in line with the idea of direct action of Anectine on brain, was that fast activity seen at the gross electrode in the hilus or in stratum granulosum prior to Anectine, was severely attenuated during the first few hours of paralysis. This attenuation permitted observation of clear theta which was obscured by fast activity prior to Anectine. If it is assumed that fast EEG activity in the hilus is associated with discharges of neural elements in the area,

then it appears that Anectine acts to reduce firing in these elements either by direct action at cholinergic synapses within the area or by action in remote junctures. Several studies have indicated that there are nicotinic receptors in the dentate hilus and the effects of iontophoretically applied nicotine are antagonized by d-tubocurarine (see Segal, 1978). There is little data available, however, concerning direct effects of succinylcholine; further research is needed to determine if Anectine alters the theta profile and if it does so via its action on cells of CNS.

In agreement with previous studies, theta frequency was reduced in the paralyzed rat when compared to pre-paralysis rates during exploratory movement. An attenuation in theta is not surprising; the conditions surrounding the presence of theta in paralyzed and moving rats are different. The meaning of this reduction in terms of the information processing state of the paralyzed rat, however, is unclear. Black and Young's (1972) studies suggest that reduced frequency does not imply disrupted information processing. They also point to a unitary basis for slow and fast theta frequencies. Vanderwolf's (1975) observations suggest that the slow theta seen here is produced by a system different from the one producing the fast theta and might be associated with a different behavioral disposition. That atropine effects on theta in the paralyzed rats of this study were not dependent on theta frequency indicates clearly that slow theta frequencies were not a function of activity in the slow-theta system described by Vanderwolf. Moreover, the presence of a continuum of different frequencies in different rats as well as within one rat suggests that

it is likely that one system, the same system giving rise to fast theta during movement of the normal rat, is operating in the paralyzed rat. These results do not rule out a two-system view of theta in behaving rats. It is conceivable that the slow, atropine-sensitive theta described by Vanderwolf is correlated only with immobility just prior to an active avoidance response and with specific segments of active sleep (Robinson et al, 1977). Since the paralyzed rat is already immobile, it is unlikely that stimulating the rat (blowing, e.g.) results in an increased tendency toward immobility (associated with slow rates); it is more likely that theta seen in the paralyzed rat is a reflection of activity in a system directly involved in the initiation and maintenance of "voluntary" actions (fast theta system). This system is still active in the paralyzed rat, that is, the rat may "intend" to move, but it's activity cannot bypass the block at the neuromuscular junction.

It is not clear why theta rates in paralyzed rats are slowed. Vanderwolf has shown that theta frequency will vary as a function of the rat's core temperature and it was seen that reduction of core temperature in one rat in this study reduced theta rates. In rats of this study, as well as other work, low theta rates were seen despite normal temperatures. And, it was noted here that theta frequency in the cooled rat would rise spontaneously despite a maintained sub-normal temperature. One possibility is that normal theta requires feedback from muscle movement. Another possibility, also speculative, is that Anectine does act centrally to block a nicotinic modulation of theta frequency.

What seemed to be a relative lack of LIA spiking seen in awake rats as well as a scarcity of sleep associated LIA suggests that the paralyzed rat was relatively alert for much of the experiment, a condition also found in studies of Whishaw et al (1976) and which could lead to a systematic bias in the types of discharge patterns observed. In the majority of cases, theta and non-theta states were easily distinguished in the raw record, and, while in many cases comparisons of discharge patterns during irregular and more regular EEG (to be described in SECTION 3) may not have reflected differences between two radically different states of arousal, they were possibly indicative of differences in brain states (behavioral dispositions) which occur during waking states of the animal.

SECTION TWO. Classification of single cell activity.

Spike trains in hippocampal and septal penetrations gave rise to a variety of different interval histograms and autocorrelogram shapes. An attempt was made to sort the histograms into a few basic categories and to determine whether the different categories tended to cluster in different anatomical loci. The classification process is described in Part A of this section; Part B provides the anatomical breakdown.

Part A. Discharge patterns of cells in the septo-hippocampal complex.

As pointed out in the General Introduction, notions of hippocampal function are intimately bound up with the activity of its projection cells. For this reason, it was important to distinguish between hippocampal cell types and to attempt to determine which types could be considered projection cells.

Septal cells were classified because it was of interest to know if certain cell types were more likely to be affected by activity of hippocampal projection cells. Also, firing patterns of lateral septal cells have not been extensively probed; emphasis has been placed on cells of the medial septal nucleus.

Method.

Basis of classification. Two circumstances are thought to give rise to differences in spike train characteristics. If anatomical factors are constant, differences in discharge sequence parameters are related to

differences in the animal's behavioral state. If physiological states are uniform, different discharge types hinge on anatomical considerations; it is assumed that different cells (not necessarily morphologically different), by virtue of a number of factors extrinsic (connections, e.g.) and intrinsic (membrane properties, e.g.) to the cell, will give rise to different spike train patterns. The latter assumption is at the basis of the classification protocol used here. Differences in firing pattern associated with differences in physiological state are regarded as different operating modes (OM's) or subtypes of the anatomical cell type.

In recording from different cell populations within a circumscribed brain area, since physiological states are usually varying, the problem arises of determining whether different discharge patterns represent different OM's of a specific cell type or the activity of different cell populations. While the problem cannot be resolved with certainty, certain considerations lead to criteria by which to distinguish, at least roughly, between the two possibilities. In some cases characteristics of the discharge itself may help distinguish mode from type (for example, the presence of complex bursts). It is also possible to record from one cell (or one population of cells) in several different states. For instance, McCarley and Hobson (1972) recorded from a clearly identified population of cerebellar Purkinje cells and examined rates and patterns of firing of these cells during different stages of the sleep-waking cycle. One of their findings was that pattern differences, measured by such first order indices as the variance, and third and fourth moments, were a direct function of

differences in rates of firing and secondary to differences in the animal's state. That is, if firing rates were low in any state, then, regardless of state, the pattern of firing was the same. What is important in the present context is that the differences in first-order measure of firing pattern were predictable from the rates of firing. For example, as rates of firing increased, the variance decreased.

Analogous relations between mean rate and first- and second-order measures of pattern were noted in the present study in which activity was recorded in different EEG states from the same cell. The shapes of the histograms associated with different states were generally similar; differences could usually be attributed to rate differences. Examples of this are seen in the autocorrelograms of Figures 29, 31, 32, and 35. These observations guided classification in the following way: if certain features of the histograms obtained from two cells were similar or if differences in the histograms could be predicted from their differences in rate, it was likely that the cells belonged to the same type. On the other hand, if differences in histograms of two cells could not be explained on the basis of mean rate differences or if the histograms were dissimilar regardless of state, the cells were assigned to different types.

Classification procedures. Time interval histograms and autocorrelograms were plotted on an X-Y plotter for each cell under spontaneous (irregular) and aroused (rhythmic or sensoreally altered) EEG conditions. Differences in histogram heights (areas) due to differences in spike counts were compensated for by adjusting ordinate scaling factors. Mean ISI's and coefficients of variation were noted beside the

histograms. A preliminary sorting of cells based on histogram shape was then by eye. A number of histogram features were used to categorize the cell:

TIH: 1. number of modes in TIH, 2. the proportion of intervals at the mode, 3. the location of the mode in time, 4. dispersion of intervals about the mode, 5. the degree and direction of skew, 6. the overall shape of the distribution.

AUTO: 1. the extent of deviation from flatness, 2. location of peaks and troughs, 3. rhythmicity of peaks and troughs.

To check the accuracy of classification, ten second segments of film of the original spike record were grouped according to type and compared to each other. Histograms and statistics for cells showing atypical records were re-examined and re-classified if an obvious sorting error had occurred. For other atypical cells, the entire segment of tape was reprocessed for the spike pair and if necessary the cell was re-classified. Characteristics of the raw discharge (complex spiking) were used as aids only in the classification of hippocampal cell types.

A majority of cells, irrespective of EEG state, gave rise to histograms that clearly belonged to different types. For other cells, however, also during both EEG conditions, the histogram differences for different cells seemed to reflect differences in OM's of a single cell type: that is, it was possible to attribute the histogram differences to rate differences. The separate status of these histograms was maintained by considering them modes or subtypes of a single broader classification. Further clarification of the distinction between a

cell type and subtype will be found in the descriptions of the different categories.

Results.

Of a total of 343 cells recorded on magnetic tape, 99 were not processed for reasons already outlined (see General Methods, p. 61). The data discussed here are based on the activity of 244 units, most of which were recorded in two's (about 166 pairs) and most of which were located in septo-hippocampal areas. The number of cells detected in each animal ranged from four to thirty; 15 cells were seen on the average. While the duration of a sample run was usually long (between several minutes to over an hour), the period selected for computer processing during either EEG state was relatively brief. The duration of the EEG state, an upper limit of 1021 spikes, and rate of firing interacted to reduce processing time to between approximately 20 seconds and 5 minutes. These factors also influenced the number of spikes counted (n) for any one of a pair of spike trains (range of about 20 - 950).

Since the upper limit of 1021 events represented the sum of counts in a two cell record, processing time and frequency count for any one channel were, of course, less than if a single channel were processed. The bias produced by this factor was greatest when one cell of the pair was rapidly firing and the other firing relatively slowly, with the slow cell statistics distorted to a greater extent. The effect of this constraining factor was attenuated, however, by the finding that increasing processing time and n by analysing one channel

at a time, for example, did not affect the overall shape of the histograms for either channel; relatively small n's and brief processing times were sufficient to characterize the probability density and autocorrelation function estimates. For a given firing mode increasing n did not produce marked changes in histogram shapes. The major effect was a smoothing of curves similar to that seen when the time resolution was effectively decreased by summing bins. Thus, although definitive interpretations of histogram irregularities were inappropriate when n's were small, it was reasonable nevertheless to use the histogram to assign the discharge pattern to a particular type or subtype. The top two histogram pairs of Figure 9 (Type A), the middle two histogram pairs of Figure 33 (Subtype C1) and the top three histogram pairs of Figure 15 (Subtype C6) are comparisons of histograms with small versus large n's (different cells) and attest to the apparent temporal constancy of discharges for three major cell firing modes (discussed below). Note that even for the smallest n's discrimination between firing sequences is straightforward.

Of the 244 cells, 12 could not be classified. The remaining cells were sorted into seven types, three of which, A, B, and C encompassed about 75% of all cells. Type B was found in relatively large numbers in both hippocampal and septal penetrations. Type A consisted of hippocampal cells, while C was dominant in septal penetrations.

In the following characterizations of the different firing modes a number of terms are included which have been used to refer to some dominant features of spike sequences. The term burst refers to a

relatively isolated brief and rapid sequence of spikes (from 2-10 action potentials in a burst with an ISI within the burst of 1-15 msec). Non-bursting action potentials are referred to here as single spikes. The occurrence of both bursting and single spikes can be regular (rhythmic) or irregular. The more regular the discharge sequence, the more constant is the interevent interval. The terms slow, moderate, and fast refer to the rate of occurrence of single or bursting spikes. Slow discharges are usually two events per second or less. Rates of 3 to 6 events per second are moderate, while fast spikes exceed a rate of 7 per second. Finally, the burst may be simple or complex. A simple burst is one in which the constituent spikes are of equal amplitude. In a complex burst successive spikes are of different amplitudes, usually decreasing.

Most of these characteristics are reflected in the shapes of histograms as well as in first-order statistics. The dominance of bursts, for example, is indicated by a prominent narrow peak at about zero time in both the TIH and AUTO since the majority of intervals are the very brief ones that constitute the burst. If the bursts are regular, then the TIH will have two peaks, one near zero, the second about a time that is equal to the interburst interval. Also, the AUTO will consist of a series of regularly recurring peaks, with the width of the peaks proportional to twice the burst duration. The coefficient of variation (CV) will be relatively high in burst sequences; the more irregular the bursts and the longer the interevent intervals, the higher the CV and the more irregular the AUTO. At the other extreme are those sequences in which single spikes are separated by constant

intervals. Here the CV will be at a minimum; the AUTO will consist of a series of regularly recurring narrow peaks.

Discharge patterns: Hippocampal penetrations. The following descriptions and figures are of two basic cell types observed in hippocampal penetrations, types A and B. Type A does not consist of subtypes. Type B includes two subtypes, B1 and B2.

The distribution of types and subtypes in each rat is shown in Table 2. The cell types, described below, were not always represented equally among the rats tested. Type B was distributed fairly evenly across rats, while about 40% of Type A cells were found in two rats, with the remaining 60% of A cells evenly apportioned.

Type A. Type A cells, slow, irregular, and with the occasional complex discharge form, corresponded to Ranck's (1973) complex-spike cell (Fig. 10). In most trains some of the bursts were complex, an important defining feature of this type (Figure 10). Not all cells classified as Type A, however, showed the complex burst pattern. A single, very slowly discharging, large amplitude spike was included in the A category. Because the behavior of this cell was similar to other complex-spike cells during EEG and because Ranck (1973) has reported that most of these cell types eventually show the complex form, this cell was categorized an A cell. It is possible, however, that this spike is generated by a population of hippocampal cells different from the population of complex-spike cells.

Events in the A sequence were usually spaced irregularly, particularly when firing rates were low. With increases in rate, brief periods of regular firing at theta rates were seen in some of these

cells (Figures 9 and 10-5). As in Ranck's work, the A cell was usually the largest amplitude cell in the record, difficult to isolate because of its varying amplitude and consequently difficult to process by computer. Statistics and histograms for this cell type should be considered approximations. Figure 10 includes several types of records that gave rise to Type A histograms. The behavior of A cells will be discussed in more detail in Section 3, which examines their activity in relation to EEG.

Type B. The B1 subtype was characterized by fast, small-amplitude, simple spikes and appeared to correspond to the "theta" cell of Ranck (1973). In hippocampal penetrations it was usually the smallest amplitude spike in a record consisting of spikes of several amplitudes (Figure 12-1); to process the B1 events required electronic subtraction of the larger amplitude spikes. Classifying the B1 cell appeared to be a relatively straightforward procedure. With the onset of theta rhythm in the EEG the B1 cells began to burst rhythmically at theta frequencies for relatively prolonged periods of time; their rhythmic patterns were easily identified at the loudspeaker. At the same time firing of A cells tended to subside (see Figures 11 and 12).

Cells of the B2 subtype fired more slowly and irregularly than subtype B1; they were also the smallest of several spikes in the hippocampal record. They were considered a B subtype because their histograms during all EEG states resembled those representing firing modes of the B cell during irregular EEG. Weak rhythmic bursting was seen in some of the cells (see Figs. 11, 12).

Discharge patterns: Septal penetrations. Septal cells fell into two dominant categories, B and C. Type C included six subtypes, C1 to C6.

Table 3 shows the distribution of septal types and subtypes of firing for each rat and indicates that the two dominant categories and their subtypes did not tend to cluster in any one rat. There were an inordinately large number of C6 patterns in rat 25 which might be attributed to anatomical factors. Rat 20 was unusual in that a majority of Types A and G cells were found in this rat.

Type B. The discharge characteristics of septal B1 cells were similar to those of hippocampal B1 cells. There was a tendency for septal cells to be slightly faster than hippocampal units, but patterns of firing, which have been described by many workers, were basically the same (Figs. 11, 12).

Type C. While Types A and B were characterized by a dominance of complex or simple bursts and a corresponding initial peak in the AUTO, the C spike sequences were distinguished by a relative paucity of rapid isolated bursting patterns. The cells fired mainly single spikes and in most cases AUTO's were marked by a brief, initial trough, indicative of the low firing probability at short intervals. AUTO's representing C sequences, aside from the initial trough, were not flat; in all subtypes there were, in some proportion of histograms, indications of rhythmic firing ranging from a rapid "pacemaker" regularity through a slower, theta-like rhythm to a still slower one Hz cyclicity. There was a tendency for faster cells to show more rapid regularities, although suggestions of slower rhythms were seen in histograms associated with rapidly firing cells.

The 84 cells subsumed under the broad C category comprised close to 60% of cells seen in septal penetrations. They were sorted into six subtypes. Distinguishing most of these subtypes from the A and B types was relatively straightforward; the distinctions between certain subtypes were not. Most cells assigned to a particular subtype retained their respective patterns across EEG states, a primary reason for maintaining their separate status. Occasional transitions from one subtype to another within a cell supported a grouping of these subtypes. It should be kept in mind, however, that some of these subtypes may represent, for reasons to be stated, the activity of different cell types.

Subtype C1.

The C1 sequence consisted of fast, fairly regular single spikes (Fig. 16-2). The initial AUTO depression was brief, lasting about 20 to 50 msec. The rest of the AUTO was relatively flat, except for pacemaker-like peaks and the occasional slow rhythm (Figure 13). The corresponding TIH's were bi- or multi-modal reflecting the presence of the nested rhythms.

Subtype C2.

The C2 subtype consisted of cells slower in rate than those of C1 or C3; n's were smaller and histograms more uneven, making the C2 histogram difficult to classify (see Figs. 13, 16). The initial trough characteristic of the C type was clearly present. The C2 cell tended to show inconsistency in histogram shape across EEG states. In some cases, when AUTO's were relatively flat, they overlapped with C1 or C3 histograms; when uneven, they were more closely allied to C4 or C5

subtypes. The proportionately larger CV's of C2 sequences suggested they were slow variants of the C1 subtype.

Subtype C3.

Spikes in the C3 sequence were more rapid than those of C1 or C2 (Fig. 16). The C3 AUTO was generally flatter, the initial trough was reduced or absent; pacemaker regularities were seen as well as hints of slower rhythms (Fig. 13). In some cases short, narrow initial peaks were seen, indicative of elevated occurrences of brief ISI's or bursts, particularly during spontaneous EEG states. Although histogram differences between C1 and C3 modes could be attributed to rate differences and possibly differences in n, the possibility that C3 was a different cell type was suggested by its relatively large CV, despite increased rates of firing.

Subtypes C4/C5.

The C4/C5 histograms (Figure 14) were the most difficult of C patterns to sort and may, in fact, represent a different cell type. The C4 cell discharged at moderate rates, similar to those of C2 (Fig. 16-4), but unlike C1, C2 or C3 cells, a type of bursting was common as indicated by an early, fairly steep peak in the AUTO. The C4 AUTO's were also marked, more than any other C subtype by peaks and troughs. During arousal EEG the peaks, rough and broad, indicated a weak and variable periodicity at a theta frequency. These characteristics linked the C4 pattern to the B firing type. A number of features distinguished C4 bursting from B type rhythmic bursting, however, the most important of which was that the initial peak in C4's AUTO appeared to arise following a brief initial trough. This was rarely seen in B

cells and reflected a refractoriness seen in most C discharges. Variable degrees of weak rhythmic bursting were seen in all C cells. Interspike intervals within the C burst were relatively long and variable; interburst intervals were also variable.

The C5 histogram appeared to represent a slower version of the C4 cell (Figure 14).

Subtype C6.

The C6 sequence, consisting of slow, irregularly spaced single spikes, was the dominant C subtype, occurring in 37% of all C cells (see Fig. 16). Its histograms, particularly the TIH, made it an easily discriminable subtype, regardless of n (Fig. 15). As with other C subtypes TIH's were multi-modal, but unlike most patterns the dominant modal peak in the TIH was not at zero and a relatively small proportion of intervals was found at the mode. The AUTO was characterized by an initial, relatively long depression indicative of low firing probabilities at the shorter intervals (50-100 msec) characteristic of C cells. Unlike C1 or C3 patterns, however, the initial depression was followed by a gradually rising, broad, rounded peak which appeared to taper off either to constant probability levels or to a second trough. These features suggested the presence of a slow rhythmicity in C6 cells, a feature hard to detect in the raw record of slowly discharging cells, although Figure 16-7 shows the oscillation quite clearly. To corroborate the observation several C6 sequences were reprocessed at a time resolution of 8.0 msec (ten times that used in other histograms). The outcome of this analysis is shown at the bottom of Figure 15. The late modes in the TIH and recurrent humps in the AUTO substantiated the

presence of a slow oscillation with a period of about 1.2 sec. Since respiration period was about 800 msec, it is unlikely that this periodicity was due to respiration artifact. Other periodicities, imbedded in the 1 Hz rhythm, were also seen.

Several factors pointed to the C6 pattern representing a very slow variant of the C1-C2-C3 continuum. The difference of the C6 TIH shape from that of C1 can be attributed to C6's slower rate; the longer initial trough might also be a consequence of the slower rate (which might in fact be due to the longer refractory period). Also, there were several fairly clear instances of a single cell showing transition from the C6 to the C2 or C3 subtype, although in the majority of cases the C6 cell retained its pattern across states. As with other C subtypes a number of rhythms were nested in the slower rhythm. Other considerations indicated that C6 was a separate type, however. Evidence for slow periodicities in other C autocorrelograms were not as evident and, while rates of C6 firing were about three times as slow as those of C1, the CV was about the same.

Four other types of discharge patterns (D,E,F,G) were observed in both hippocampal and septal penetrations comprising in all about 17% of the cells processed. These will be briefly described.

Type D discharges, seen mainly in septal penetrations, were very rapid, firing in single spikes with occasional theta-type bursting; it was conceivable that this firing mode represented the fast extreme of the B1 subtype. This type was not considered a B subtype, however, because B histograms of any state did not resemble the D histogram and vice-versa.

The Type E sequence was recorded primarily in neocortex. It consisted of fast, very regular, single spikes easily identified in raw records and histograms. Because of the constancy of its discharge characteristics within and between states, the E cell was often not recorded and is therefore underrepresented.

The F cell, distributed equally between hippocampus and septum, was a moderate cell with a relatively large CV. Some of its histograms were similar to those of C1 or C2. A fairly strong regularity with a period of about 800 msec, distinguished the F type from other subtypes. Since respiration was set at 70 strokes per minute, respiration artifact may have been the source of the pattern.

About one-half of Type G cells were seen in the septal pass of one rat (#20); histograms were very similar to C6 subtypes. A prominent, very narrow peak at zero in the G histograms reflected the presence of a number of isolated bursts.

Part B. Anatomical location of different cell types.

The aim here was to determine if the different electrophysiologically defined cell types tended to cluster in different portions of the septo-hippocampal complex.

Method.

Histological procedures necessary to prepare microscope slides of stained brain sections have been briefly described in the General Methods. Slides containing sections with critical portions of the microelectrode track were placed on an enlarging projector and the

magnified image traced on paper. The tracing provided a good estimate of the anterior-posterior and medial-lateral position of the electrode. For several reasons the tip of the track could not be considered an adequate reference point for the determination of the dorsal-ventral position of different cells encountered along the track. In some cases more than one penetration per site resulted in the appearance of several closely adjacent tracks; in other cases the track was seen to pass through the base of the brain. In still other instances the coronal plane section was not perfectly parallel to the track which meant that the tip of the ending may have been overlooked, and, in general, the duration of storage of brains in formalin was variable leading to a variability in shrinkage.

To provide a more accurate determination of tip depths it was necessary to supplement the anatomical findings with electrophysiological observation. The following criteria, taken together, helped established a number of reference levels: 1. the appearance of the fast record, 2. the fast activity depth profile, 3. the theta wave depth profile (hippocampal penetrations), 4. the presence of responses to electrical stimulation of contralateral hippocampus, and 5. the tip of the track. The reference levels were noted on the tracings and in conjunction with the micromanipulator depth readings were used to compute the position of each cell.

Results.

Location of cells in hippocampal penetrations.

Brain sections at the level of the hippocampus were examined

for 15 of the 16 rats. In eight rats with more anterior placements there were two separate hippocampal probes, medial and lateral; in seven rats with more posterior penetrations, there was one probe per rat. Histological evidence for 22 of the 23 tracks left by the probes was found and Figure 17 shows representative anterior and posterior appearances of these tracks for four rats. Section D of Figure 17 provides an instance of a track which required high power magnification to be seen. That the faint line seen in section D was in fact a portion of the microelectrode track was substantiated by its appearance on adjacent sections and by indications of the track entry point. The unlocated track was for a rat with a single posterior probe. The positions of the electrode tip were estimated from the electrophysiological signature which closely resembled that of a posterior probe in a different rat. In almost all cases some portion of the track was seen in the hippocampal complex. In two rats the laterally aimed electrode passed through fimbria. In one rat only neocortical portions of the track were seen.

In Figure 18 the results of estimation of the loci of 91 of a total of 96 cells of hippocampal penetrations are summarized. The depth of cells in more posterior hippocampal penetrations were more difficult to reconstruct than the anterior ones because depth profiles were more complex and not as clear; the thinness of corpus callosum in posterior lateral brain created some uncertainty about the neocortical versus the hippocampal locus of a dorsal cell. Cells in the dentate hilus were also difficult to localize with precision. As the figure shows the large majority of cells were situated in pyramidal and

granule cell body layers of the hippocampus (.88). It was not possible with the methods used here to resolve differences in location of A and B cells as indicated in the work of Fox and Ranck (1975). All subareas of Ammon's horn were sampled (CA1, 2, 3, 4) in dorsal and/or ventral hippocampus. The largest proportion of cells were located in dorsal anterior CA1.

Location of cells in septal penetrations.

Brain sections at the level of the septum were examined for 15 rats and septal portions of the microelectrode track were found for each rat. Photomicrographs of four representative sections in four different rats can be seen in Figure 19. The outcomes of the estimation of the location of 144 of 148 cells encountered in septal probes are schematically summarized in Figure 20. A substantial proportion of cells (.88) were found in the lateral and medial regions of the precommissural septum. The remaining cells were found in the anterior continuation of the hippocampus (HIA), cingulate cortex, and in the accumbens area. Cells located in the LSN were found mainly in the dorsomedial portions.

Relation of cell type to anatomical locus.

A rough idea of the general distribution of different cell types can be gained from Figures 18 and 20. Table 4 furnishes a numerical breakdown of the anatomical locations of all cell types and subtypes. As previously noted, and as is made clear from these summaries, hippocampal cells fell into two main categories: A and B, while B

and C modes were dominant in the septum. Those C modes found in hippocampus were usually recorded from posterior portions. In septal penetrations Type B cells were heavily concentrated in the medial septal nucleus (MSN). That Type D, which showed occasional weak bursting, was also found primarily in the MSN, supports its association with the B cell type. Subtypes of C were generated by cells located mainly in the lateral septal nucleus (LSN). Figure 20 indicates a rostro-caudal segregation of C firing patterns seen more clearly in Figure 21, which compares relative proportions of different C mode cells in two rostro-caudal planes. As the figure shows C4, C5, and C6 subtypes were dominant caudally. Those C6 cells that appeared in the more anterior sections (3.0 to 3.4) were found mainly in the anterior continuation of the hippocampus. Subtypes C1 and C2, on the other hand, were more likely to be seen by an anterior electrode, which supports the assumption that a common cell type gave rise to both subtypes. There is also a suggestion in Figure 20 that C2, C3 occur in more lateral areas, while C4 and C5 tend to be found along the LSN midline.

Discussion.

Discharges of cells in the septo-hippocampal system were sorted into three major categories, partially on the basis of discharge form and on the basis of pattern. The distinguishing characteristics of Types A and B were relatively easy to discern in the raw record; histograms confirmed visual and auditory impressions. These types, in hippocampal penetrations, corresponded in many respects to Ranck's

(1973) "complex spike" and "theta" cells: amplitudes of the A cells were generally larger than those of B cells, the B cell rates were faster, and both types were found in the vicinity of the pyramidal and granule cell layers. Although few cells were recorded in the dentate area, the majority of those seen were B types, more clearly isolated than the B cells of Ammon's horn, a finding also consistent with Ranck's data. Both A and B cells were also seen in the postero-ventral hippocampus in the present study, a finding which provides an anatomical extension of Ranck's findings. In addition, those C patterns that were seen in hippocampal penetrations were primarily associated with cells in posterior hippocampus.

The similarities between the present observations and those of Ranck, made in the chronic, behaving animal, is some indication of the normalcy of the paralyzed preparation used here. More importantly, the electrophysiological identification of two different cell types suggests, as does Ranck's work, that they originate in two different populations of cells in the vicinity of the pyramidal cell layer: the slower, larger-amplitude complex spike may be associated with the hippocampal projection cell, while the smaller B spike may be generated by the basket cell, an interneuron found in proximity to the pyramidal cell. It should be kept in mind that the criteria used here for distinguishing between cell types were not stringent; behavioral correlates of the cell were, of course, not available. It is conceivable, even likely, that some proportion of A cells were identified as B cells and vice-versa. Considering that many of the B cells were the smaller cell in the record, it is more likely that A cells were mistaken for B cells.

The sorting of cells into a third major category, C, was considerably more difficult to achieve. The discharge form was not unique, patterns of firing were not easily recognized or distinguished, either visually or aurally, and in some cases, irregular trigger levels may also have contributed to the diversity in C cell types. The C category, seen mainly in septal penetrations, was discerned primarily on the basis of histogram shape. Its dominant characteristic was an initial trough in the AUTO of variable duration, signifying a lack of the type of bursting seen in A and B cells.

The six different C subtypes described here may represent activity of different cell types. Subtypes C1, C2, and C3 shared a number of electrophysiological characteristics which suggested their origin in a common cell type. The anatomical finding supported a grouping of C1 and C2 cells which were found primarily in the most anterior and lateral portions of the septum sampled here. The C3 discharge pattern was seen more posteriorly, with some cells situated in the medial septal nucleus. The C4 and C5 firing subtypes arose primarily from cells lying in more posterior, midline portions of the lateral septal area. These cells showed the greatest amounts of a theta-type regularization of discharges and may constitute a different cell type. While the C6 subtype was found throughout the lateral septal area, there appeared to be a greater likelihood of its occurrence in more posterior septum. In the most anterior portions of septum it was concentrated in an area corresponding to the anterior continuation of the hippocampus.

It is difficult to compare findings of C cell firing patterns to those of other authors. Most workers have focussed on B firing modes in the septum and their relation to the hippocampal theta rhythm. Descriptions of firing patterns in the lateral septum have been brief and non-quantitative. Most have stated that lateral septal units do not discharge rhythmically (McLennan & Miller, 1974; Wilson, Motter, & Lindsley, 1976). Ranck has described lateral septal unit discharges in relation to behavior and mentioned that four neurons (out of 65) gave some hint of a theta rhythm, all of which were located at the level of the fornix (Ranck, 1973). These findings support the present ones in which cells showing the most obvious rhythmicity of discharges were situated more posteriorly (subtypes C4, C5). In a later paper (Ranck, 1976) the discharge characteristics of LSN cells during behavior of the rat were described in more detail. It is clear that the cells called "approach-orient" cells by Ranck, which were the dominant cell type in the dorsal LSN, correspond closely to the C6 subtype of this study. Ranck described these cells as slowly firing cells in which the overall rate of firing did not change appreciably as a function of behavior, although the cells did show transient increases corresponding to certain changes in waking behavior. As in most other studies, however, the patterns of firing of these cells were described as irregular. In the present study AUTO's revealed that all cell types in the LSN fire rhythmically at some time; their rhythmicity would not have been detected by inspection of the raw record alone. Autocorrelograms, necessary to disclose it, made it clear that cells throughout the lateral septum are capable of firing rhythmically, albeit weakly and

variably. That some lateral septal cell types can discharge in theta rhythm supports an idea of McLennan and Miller's (1974) that rhythmic activity of lateral septal cells may contribute, via their projection to the medial septal nucleus, to the rhythmicity of medial cells. More will be said on this point in Section 4, Part B.

The empirically obtained proportions of occurrence of the different discharge types and subtypes within a given brain area very likely were not an accurate estimate of actual proportions of cell populations giving rise to the different patterns. There are a number of sources of sampling error inherent in microelectrode recording techniques which have already been discussed. Also, since the overriding aim of this study was not a classification of cells, sampling was selective. Type E, for example, was often not recorded. On the other hand, hippocampal B cells were almost certainly overrepresented. While proportions of types within a brain area are misrepresented here, however, it is still possible to use the data to describe differences in firing patterns as a function of different anatomical loci. Thus, while the proportion of C cells in septal passes probably was not accurately reflected in the data, the fact that C cells were more likely to be seen in septal penetrations, is very likely a reflection of their greater occurrence in the septum.

The finding that different cells (presumably of the same type) showed similar discharge patterns during brief or relatively longer time periods and that a single cell retained its discharge pattern in time, indicated the presence of stationarity in discharge sequences during sample runs. These findings suggest that observations of

different discharge types were not a function of departures from stationarity. The presence of stationarity was indicated by a number of other factors and observations. Sample runs with large deviations from stationarity, usually detected by aural monitoring of spikes, and which seemed to be due to electrode movement, were rejected for analysis. Loose criteria of stationarity were fulfilled by sampling unit activity during relatively constant EEG states. When recording from the same hippocampal cell over a large number of sample runs, pattern changes between runs were negligible, although rates did change. The same was true for septal cells recorded early and late in an experiment after a number of intervening runs. Also, differences in C subtypes were associated with rostro-caudal and possibly medial-lateral differences in electrode tips positions. If the differences were due to departures from stationarity, it would be expected that type differences would relate to a dorsal-ventral dimension. Ranck (1976) has found differences in lateral septal cell types which corresponded closely to anatomical findings of differences in cell types between dorsal and ventral portions of the LSN (see Fig. 2B). These differences were not seen here for the simple reason that cells in more ventral portions of the LSN were sampled only rarely. In most cases, the electrode was in midline portions of the LSN and as it moved ventrally, it infringed on cells of the MSN.

That anatomically distinct cell types gave rise to different signatures in the AUTO and that regardless of state a given cell type retained its essential characteristics in the AUTO has also been noted by Wilson et al (1977) and has led these workers to propose that

"statistical analysis of neuronal firing patterns may prove to be a powerful tool in the neurophysiological identification of functional cell populations".

SECTION THREE. Unit activity and EEG.

In Section One hippocampal EEG patterns were described, while in Section Two an analysis of cell types in the hippocampo-septal circuit was provided. The present section considers the nature of cell activity during EEG rhythms. Of particular interest is the hippocampal A cell, since its activity furnishes a direct indication of hippocampal output. The methods of monitoring unit activity and EEG have been discussed in the General Methods.

Results.

As noted in Section Two, it was possible to distinguish between two basic hippocampal cell types: Type A, which corresponded closely to Ranck's complex-spike cell and Type B, whose activity resembled that of Ranck's theta cell. The activity of B cells, relatively easy to characterize, will be considered first.

B cells. In most cases, during irregular EEG, B cells discharged in simple, irregular bursts. With the appearance of theta (spontaneous or induced), rate of discharging increased and bursts became rhythmic, apparently time-locked to the theta waves. Figures 22 and 23 (cells 20-12A, 26-1, 27-7) show changes in theta cell activity in a transition from LIA to stimulation-induced theta. Median firing rate during non-theta was about 10 spikes/sec rising to about 13 spikes/sec during theta. The tendency for B cells to fire most vigorously during the clearest periods of theta, usually during initial post-induction periods of theta, is detected in Figure 22 (cell 20-12A).

A cells. Discharge characteristics of A cells have been described in Section Two. In most cases A cells were large amplitude, slowly discharging cells, which occasionally burst in complex fashion. For some cells sampling times were brief and complex spikes were not seen; slow rates of firing, patterns of firing, and large amplitudes suggested their identity with p. cells.

The behavior of A cells during non-theta was relatively straightforward. As with B cells, discharges were irregularly spaced, although rates were considerably slower than B cell rates. The form of the discharge was either single, complex, or a non-complex burst consisting of spikes of several amplitudes irregularly intermixed ("mixed" burst). All three types of events could occur during different segments of LIA at the same electrode tip. While interevent intervals during LIA were rarely constant, discharging was sustained and homogenously distributed. The mixed burst was often seen during LIA; the occurrence of this burst in many cases was simultaneous with large amplitude LIA spikes (see Figs. 10, 23, 26).

During theta, A cell activity became more difficult to characterize. As in studies of Green et al (1960) and Ranck (1973), there was generally speaking an inhomogeneity of firing during what appeared to be a uniform period of theta. Despite this variability, the recurrence of a number of phenomena suggested some systematic processes at work. The activity of A cells appeared to be contingent on two broad factors: the state of the EEG just prior to a transition to clear theta and the time since the transition originated. If EEG patterns just prior to a spontaneous or, more often, to a sensory-induced change to

theta were not far different from those appearing during the clear theta segment, then unit activity changes were also not great (Fig. 24, cell 31-10). When the theta segment was clearly distinguishable from the preceding EEG pattern, then distinct changes in unit activity were also noted. In such cases the temporal distribution of unit events in theta depended on the time since theta initiation. In most instances, the immediate correlate of a spontaneous or stimulus-induced change was a total suppression of A firing. Discharges of B cells were occasionally observed during this initial period (Figs. 22, 23). After a variable time period recovery of A cell firing was seen with theta still visible in the EEG (Fig. 25, cell 20-3). In some cases there was total suppression until LIA reappeared in the EEG. The first cells to recover were usually simple or complex. Mixed bursts were suppressed throughout theta, recovering only when LIA spikes were reinstated (Fig. 23). During those periods when A cells discharged during theta, median rates were generally higher than rates during LIA.

Patterns of A cell firing during theta could be regular or irregular. In Figure 22 (cell 20-12) an A cell is seen firing during theta, in phase with the negative portion of the theta wave, although not firing on every wave. Figure 26 (cell 26-2) shows clearly rhythmic discharges of another A cell this time in the recovery period following sensory input. Note that the onset of the long rhythmic barrage appears to be associated roughly with a deterioration of theta. Details of the firing of another complex cell in a post-sensory recovery phase are shown in Figure 27-1 (cell 27-7). Here it is seen that as theta slows, small amplitude complex cells and simple cells begin to

discharge; with further deterioration of theta, other complex cells of different amplitudes are recruited, firing in semi-regular fashion, without time-locking to the theta wave. Additional examples of the tendency for A cells to discharge as theta clarity diminishes are seen in Figures 24 and 25.

The presence of different amplitudes at different instants during theta was a common observation (see Fig. 27). It should be remembered that pyramidal cell bodies are packed together extremely tightly, probably more so than in any other brain area. The probability of seeing many cells at one tip is consequently high and, while on the one hand it is a source of noise, it also provides important information about the timing of firing of adjacent cells in different states.

One more impression is worth mentioning. It seemed that in the majority of cases where A cells could be clearly distinguished, a decline in A rate during theta was observed. In those cases where it was difficult to evaluate if A or B cells or both were at the electrode tip, then an increase in firing during theta was more likely. This observation is mentioned here because there are several examples in the published literature which are interpreted as an increase in putative complex-spike cell firing during behavior and associated theta, but which show two or three cells at the tip, one of which might be a B cell.

C cells. The discharges of C cells did not show the clear changes in rate or pattern seen in A or B cells, even when EEG states were strikingly different. There was, however, a clear tendency for slowly

discharging cells (C5, C6) to decrease in rate while more rapid cells increased in rate in the transition from irregular to regular EEG patterns. This trend was true for hippocampal cells as well: A cells decreased in rate, while B cells increased during theta.

Discussion.

The results with respect to activity of B cells during theta and non-theta states agree with those of Ranck as well as other researchers who in some descriptions of hippocampal cell activity have probably referred to the B cell or theta cell. However, in Ranck's work rates of dorsal hippocampal theta cells during irregular and regular EEG were substantially faster than those seen here. There are at least three reasons for this discrepancy. One is probably minor and relates to differences in spike counting methods, visual in Ranck, electronic here. A second, more important reason is related to the observation that slow-wave theta frequency is reduced in the paralyzed rat. It follows that discharge rates of theta cells will also be slower. A third reason has to do with factors related to homogeneity of discharge rates and patterns during the particular time segment in which spikes are counted. It appears from Ranck's descriptions that spikes were counted during relatively brief periods of uniform behavior/EEG states. In the present study the period during which spikes were counted was guided mainly by EEG and contained more variability in B cell activity.

With respect to the activity of complex-spike cells (or A cells here) the present study agrees with a number of salient observations made by Ranck (1973):

1. Transitions to states of "unaroused motionlessness" (non-theta states) such as quiet waking (standing or lying), drowsiness, light or deep slow wave sleep were associated with increases in firing of complex spike cells sustained homogenously over the period of motionlessness. In this study transitions from theta to non-theta states were accompanied by increases in firing of A cells, maintained evenly over the period of irregular EEG.

2. Interruptions of a motionless state, either spontaneously or through sensory disturbance, were correlated with a decrease in complex-spike cell activity. In this study spontaneous and induced transitions from LIA to theta were accompanied by cessation of A cell activity. It was also noted here that if the rat was in an aroused state (theta), then further arousal did not significantly alter A cell activity. Similar findings have been reported by Lidsky et al (1974) and by Mays and Best (1975).

3. In Ranck's work the same cell that showed sustained homogenous firing patterns during the course of some type of motionless behavior, while declining in rate when the rat was aroused and remaining quiescent during most aspects of waking behavior, would increase in rate when the rat was engaged in a specific type of activity (the behavioral correlate of the cell). Of course, it is impossible to compare present findings to these, but it was noted here that A cells, while inactive during some portions of theta, were active during other segments.

The mixed burst seen here requires some discussion. Its appearance has not been reported extensively, although there are

suggestions of its appearance in other work (O'Keefe & Nadel, 1978, pp. 150-152). That similar phenomena have only been rarely reported can be attributed to at least three reasons. First, others may have neglected to report the phenomenon in the belief that it represented an injury-induced discharge. It seems unlikely that this is the case because it could occasionally be seen as a very small amplitude background event during the LIA spike, while the electrode was registering events of larger amplitude, such as complex spikes. Further, the appearance of mixed bursts was contingent on the EEG state. Second, it is possible that mixed bursts are unique to paralyzed animals. That this is not the case is indicated by its consistent occurrence in several chronic animals tested here. The occurrence of mixed bursts was also associated with LIA in these animals and began to appear when the rat assumed a quiet sitting or awake, lying posture. A third possibility is that others have simply not reported these phenomena believing them to be symptomatic of poor isolation or noise. In fact, the mixed bursts do appear to be noisy and initially, they were diregarded here. However, repeated observation led to the conclusion that such events in an area of tightly packed cells, while noisy, were also informative, particularly since the "noise" was correlated with EEG state. The mixed burst suggested that adjacent cells in s. pyramidale were firing together during LIA, particularly during the LIA spike. During theta different amplitudes were seen at the microelectrode tip at different instants in time, indicating desynchrony of discharge.

The appearance of A cell activity during strong theta, weaker theta, and irregular activity in this and other studies suggests some notions about how A cell activity might be organized during these states. These notions relate to the factors which set the probability of a single A cell firing as well as the probability that a number of adjacent A cells will discharge in concert or separately.

During strong theta the probability of A cell discharge appears to be quite low. It is likely that the source of inhibition is the B cell which tends to discharge at highest rates during theta and it is possible that the B cell is driven during theta by the rhythmically bursting cells of the medial septal nucleus. It has been reported (Ranck, 1973) that during high-frequency theta hippocampal B cells discharge at their highest rates, occasionally losing their rhythmic bursts and firing in a continuous stream, suggesting uninterrupted inhibition on A cells.

The periods of p. cell firing during select portions of theta might be attributable to a number of interacting factors: a slowing of the rate of septal driving (slowing of theta), a decline in basket cell efficacy due to repetition (Finch & Babb, 1977), potentiating increases in excitatory input, and a post-inhibitory rebound. Type A cells may discharge in phase with the theta wave during this segment and at fairly high rates for brief periods of time, but it is likely that adjacent A cells do not fire together. With the disappearance of theta, the probability of sustained A cell firing increases. The probability of the LIA spike also increases and the firing of adjacent A cells tends to become more synchronized.

On the surface, the present data, in conjunction with those of O'Keefe and Ranck, provide some justification for the old notion that the hippocampus is relatively "inhibited" during theta. There are some clear exceptions to this view. One example is Fujita and Sato's (1964) intracellular work in which p. cell spikes (simple and complex) are seen riding on top of rhythmically recurring waves of depolarization. It must be emphasized, however, that Fujita's studies were done in Nembutal-anaesthetized rabbits in which theta frequency was between 2-4 Hz (theta was elicited by sciatic nerve stimulation). It is possible that during such low-frequency theta inhibition on pyramids is attenuated, allowing other processes such as rebound excitation, etc. to exert a dominating influence. Recent unpublished manuscripts by Bland et al (1978) and Andersen et al (1978) have described prolonged rhythmic firing in identified pyramidal cells during theta in urethane-anaesthetized rabbits. Urethane-treated animals show slow theta. Vinogradova (1970) has recorded from unidentified hippocampal cells in restrained rabbits and has observed rapid discharging of elements, presumably during theta. Since she usually rejects complex spike cells from her sample, believing they represent injury potentials, she may be favoring the selection of the basket cell (see CIBA Symposium, 1978, p.311). Also, her rabbits were highly aroused, a factor which might also bias towards theta cell selection. Still, it is unlikely that all, or even most, of the cells observed in her studies are basket cells, given the ratio of basket to pyramidal cells in the hippocampus. The fact that her rabbits were immobile, however, implies slow theta (see Kramis et al, 1975), during which pyramidal cell discharge may be

favored. Taken together, the discrepancies between rabbit and mobile rat findings may be due to species differences, the recording of unnatural slow theta, or biased sampling in which theta cells are favored.

Both Ranck (1973) and O'Keefe and Nadel (1978) have concluded that during theta the pyramidal cell is in an inhibited state. O'Keefe & Nadel (1978, p. 152) stated:

> From the physiological point of view LIA appears to represent a state during which the theta mechanism is inactive. Theta, on this view, represents an active inhibitory process which locks off the soma and dendrites of most of the pyramidal cells, allowing only a few to fire at any given time.

and Ranck (1973, p. 518) has asserted:

> Two modes of inhibition are thus suggested, a global inhibition during the theta rhythm and a localized lateral inhibition of neighbouring complex cells.

Despite these basic observations and conclusions O'Keefe & Nadel (1978, pp. 167-168) were led to say this about Grastyan's conclusions:

> ....it follows directly from their argument (Grastyan et al, 1959) that theta represents an inactive state of the hippocampus... recent hippocampal physiology indicates that this conclusion is almost certainly wrong.

It appears that both O'Keefe and Ranck (and other chronic unit workers) emphasize the significance of p. cell firing during theta in the definition of hippocampal behavioral function, despite the overall reduction in p. cell activity during alert waking behaviors. Of course, for the chronic unit worker, the important correlate of unit firing is behavior, not EEG. If the cell shows a phasic change in response during a phasic shift in behavior, then there is cause to believe that the cell participates in the behavior, regardless of EEG

state. This basic view is not readily applied, however, to unit patterns in the hippocampus. In sensory and motor systems, phasic changes in single cell responses during discrete changes in sensory-motor function are expected. In the hippocampus, while there are cells which respond to punctate stimuli, the firing of cells appears to cut across different behavioral categories (EEG state). For example, one dominant type of behavioral correlate of the complex-spike cell in Ranck's studies was the "approach-consummate cell". The cell discharged at high rates when the animal approached food (theta) and then ate the food (LIA). Firing was present during both states (behaviors). O'Keefe and Nadel's spatial cells also fired during different behaviors. What this suggests is that the EEG may not be a very informative measure of essential processes involved in behavior or that one is dealing with a continuum of neural events which runs parallel to a continuum of change in some modality of behavior. This is suggested in the data of the paralyzed rats here in which some p. cells fired during weaker theta in transition to irregular EEG.

The next and final section attempts to assess the significance of activity in the hippocampus during theta and non-theta by examining cross-correlograms based on simultaneously recorded activity of units in dorsal CA1 and in the lateral septum during the two EEG states. If the hippocampus is involved in the control of behavior during these states, then the message carried by the activity of pyramidal cells should be capable of influencing the activity of target cells in the LSN. Differences in output associated with state indicate that the EEG is an important indicator of information processing.

SECTION FOUR. Hippocampal influence on septal neurons.

Part A. Stimulation induced.

During acute experiments septal target cell responses to contralateral hippocampal stimulation were periodically sampled. A principal reason for this procedure was to obtain electrophysiological indication that target cells were in the septum, possibly connected to cells at the hippocampal microelectrode. The stimulating electrode (which also served to monitor EEG) was situated contralaterally to minimize the risk of damaging cells in the vicinity of the hippocampal microelectrode, but the effects of contralateral stimulation were similar to ipsilaterally produced effects in pilot studies (see General Methods). They were also similar to responses reported in the literature and it is mainly the results of contralateral stimulation that are summarized in this section. The outcomes of these studies also provided a basis for interpreting hippocampal influences on the septum during "natural" states.

Methods.

The stimulating electrode was in left anterodorsal hippocampus (the tip sites are shown in Fig. 4) while the micropipette was in the contralateral right septum (see Fig. 28). Evoked responses of a proportion of spontaneously active cells along each track were sampled, usually at the termination of the recording of the activity of pairs of

cells in hippocampus and septum. Biphasic pulses (.5 msec) were applied at slow rates (about 1 per 2 sec) to avoid inadvertent elicitation of abnormal epileptiform responses. It had been seen that rates as low as 4/sec could yield prolonged afterdischarges.

Results.

Septal unit responses were consistent both with respect to response type and spatial distribution. The response pattern seen most frequently is depicted in Figure 28A. It consisted of a multiple spike burst occurring at a latency of 20-25 msec followed by a long inhibition of activity ranging from 100 to 800 msec. With ipsilateral stimulation in pilot studies responses were similar, with briefer latencies in the range of 15 to 20 msec. Some latencies were as short as 12 msec with stimulation of dorsal hippocampus. Figure 28D shows a cell in the dorsomedial portion of the LSN activated by ipsilateral dorsal CA1 stimulation. The accompanying slow activity at the microtip is also shown. Note that spikes occur during the negative portion of the wave.

The E-I sequence was seen in anterior as well as more posterior septal placements, with corresponding differences in latencies. An additional short-latency component (10 msec) was recorded when the pipette tip was in more postero-dorsal aspects of the midline lateral septum (Fig. 28C).

In these posterior sections (Fig. 28B, C) the E-I response type was localized to that portion of the LSN lying close to the midline (about 0.3 mm from the midline). It was not detected in three tracks

lying more laterally, nor was it seen in sites dorsal to the corpus callosum (cingulate cortex) or in the MSN (see Fig. 28 - areas outlined by heavy black borders). Cingulate responses were seen with ipsilateral stimulation, however.

In more anterior areas (Figure 28A) the response was confined mainly to a region corresponding to lateral septum. The E-I sequence was seen irrespective of stimulation locus and was maximal in an area located about one third of the way between the ventral limit of the corpus callosum and the dorsal border of the MSN. While in most cases cells in the MSN did not respond to electrical stimulation, there was an occasional cell group which was suppressed for brief periods following stimulation. In one instance, a clear burst of 8-10 spikes was evoked at a latency of about 40 msec at a site indicated by the X in Figure 28B.

Discussion.

In agreement with recent anatomical and electrophysiological work, stimulation of dorsomedial zones of the hippocampus evoked responses in a restricted zone of the septum, the dorsomedial portion. Clear responses were also detected in an area corresponding to the LSN at the level of the anterior continuation of the hippocampus, with latencies slightly longer at the more anterior septal sites. Responses were not detected in more lateral areas of the LSN, lying outside the predicted terminal zone of sites stimulated. It is likely that stimulation of more lateral hippocampus would have elicited responses in lateral areas. Except for occasional hints of a brief initial inhibition, responses were not detected in the MSN.

The basic response type, initial excitation followed by long periods of spike inactivity, also corresponded with previous reports in the literature. Latencies to the initial excitation were not, however, in agreement with previous work. While other studies in the rat (DeFrance et al, 1973b; McLennan & Miller, 1974) have reported latencies ranging from 4 to 7 msec to initial excitation, latencies here were about 12 to 20 msec with ipsilateral stimulation and 20 to 25 msec with contralateral stimulation. These latencies are more in line with those reported by Edinger et al (1973) in the cat.

Reasons for the latency discrepancy are not immediately obvious. Histological analysis of septal microelectrode placements, septal electrophysiological profiles as well as septal response topography to electrical stimulation showed unequivocally that cells in the LSN were being sampled. The slow wave responses to ipsilateral electrical stimulation indicated that spikes evoked at the modal latency of 15 msec were due to excitation and not a delayed rebound from inhibition. This was supported by observations that repetitive stimulation led to repeated elicitation of initial excitation with increases in duration of subsequent inhibition. Had the spikes been a consequence of rebound inhibition, repetitive stimulation should have suppressed cell activity.

Although it is unlikely that differences in elements recorded from were at the basis of the latency differences, there is a possibility that differences in elements stimulated may account for some of the discrepancy. In the studies of DeFrance et al (1973b) and McLennan & Miller (1974), fibres were stimulated in most cases. DeFrance et al

did report stimulating CA1 and CA3,4, but it is not clear where the stimulating tips were placed, since histological details were not reported. In one study DeFrance et al (1971) reported stimulating posterior CA1 alveus, suggesting that electrode tips reported to lie in CA1 or CA3 were in contact with alvear fibres passing across the areas. It has been pointed out that the fimbria-fornix-alvear system carries fibres originating in a number of different zones. Fibre diameters and /or degree of myelinization may differ within the mass of the fimbrial system. It is conceivable that early responses recorded in the LSN by these research groups activated fibres originating in cells other than those stimulated in the present study. Conversely, stimulation of cell bodies in this study may have activated fibre systems (smaller diameter, higher threshold, less developed myelination, and slower conduction velocities) than those tapped in the other studies. An explanation of this type is supported by the fact that DeFrance et al (1973b) reported latencies of 4 msec to peak of extracellular negativity in the LSN of the cat following fimbrial stimulation. Edinger et al (1973), stimulating hippocampal cell bodies in the cat reported latencies ranging from 10 to 30 msec.

It is also possible that only polysynaptic responses were recorded in this study, similar to those seen in more postero-ventral sites in other work. Since the responses reported here were seen in all dorsomedial zones of the LSN, this possibility is not likely.

The longer latencies of responses with contralateral stimulation as opposed to ipsilateral stimulation can be explained by assuming that contralateral routes to the LSN are longer and perhaps multi-

synaptic. Figure 4, which shows the sites of contralateral macro-electrode tips, indicates that in most cases cells in the dentate region were activated. The response evoked in contralateral LSN was probably relayed through junctions in CA3 and CA1 which would account for the longer latencies and the multiple burst response.

The short-latency population spike at dorso-caudal levels of the septum might be interpreted as a fibre response. The micro-electrode tip at those levels may have been in relatively close proximity to discrete fibre bundles passing from or towards septum or other areas.

Pure inhibitory responses such as those described by Edinger et al (1973) in the cat and by DeFrance et al (1973a) in the rat (but not by McLennan & Miller, 1974) were not detected in the present study. It is possible that the parameters of stimulation used were inappropriate for the detection of some of these effects (see DeFrance et al, 1973b), but the main concern of this study was to confirm some degree of connectivity. Extensive parametric tests were not done in either the pilot experiments or in the main experiments since it was desirable to avoid any residual stimulation effects.

Part B. Influence on septum during naturally occurring EEG.

Determining effects of electrical activation of one group of cells on its targets is a routine electrophysiological procedure. As noted in the Introduction and in Part A of this section both stimulation-response and topographical relations of septal cells to hippocampal elements have been fairly well established. A characterization of the

biases imposed by one area on another under natural conditions is considerably more difficult to achieve. A primary goal here was to determine whether such naturally occurring effects could be detected.

The cross-correlational analysis has been the most frequently used method of detecting a functional relationship between the firing of two neurons. The papers of Moore, Segundo, Perkel, and Gerstein (see Moore et al, 1966; Perkel et al, 1967a,b) have been particularly instrumental in the formulation of the mathematical basis for the CC as well as the application of the technique to the analysis of spike train pairs. The development of these statistical methods has relied to a large extent on observations of spike activity generated by cells of the marine invertebrate, Aplysia californica. The relatively simple arrangement of neuronal networks in this snail has permitted precise descriptions of the anatomical as well as electrophysiological nature of connectivity between certain classes of cells. It has been possible to record intracellularly from two separate neurons whose synaptic interactions are known and to observe how their rudimentary interactions are reflected in the CC (Moore et al, 1970). It has also been feasible to assess the effects of electrically driven changes in presynaptic firing patterns on the fundamental synaptic influence exerted by the pre-synaptic on the post-synaptic element (Bryant et al, 1973). Observations derived from these studies have provided an important guide to inferring the nature of synaptic interactions between two cells (as well as other characteristics of the network in which the cells are imbedded).

On the whole, studies correlating the activity of neuron

pairs in mammalian nervous system were not aimed at inferring the nature of trans-synaptic interactions between two anatomically separate brain areas (for an exception see Oomura et al, 1967). Most studies have employed a cross-correlational analysis to investigate the relative synchronization of discharges during a particular brain state (Creutzfeldt & Jung, 1961; Holmes & Houchin, 1966; Li, 1959; Noda & Adey, 1970). In other studies, which have been aimed at describing synaptic interactions (Arnett, 1975, 1978; Dickson & Gerstein, 1974; Eckhorn & Popel, 1972; Rodieck, 1967; Stevens & Gerstein, 1976; Wilson et al, 1977), the neuron pairs recorded from have been closely adjacent, typically registered by the same electrode and confined to one nuclear region.

Noda and Adey (1970) suggested that with increasing distance between elements, the likelihood of strong effects in the CC diminishes. Dickson and Gerstein (1974) have verified this empirically; 93% of CC's based on cell pairs recorded at the same tip showed effects, while 35% showed effects when two separate microelectrodes were used. The same trend is seen in the findings of Wilson et al (1977).

Findings of primary effects suggestive of direct synaptic impingement of one neuron on the other have been rare; much more common is the finding that the two elements are affected by a common input. In the study of Dickson & Gerstein (1974), in which cells sampled were close together, less than 5% of CC's indicated trans-synaptic effects while 50% of CC's showed features indicating shared input. In view of the suggestion that in the plot of the CC wide peaks straddling the origin are more likely to appear when recording from closely adjacent elements (Dickson & Gerstein, 1974), their results are not surprising.

It might be predicted that, in mammalian brain, the probability of locating with microelectrodes two cells in separate regions that are interconnected synaptically is extremely low. In Aplysia, there are few cells, the network they create has been well worked out, and the cells, usually large, are easily penetrated and held by the intracellular microelectrode, under visual guidance. None of these conditions hold in the mammal. In order to initiate investigation into discharge relations between separate areas in the mammal, it is necessary to assume that the activity of the two neurons is representative of activity of homogenous neuronal pools of which they are part. This is not an unreasonable assumption (see discussion by Milner, 1970, p.69 with respect to spinal motoneurons). A single axon projecting from a hippocampal cell probably branches considerably in the septum, forming vast numbers of synaptic contacts with the tangle of dendrites associated with a large number of septal cells. That synaptic contacts of hippocampal axons on septal cells are axodendritic was reported by Raisman (1969). Activity in a single axon will influence a large number of septal cells and activity in a number of axons coming from the hippocampal pool will influence a single septal cell. Assuming this type of network structure, the presence of a transsynaptically-induced correlation between two individual cells signifies the interaction between neuronal pools.

Another troublesome point concerns the assumption of stationarity. The representation of activity in time by means of an averaging process requires that different segments of time over which activity is observed contain the same rates and patterns of activity. In neuro-

physiological work major contributions to non-stationarities arise from fatigue or degeneration effects which take place over the course of long experimentation in acute preparations. The presence of stationarity can be assessed by rigorous or by relaxed methods. In most studies, including this one, tests for the presence of stationarity have not been rigorous. The lack of strict procedures in this study can be justified by assuming that the continuing presence of clear hippocampal theta, necessary to the collection of spike data, was a prime indicator of the rat's state. Also, spike data was not collected over long time periods; the activities of cell pairs were compared during different EEG states, brief in duration, and histograms and statistical data indicated that activity of cells did not vary as a function of time segment.

Although restricted sampling time reduced problems associated with non-stationarity, it raised another type of problem for the CC's of this study: the number of spikes counted were small, particularly since rates of firing were slow in both channels. The issue of stationarity arises partially because to obtain reasonably smooth histograms of neural activity, a fairly large n is required which in turn requires that sampling extend over long periods of time, unless firing rates of both cells in the record are fast. What constitutes a large enough n seems to be an empirical matter. In many studies it appears that neither channel should contain less than 400 to 500 spikes. The CC's in Wilson et al (1977) are based on spike counts considerably less than this and the invertebrate work of Kristan (1970) indicates that if transsynaptic effects are strong, very few spikes must be accumulated.

Of course, the presence of powerful effects can be detected without the CC.

There are other problems associated with the CC: the cumulative influence of successive hippocampal spikes on septal events cannot be determined (Bryant et al, 1973). Also, whether some CC feature departs significantly from the average histogram height is in most cases evaluated by subjective methods since there are no statistical tests available to perform this task (Bryant et al, 1973; Moore et al, 1970).

Particularly severe are the problems associated with the interpretation of peaks and troughs, even if their significance could be assessed statistically. External influences on interacting neurons can obscure the fundamental effect in the CC and the same histogram feature can be related to a number of different physiological mechanisms. A histogram peak, for example, could arise because of direct synaptic influence or because the neurons recorded from are activated by a common source with one neuron activated first because of a shorter conduction distance.

Despite the potential difficulties in detection, characterization, and interpretation of CC irregularities, it was felt that the application of the method to more complex forebrain circuits might yield information that potentially could advance understanding of which EEG states (behaviors) are accompanied by hippocampal output to septum and help characterize the nature of hippocampo-septal interactions during these states. Even negative results could contribute to an assessment of the viability of the technique in the analysis of complex brain circuits.

Methods.

As pointed out above, slow firing rates and brief sampling times often resulted in the accumulation of relatively small numbers of spikes. As a consequence, the CC was potentially susceptible to isolated and occasionally artifactual temporally related events. To detect and avoid such cases, the raw records for those cells which generated signs of apparent synaptic interaction in the CC were re-examined and a number of sweeps containing the effect were photographed (Polaroid) with the hippocampal spike triggering the sweep. In some cases the effect was re-examined with the aid of a computer program which generated a post-stimulus histogram (PST) with the hippocampal channel acting as stimulus and the septal channel as response. In this analysis hippocampal bursts, simple and complex, were treated as single spikes. In both types of re-analysis sampling of activity was more restricted than during the cross-correlational analysis in which sampling occurred during relatively long segments of theta and non-theta activity, with the two EEG states occasionally overlapping. Sampling was confined to a particular EEG state and was also guided by the rate and pattern of firing of A cells during the EEG segment. In some cases segments of cell activity associated with a long period of theta, and which had been originally processed for the entire period, were divided into a number of smaller segments and a separate post-stimulus histogram was generated for each segment. This analysis provided an index of the variability of cross-channel influence (stationarity). A third type of analysis, performed for a small number

of cells, furnished a clear display of the variability of septal activity given the occurrence of a hippocampal event. In this analysis spike pairs were again computer processed with the hippocampal event serving as stimulus and septal spike as the response. The program output was a raster display of dots with each successive horizontal line of dots representing the septal responses following a particular stimulus. All three procedures, photograph of raw data, PST, and dot display, while not ruling out the possibility that CC effects were other than trans-synaptic, verified that the effect in the CC could not be attributed to gating errors or other spurious events.

Other factors which produce irregularities in the CC fall under the heading of secondary effects already discussed in the General Method section. Briefly, these are the CC features which reflect periodicities in the individual spike trains. Cross-correlograms in which peaks and troughs could be attributed to secondary effects, as seen in the AUTO's, were excluded from analysis here. Those histograms which showed little effect or uninterpretible, ambiguous features during both EEG states were also not considered.

Histograms with outstanding features to the right of the origin during either EEG state were sorted into several different categories and were re-classified by an experimentally blind, independent observer. Agreement between the two categorizations was 81%.

Results.

Out of approximately 166 pairs of spike trains sampled in 16 rats, the simultaneous activity of about 37 pairs in 11 rats gave rise

to CC profiles suggestive of synaptic interaction during one or both of the two EEG states sampled.

Two basic hippocampo-septal effects were observed (septum to hippocampus effects are not considered in any detail here): in some cases the activity of A cells was followed by a time-locked acceleration of septal discharges (PEAKS); in other cases a slowing of activity was seen (TROUGHS).

Histograms with peaks were sorted into three separate categories: 1. those showing early single or multiple peaks ("initial peaks"), 2. those in which the early peaks were followed by troughs which were occasionally followed by "rebound" peaks ("peak-trough"), and 3. those in which the early peaks were mirrored by a peak on the left half of the histogram ("symmetry"). The third category of peak histogram consisted of those CC's in which the left and right halves of the histogram were approximate mirror images of each other. Typically, the symmetrical early peaks were situated close to the origin and were followed by troughs and rebound peaks. The symmetrical CC's were placed in a separate category because they were thought to represent the lack of trans-synaptic effect of CA1 on LSN; the symmetry of early peaks was indicative of septal and hippocampal units firing very closely together as a consequence of shared input.

Histograms with troughs on the right hand side were of two types: in one case the trough was immediate, occasionally followed by a rebound peak ("initial trough") and in other instances the trough was late, also sometimes followed by a rebound peak ("late troughs").

These two dominant effects, PEAKS and TROUGHS will now be considered in turn. Examples of peaks are presented in Figures 29-32 inclusive; troughs are seen in Figures 33-35.

Acceleration of septal firing: PEAKS.

Table 5 summarizes the types of CC effects generated by the activity of 37 cell pairs during irregular (LIA) and regular (theta) EEG (74 histograms in all). As the table shows, out of a total of 48 histograms which suggested primary effects, in 29 (60%) early peaks (initial peaks as well as peak-trough) were the dominant feature. In general, the peaks were fairly broad, lasting in most cases from 40 to 60 msec. Secondary peaks were seen in about 33% of peak histograms (Fig. 31, cell 27-15 during LIA).

The latency to peak onset was variable, ranging in duration from 10 to 200 msec. In eight cases (28%), peak onset was reached within 13 msec. In three instances it was reached in 14 to 27 msec. In seven cases (24%) it occurred within 28-41 msec. In six cases it ranged from 75-100 msec. In three cases latency to onset ranged from 130-155 msec, while in the two remaining cases it occurred at about 200 msec. Thus, in 18 (62%) instances, latency to onset was reached in 40 msec, while in the remaining 11 histograms onset latency occurred between 75 and 200 msec.

It is legitimate to inquire whether the longer latency peaks represented primary effects. The CC's with such effects were included because their features were not obviously due to secondary effects; possibly they reflected polysynaptic long-latency effects of CA1 on septum or shared input with one branch of input arriving at CA1 much more rapidly than the LSN branch.

Figure 29B provides an example of a long-latency peak (90 msec) in the CC during LIA. During theta the peak is attenuated and there is a slight suggestion in the peaks at the origin that the cells are firing together. There is also an indication of a delayed trough during theta, particularly evident in the PST of Figure 29C3. The location of the septal cell involved in the long-latency peak of Figure 29 was ventral septum adjacent to the MSN border (Fig. 29A) suggesting that other septal cells giving rise to CC's with long-latency peaks might also be situated more ventrally. In fact, this appeared to be the case. If the anterior hippocampal continuation zone is excluded, then of 16 histograms with short-latency peaks (less than 40 msec), 11 (69%) were situated in dorsal zones of the LSN. Of nine histograms with long-latency peaks, only three (33%) were in dorsal portions.

Examples of short-latency peaks during LIA are seen in Figures 30 to 32. In the raw record depicted in Figure 30A it is seen that a burst in the hippocampus during LIA is followed by a burst in the septum. The short-latency, long-lasting peak in the CC to the right of the origin reflects this relationship. A small, very narrow peak is also seen at the left of the origin suggesting the possibility that during some portion of the LIA run the cells fired together. The tendency for firing together is seen more clearly in the CC during theta. It is possible that brief periods of theta were sampled during LIA.

During LIA, spike trains of cell pair 27-15 (Fig. 31B) yielded a CC quite similar to that of cell pair 26-1 of Figure 30. These cell pairs came from different rats. Again, the broad peak in the CC can be

accounted for by the long burst in septum which follows the hippocampal burst. It is interesting to note that the septal cell of Figure 30 (C4 subtype) and that of Figure 31 (B2 subtype) both showed weak theta bursting during slow wave theta, suggesting that there may be excitatory impingement of CA1 cells on septal cells which burst rhythmically during theta. However, as can be seen in the figures the excitatory effect was seen mainly during LIA; during theta the initial peak is attenuated.

Figure 32A (cell 27-8) provides an example of an early peak in the CC during LIA which is still present during theta; the latency to the peak increases slightly during theta.

Slowing of septal discharge rates: TROUGHS.

In 19 of 48 (40%) histograms a time-locked slowing of septal rates was noted. In ten cases the troughs were immediate. The "initial troughs" were generally of long duration, ranging from 40 to 230 msec, with the average trough 100 msec in duration.

In Figure 33 (cell pair 20-3) the interaction between the hippocampal and septal cell resulted in a 100 msec initial trough during LIA, an effect seen in the CC (Fig. 33A) as well as in the raw data (Fig. 33C). The hippocampal cell of Figure 33 is identical to that of Figure 29; in one case (Fig. 33) it is interacting with a dorsolateral cell in the LSN, yielding a short-latency trough during LIA; in the other case (Fig. 29) it is interacting with a medioventrally situated cell resulting in a long-latency peak, also during LIA. The interaction during theta for these cell pairs is roughly the same and is diminished compared to LIA.

Figure 33D presents the outcome of a dotgram analysis for spike pair 20-3 which provided a good example in that S/N ratios were excellent in both channels and changes in EEG patterns were clear. The figure shows activity of septal cells during successive hippocampal spikes as a transition from clear LIA to spontaneous theta occurs. During LIA an early suppression of septal activity appears to dominate. Generally, during theta, the early inhibition is attenuated as spikes are seen during the inhibitory period. However, there appears to be a late suppression (reflected also in the CC) which varies in latency and duration.

Two more examples of "early trough" histograms are provided in Figure 34, in both cases during LIA. For both these cells the spike count in the hippocampal channel during theta was too small to allow construction of histograms.

"Late troughs" appeared in 9 of the 48 histograms (19%) showing effects; five of these late troughs were from one animal (#20). Late troughs were clearest during LIA. In two cases of late troughs during LIA very slight peaks preceded the troughs.

The final figure of the series, Figure 35, shows a late trough during LIA which is altered to an effect classified as an initial peak during theta, although the peaks at either side of the origin suggest that shared input may have been partially responsible for the effect.

Late trough duration ranged from 90 to 200 msec with a mean duration of 136 msec. Latencies to trough onset ranged from 14 to 128 msec with a mean onset latency of 58 msec.

Transitions from LIA to theta. As seen in Table 5 about 81% of the histograms showed "primary" effects during LIA while 49% showed primary effects during theta. A z-test for correlated proportions described in Ferguson (1971) indicated that the difference between the two proportions was significant at the .02 level. Nineteen of the 37 pairs showed a transition from an effect in LIA to a reduction in the effect during theta, while 11 pairs showed effects in both. In the CC's associated with the remaining seven pairs there was no detectable relationship during LIA, while during theta an effect was seen.

Further analysis of the transition from LIA to theta showed that of the 13 CC's with early peaks during LIA, in nine the effect was attenuated during theta (Figs. 29 to 31 inclusive). In three of the remaining four cases a peak was also seen during theta (Fig. 32). Of four CC's showing peak-trough effects in LIA, two were attenuated and two showed peak-trough effects during theta as well.

Of the eight CC's with initial troughs during LIA, six showed reduction of the effect during theta (see Figs. 33, 34). The remaining two cases were transformed to a late trough and early peak. Finally, of the five CC's with late troughs during LIA, during theta two were attenuated, two showed peaks and in one the late trough was repeated.

In sum, it appeared that the majority of the changes from LIA to theta consisted of an attenuation of the LIA effect, regardless of the CC effect during LIA. Moreover, most of those pairs in which the effect was not attenuated, showed early peaks during theta.

Relation of CC effects to cell type and anatomical locus.

Primary effects in the CC appeared to be independent of the septal autocorrelogram shape (cell type or subtype) and the anatomical location of cells; that is, AUTO's with initial troughs or peaks could yield either initial troughs or initial peaks in the CC. This was seen both in comparisons between different cell pairs as well as within a single cell type; the same cell types interacting in two different states could give rise to different effects in the CC. Figure 29B, for example, shows that although individual AUTO's were not strikingly different between states, the CC's during LIA and theta are different. On the other hand, Figure 34A and B, shows that two different septal cell types interacting with an A cell could give rise to similar cross-correlogram effects.

Discussion.

About 22% of all cell pairs sampled gave rise to CC's with features suggestive of synaptic interaction between hippocampal neurons and their septal targets. In itself and relative to other studies, a proportion of this magnitude appears high, although it is difficult to compare these findings to other studies since the criteria for establishing which CC features are indicative of primary effects are relatively vague and may vary from study to study, particularly if transsynaptic distances vary. In this study several CC's contained features whose latencies suggested that their interactions may not, in fact, have been primary. If the cell pairs which gave rise to CC's containing relatively late peaks and troughs are excluded, the result is that

about 15% of the 166 cell pairs recorded from resulted in primary features in the CC. Given that septal to hippocampal influences were in the main disregarded here, this, too, seems like an inordinately high ratio. If these features do represent primary synaptic effects of the hippocampus on the septum, this would mark the first report of such effects in a closed-loop limbic circuit of the mammal under natural conditions.

While other studies suggest that primary effects between separate areas should be rare, a number of considerations, some already alluded to, indicate that their presence should be expected in a small proportion of pairs. First, detection might be expected if it is assumed that the cells giving rise to these effects belonged to neuronal pools containing cells engaged in similar activities. Second, in most cases where effects were found, the hippocampal cell corresponded to a CA1 projection cell while the septal cell was in most cases located in a portion of the lateral septal region reported to receive the terminals of the projection cell. The third, and strongest, factor lies in the selection of sampling times. In other studies, the prolonged sampling of spike train pairs may have cut across meaningful functional segments, thereby averaging out effects confined to a particular segment. It was noted here that during some segments cross-relations were different than during other segments (irregular vs regular EEG). By keeping sampling times brief and within these electrographic segments, effects in the CC may have been maximized.

As might be anticipated from the results of electrical stimulation studies, the majority of histograms contained initial peaks. It is clear, however, that these CC's did not duplicate the response

patterns seen with electrical stimulation. With electrical stimulation excitatory-inhibitory response sequences were evoked in the septum with latencies to initial excitation ranging from 15 to 25 msec. In the CC's initial peaks were typically broad, spanning 50 msec or so, and in most CC's (62%) the peak onset took over 25 msec to develop. In relatively few cases were initial peaks followed by troughs. That peak latencies were variable on the long side and that peaks were broad weakens an interpretation of these effects as being monosynaptic.

The assumption that natural peaks should precisely mimic artificially created peaks may be unwarranted, however. Electrical stimulation will produce an unnaturally synchronous activation of many neural elements which in turn will result in synchronous post-synaptic potentials and action potentials at the recording site. The rise time of the EPSP will be fast, relative to the naturally occurring one, leading to a briefer response latency. If there are recurrent collateral inhibitory circuits in the target zone, the synchronous activation of the cells which excite them will produce a powerful feedback inhibition. It is highly improbable that at any given moment patterns of activity during normal states approach the synchronicity produced by electrical stimulation. Also, at different moments in time different elements within the pool will fire leading to a relative dispersion of action potential arrival times. The presence of such factors will lead to increased variability in response latencies, increased latencies and durations of peaks and relatively weak recurrent inhibition. Taking these factors into consideration, the simplest type of mechanism that could underly the short latency peaks of this

study is a monosynaptic excitatory effect of CA1 projection cells on target cells in the septum (Fig. 36, pathway 1).

Given the tendency for septal cells involved in longer latency CC peaks to lie more ventrally, it is possible that the longer latency effects were mediated via polysynaptic connections. The intermediary neuron may be situated at the level of the septum and/or within the hippocampus itself. It might be recalled that area CA1 can influence lateral septal cells either directly or through a relay in the subiculum (see Fig. 36, pathways 2 and 3).

Other explanations are possible, most of which arise from an introduction of inputs to areas CA1 and LSN originating in a common source. Such inputs may induce primary correlations as well as secondary effects, both of which will be complicated by possible differential arrival times of input. For example, if activation of pathway 6 and 7 in Figure 36 either excites or inhibits the elements recorded from, then peaks about the origin will develop in the CC. Depending on the timing of inputs to the two regions, the peaks may be diplaced to the right or to the left of the origin. Other effects will be seen if the common input excites one zone while inhibiting the other.

In the case of areas CA1 and LSN there are at least two possible sources of common input: brainstem and CA3 (see Fig. 36, broken lines). If the brainstem site is active, its output will probably arrive at the septum before the horn, in which case the peak should be displaced to the left and would not be of concern here. Output from CA3 will reach area CA1 before it reaches the LSN, however, and it has

been demonstrated that CA3 is excitatory to both CA1 and to LSN. Shared input from CA3 would also help explain the variability in peak latencies as well as the long peak durations.

It should be recognized that whether the CC peaks represent monosynaptic effects of CA1 on LSN or common input from CA3, the peaks still reflect the presence of output from horn cells (CA1 or CA3) which produces an effect in the target zone. That is, in order for an effect to exist because of common input from CA3, area CA3 must produce an effect in both CA1 and LSN. Thus, the possibility that common input from CA3 is responsible for at least some of the peaks, does not prevent interpretation of CC's in terms of hippocampal output. The problem is one of deciding whether the peak represents CA1 or CA3 output to LSN. This would not be true, of course, if the source of the cross-correlation was brainstem common input.

There is a suggestion in the data that horn A cells are capable of exerting an excitatory influence on septal cells which burst weakly at theta frequencies (Figs. 30, 31). Superficially, such a finding is in support of an implication of McLennan and Miller's idea that during theta, activity of hippocampal pyramidal cells will drive lateral septal cells, resulting in the rhythmic activity necessary to maintain theta. The CC's indicate, however, that the excitatory influence is exerted mainly during non-theta states. During theta, the synaptic coupling between hippocampus and septum appears to be disrupted. The occasional presence of symmetrical peaks about the origin during theta is most likely attributable to non-synaptic entrainment of rhythmically bursting cells in both areas. The significance of such entrainment for

function is not clear, but it suggests a state of reduced information processing.

Considering the rarity of inhibitory effects in septal cells with electrical stimulation of hippocampus in the rat, it is surprising that in about 40% of all CC's with apparent primary effects, initial or delayed troughs were present. Some of DeFrance's findings with respect to the distribution of inhibition in the septum are useful in accounting for the large proportion of inhibitory troughs. As mentioned in the Introduction, DeFrance et al (1973b) found that the intracellularly recorded response of a septal cell to stimulation of hippocampal elements differed according to intensity of stimulation. At high intensity an initial EPSP and action potential was seen followed by a hyperpolarization or IPSP. With decreased intensity initial spikes were absent, although the EPSP was still present as was the IPSP. With still lower intensities, the EPSP also disappeared leaving the IPSP. These outcomes indicated that at lower intensities the cell impaled was no longer in the excitatory focus present during high intensity stimulation, but was still influenced by a widespread inhibitory surround produced by excitation of adjacent elements. Recording from a single hippocampal neuron in conjunction with a single septal cell can be compared roughly to stimulating a group of horn elements at very low intensities, so that only a few horn cells are activated. Under such conditions the excitatory field will be narrow and the chances that a septal microelectrode will be outside the excitatory field and adjacent to a cell in the inhibitory field relatively high.

Such considerations enhance the likelihood that troughs were attributable to a trans-synaptic inhibition of hippocampal cells mediated by septally located short interneurons. As with peaks, a host of alternative explanations are possible.

Regardless of the precise interpretation assigned to CC features, it is clear that in a large majority of cells the features differed during different EEG states, while at the same time for a number of cells sampled, features within an EEG state remained constant. In most cases transitions to theta were accompanied by an attenuation or break up of primary effects. The most obvious example of this attenuation were those cases in which discharge rates of hippocampal cells were reduced to near-zero during theta. In some cases the effects seen during LIA were abolished during theta, even though hippocampal cells continued to fire. In still other cases the effects seen during irregular EEG were converted to peaks about the origin during theta.

Disregarding reductions in A cell spike rates during theta, there are a number of interpretations of such changes. One interpretation is in terms of possible changes in the synchronization of CA1 pyramidal cells as a function of state. Noda and Adey (1970) have demonstrated, with the use of CC's, that a greater synchrony of activity exists in behavioral states and neocortical EEG periods associated with hippocampal LIA activity. In the present study it was seen that the LIA spike was associated with a mixed burst which appeared to consist of the simultaneous discharge of a number of cells. The mixed burst was never seen during theta. Instead, it appeared as if

different cells (as judged by the different amplitudes) would discharge at different instants during a segment of theta. This observation is supported by a number of other studies (Hill, 1978; O'Keefe & Nadel, 1978; Ranck, 1973).

A greater degree of synchrony during LIA would lead to a spatial and temporal summation of excitatory and inhibitory influences on septal target cells thereby facilitating disclosure of such effects in the CC. Desynchrony during theta would lead to a simple algebraic attenuation of the effect during LIA; while each neuron in the horn would still have the same effect on septal target cells, there would be inadequate summation of effects to drive or inhibit those cells.

The present data do not contradict a different kind of interpretation: desynchrony during theta does not represent attenuated processing, but a different state of processing in which the organization of hippocampal output to septum is altered. One consequence of such an alteration might be that different populations of septal targets are influenced during theta, perhaps because of different convergence patterns on septal cells. To illustrate, consider a speculative model. During LIA closely adjacent output cells, in one functional field of the horn such as a lamella, will fire in synchronous clumps and converge on and influence one population of septal targets. During theta adjacent cells in the same lamella will be desynchronized with respect to each other, but relatively synchronized with cells in other lamella. In this case different populations of septal cells may be influenced because of different convergence patterns. There is little data to support this particular mechanism,

but it serves to illustrate one of a number of ways in which altered organization of firing of hippocampal cells might lead to effects on different septal populations.

Other explanations in terms of external influences are possible. For example, during theta, projection from a third element to the LSN may bias the state of the septal cell differently during LIA and theta so that the same horn output will be interpreted differently by the septum.

Although there are a number of ways of viewing the present data, it appears that the most parsimonious interpretation of CC features is in terms of a direct influence of horn cells on the septum. This influence is relatively great during irregular EEG, becoming attenuated as EEG becomes more regular. Recent findings which show that evoked responses in hippocampus are largest during LIA states are consistent with this interpretation. Segal (1977), stimulating in contralateral hippocampus, sampled evoked responses in CA1 of freely-moving rats during slow-wave sleep (LIA), quiet awake periods (waking LIA of Vanderwolf), and theta-related behaviors. He found that the initial component of the response, which was equally large during the two LIA states, was significantly greater than the response during theta. Winson and Abzug's (1978) findings were similar with respect to population spikes evoked in CA1, CA3, and the dentate areas following ipsilateral perforant path stimulation in moving rats. While these authors did not sample Vanderwolf's LIA, they did find that responses were largest in all three regions during the LIA of slow-wave sleep.

The findings of Winson and Abzug in conjunction with those of Segal, suggest that LIA, whether it be associated with SWS or quiet immobility, reflects a state wherein neuronal transmission within hippocampal circuits appears to be facilitated. The present findings provide evidence of a continuing facilitation of transmission of activity during LIA to regions lying outside the hippocampal formation.

FINAL DISCUSSION

In most investigations of hippocampo-septal relations, the emphasis has been on the influence of septal neurons on hippocampal EEG and unit activity; the role of medial septal cells in the production of hippocampal theta has been of enduring and special interest. This study is unique in its attempt to characterize the effects that hippocampal cells might exert on septum during theta as well as non-theta states. It is also unique in its use of cross-correlational techniques to measure trans-synaptic effects resulting from simultaneously recorded "natural" activity in the two areas.

Cross-correlation of single unit activity has not been applied to an analysis of complex mammalian forebrain circuits and, for reasons outlined in section Four, the risk of obtaining uninterpretible results in such circuits is high. Nevertheless, the technique was used in the hope that it would cut through some of the ambiguities in interpretation of the hippocampal EEG literature. While many of the anticipated difficulties were encountered, there were a sufficiently large number of CC's indicative of primary synaptic effects of horn cells on lateral septum, to warrant continuing exploration of the approach in the analysis of complex circuit function.

Some twenty years ago Grastyan suggested, on the basis of stimulation and lesion evidence, that the hippocampus was inhibited during theta. More specifically, he proposed that during non-theta

EEG, there was output from hippocampus which acted to suppress a neural zone which generated orienting movements. During theta, the hippocampus no longer transmitted inhibitory information to the orienting system. He was aware that his evidence was indirect and suggested that observations of single cell activity during EEG might furnish the "final solution". A fairly large body of evidence consistent with Grastyan's position has accumulated since his initial probes (see General Introduction), but much of this evidence has also been indirect. The present findings provide the first direct indications that neuronal transmission from hippocampus to septum is attenuated during theta. Not only is overall firing in hippocampal projection cells reduced during theta, as seen in Section Three, but the firing that does take place during theta is less likely to influence cells in septum than is firing during irregular EEG.

The placement of the hippocampal microelectrode in CA1 permitted a monitoring of direct output of CA1 to LSN and provided indirect indication of the state of CA3 output. It is difficult to distinguish between the two possibilities in the CC's, although rounder peaks may be indicative of CA3 output. Future studies in which select elements are destroyed may discriminate between these two alternatives. For example, it may be possible to interrupt the connections between CA1 and subiculum or to selectively destroy subicular cells, thereby eliminating the possible mediation of observed effects through subiculum. Selective destruction of CA3 cell bodies with kainic acid might rule out mediation of effects by CA3 output. At the same time, however, it will severely distort CA1 activity. Alternatively, kainic

acid can be applied to CA1 and the hippocampal microelectrode placed in CA3. This procedure would focus on CA3 output and is particularly important because there are indications in the literature that CA3 cells may behave somewhat differently from CA1 cells during theta. Winson and Abzug (1978) have found that, during theta, population spike amplitudes in CA3 were more variable than those of CA1. Ranck (1973) has recorded from cells in dorsal CA3 in behaving rats. He found that overall patterns across the sleep-waking continuum were similar to those of CA1, but his behavioral descriptions indicated that CA3 cells were more apt to fire during theta. Again, however, the dominant effect was suppression.

It is also important to recall that the lateral septum is only one of several targets of the pyramidal cell. There is the possibility that during theta, output to the LSN is attenuated, but that output to other zones is strengthened. This is unlikely with respect to CA1 output to subiculum or CA3 output to CA1 or LSN since activity in these pathways was monitored indirectly. That subicular output via the post-commissural fornix to the mammilary bodies and anterior thalamus might increase in strength during theta must be considered. If increased effects during LIA are in fact due to an increasing synchronization of firing in adjacent elements, then it follows that all hippocampal elements regardless of destination should exert greater effects during irregular EEG activity. Again, this question requires further observation for its resolution.

Future studies might also attempt to evaluate cross-correlations in a greater number of EEG states. It would be informative to

determine whether cross-correlations during slow-wave sleep differed from those based on awake LIA. Comparisons between active-sleep theta and awake, moving theta would also be of interest. Ideally such studies would use chronically-prepared rats which could provide answers to questions about the nature of the correlation between "spatial" cells, for example, and their septal targets during different behaviors. With refinements in technique, it should be possible to obtain interpretible results from arrays of three microelectrodes, one in CA3, a second in CA1, and a third in the LSN. The variations on this experimental theme are, of course, close to infinite. Initially, the implementation of such techniques would be enormously time-consuming. In the long run, however, the information they yield could potentially contribute to a more profound comprehension of the involvement of hippocampal circuits in behavior.

The present study represents what must be considered a preliminary attempt to determine if significant information about functioning in complex circuits can be obtained by examining the correlations of cell firing in the circuit during natural states. The results are tentative, requiring verification and extension. Keeping this in mind, let us briefly consider their implications for views of hippocampal function in behavior. The most straightforward implication is that it is during behaviors associated with LIA that the hippocampus is most active. Views which emphasize hippocampal involvement in those behaviors correlated with theta are weakened. Although Vanderwolf's position assumes only that during theta the hippocampus is receiving information about movement, if the hippocampus is not utilizing this

information to modulate ongoing behavior, then it must be concluded that its functions during movement are limited. O'Keefe and Nadel's position is also weakened since diminished output during theta implies that when the animal is moving through space, its map is not being utilized to direct ongoing movement. Since O'Keefe explicitly assumes that formation of the map is heavily dependent on entorhinal input during theta, the additional findings that entorhinal transmission through hippocampal circuits is maximal during LIA (Winson & Abzug, 1978), makes his position uncertain.

One view which is consistent with the evidence presented here is the response inhibition view or some variant thereof. During LIA the rat tends to be motionless or engaged in small-amplitude automatic movements; broad-ranging movements are absent, suggesting that the hippocampus might be involved in inhibiting such movements. The response inhibition view has run through the hippocampal literature in some form for many years and fits a good portion of the literature. Ranck's (1973) formulations with respect to dorsal hippocampal function, although not presented under the rubric of response inhibition, are consistent with such a model:

> Hippocampal transformations would seem to help solve such problems as how to sequence various automatic behaviors appropriately, how to sequence automatic and non-automatic behaviors appropriately; how to test the appropriateness of an automatic behavior or sequence and stop or change it if needs be, how to shift from one behavior to another...(p. 524)

The functions of sequencing, shifting, testing, changing, and stopping can all be taken to imply that ongoing behavior patterns require suppression for variable time periods, allowing other behaviors

to surface. Suppression of behavior in such circumstances, as is evident from the literature, is accompanied by LIA. The transition from an approach movement to a consummatory act, for example, is accompanied by a change from theta to LIA. If the rat actively explores its environment, stops momentarily to sniff at a novel object, and then continues exploration, the EEG will show periods of theta with momentary LIA sandwiched in between. It might be during this type of irregular EEG activity that hippocampal modulation of behavior is maximal. This conclusion is directly opposed to that of Altman et al (1973) who suggested that during LIA the hippocampus was most inactive and most active during SIA. Hippocampal evoked response studies, in which responses were measured during behavior, and the present results indicate that Altman et al's conclusions were premature. It is possible, however, that these workers were accurate in their surmisal that theta represents activity in a "braking" system without its engagement. For example, the firing of p. cells during theta which precedes a consummatory response (see Ranck, 1973) may represent a state of readiness for the cessation of the response accompanied by the theta. During the theta behavior firing may result in relatively weak output, sufficient only to slow ongoing movement. This preparation might act to ensure that behavioral shifts are "delicately controlled" rather than jerky. Maximal inhibition is achieved during LIA. This type of scheme is appealing because it assigns a role to the strong recurrent inhibition in the hippocampus which is similar to one of its roles in ventral horn motoneuron pools. That is, it allows for a smooth modulation of behavior (in this case inhibition) without constant feedback about the state of ongoing movement.

The above is not intended to provide a definitive view of hippocampal function in behavior. It is meant to show, with reference to a specific example, how knowledge of the state of hippocampal neuronal transmission during different EEG rhythms may guide the observer to the types of behaviors controlled by the hippocampus. A corollary conclusion is that chronic single unit studies must also take into consideration the state of information flow; that is, the information signalled by the firing of a single cell is not only dependent on rate and patterns of firing, but also on what surrounding cells are doing and how many are active. The firing of a single cell during a theta-related behavior may not be as informative as the firing of the same cell during LIA. Ideally, recording from units with arrays of microelectrodes might circumvent this problem to some extent; practically, this is extremely difficult to implement. Thus, careful monitoring of EEG activity in chronic unit studies might be useful in assessing the significance of firing during behavior.

Assume for the moment that future study will show that hippocampal effects on all its major targets are attenuated during theta. Would such a demonstration imply that the hippocampus is relatively "non-functional" during theta? In an important sense the answer is yes; it is during output transmission that the hippocampus (or any brain area) fulfills its critical role since it is then that its functions are brought to expression in behavior. But, activity during theta might represent function in a passive or input sense; the presence of theta may create a background state which allows a select processing of environmental inputs, for example. This is a restatement

of a dominant view of the physiological role of theta, used recently by O'Keefe and Nadel (1978) in the most well thought-out statement to date of how theta activity might regulate the functions of the hippocampus. In this view, theta oscillations, representing alternating excitability cycles, maximize input processing during the excitability peak. Phase shifts and amplitude modulations during theta permit a systematic selection of which hippocampal elements will be activated during the excitability peaks and it is this theta-determined distribution of excitatory foci in CA1 or CA3 that constitutes the "map".

On the basis of the present evidence it is impossible to comment on the map theory or on any theory which imputes an input function to the hippocampus during theta, whether it be the processing of time, space, or other environmental regularities. What the present evidence is capable of commenting on is the nature of those behaviors that make use of information gathered by the hippocampus. For example, O'Keefe and Nadel have proposed that environmental events which don't match the internal map result in firing of p. cells which activate motor circuits controlling exploration. The present findings indicate tentatively that firing of p. cells during exploration may be relatively ineffective in the direct modulation of that behavior and that such firing represents the decoupling of pyramidal cells from circuits controlling behavior. It is more likely that p. cell discharge during uncertainty leads to a decline in ongoing behavior.

Figure 1. A. Dorsolateral view of exposed hippocampus and septum of rat. The neocortex has been torn away as far laterally as the rhinal fissure. The corpus callosum has been peeled away. The caudate and thalamus have been removed from the right hemisphere to show the ventral tip of the hippocampus. Two sections through the hippocampus are schematically depicted, oriented at a lamellar plane. The slice labelled X has been magnified and labelled in Fig. 2A to show the internal structure of the hippocampal formation. Several fibre systems carrying hippocampal efference are also depicted: the fimbria at the lateral edge, the dorsal fornix, schematically represented by the dashed line along the midline, and the alveus, represented by dashed lines running along the surface of the left hippocampus.

B. Sagittal section of rat brain about .240 mm from the midline (adapted from König & Klippel, 1963). The septal area is stippled. The figure attempts to show the approximate trajectory of the components of the fornix system.

Abbreviations: AC, anterior commissure; Dorsal Comm, dorsal hippocampal commissure; LS, lateral septum; MS, medial septum; nDBB, nucleus of the diagonal band; POF, postcommissural fornix; PRF, precommissural fornix; Ventral Comm, ventral hippocampal commissure.

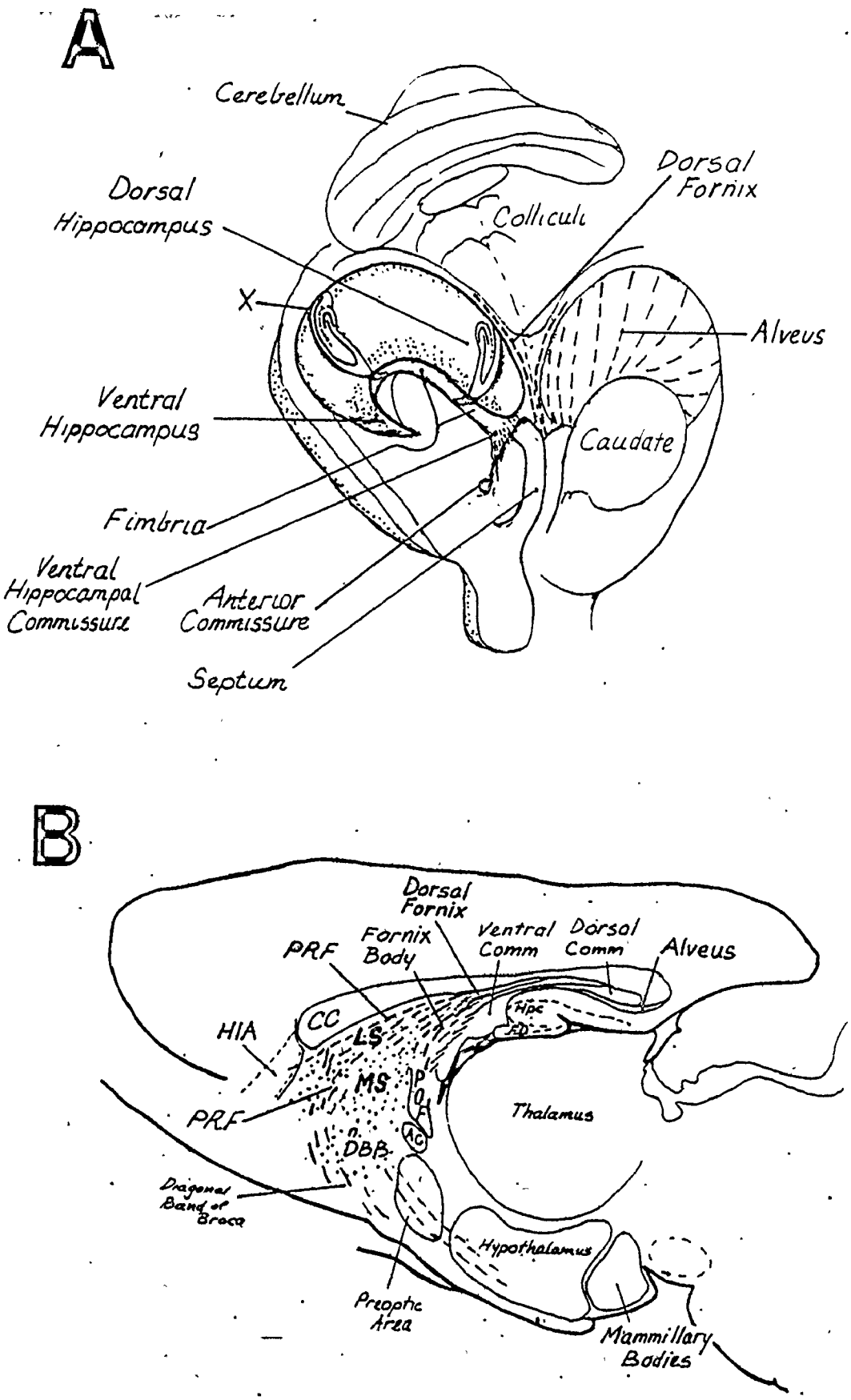

A
Cerebellum
Dorsal Hippocampus
Colliculi
Dorsal Fornix
X
Alveus
Ventral Hippocampus
Caudate
Fimbria
Ventral Hippocampal Commissure
Anterior Commissure
Septum
B
Dorsal Fornix
Fornix Body
Ventral Comm
Dorsal Comm
Alveus
PRF
Hpc
HIA
CC
LS
MS
PRF
POA
AC
DBB
Thalamus
Diagonal Band of Broca
Hypothalamus
Preoptic Area
Mammillary Bodies

Fig. 1

Figure 2. A. Horizontal section of hippocampal formation at level of section labelled X in Figure 1A. The section illustrates a number of features: 1. the relationship of the hippocampus to the cortical systems in which it lies. Caudally entorhinal, parasubicular and presubicular cortices are found. 2. Hippocampal divisions (subiculum, Ammon's horn, and the dentate area) are separated by arrows. 3. The major cell fields of Ammon's horn, CA1-4 (CA=Cornu Ammonis). 4. The different layers.

B. Coronal section through anterodorsal hippocampus showing CA fields. (Figure 13A, B shows photographs of histologically prepared coronal sections.)

C. Coronal section through anterior septum showing some dominant nuclei.

D. Coronal section through mid-portion of septum.

Abbreviations: AC, anterior commissure; ACC, nucleus accumbens; Al, alveus; BST, bed nucleus of stria terminalis; CC, corpus callosum; DBB, nucleus of diagonal band of Broca; FD (b), buried blade of dentate area; FD (e), exposed blade of dentate; g., granule cell layer of hippocampus; HIA, anterior continuation of the hippocampus; lac., lacunosum layer; LS, lateral septal nucleus, dorsal (D), intermediate (I), and ventral (V) divisions; MS, medial septal nucleus; mol., molecular layer; or., stratum oriens; p., pyramidal cell layer; PARA, parasubiculum; PRE, presubiculum; rad., stratum radiatum; SH, septohippocampal nucleus; SUB, subiculum.

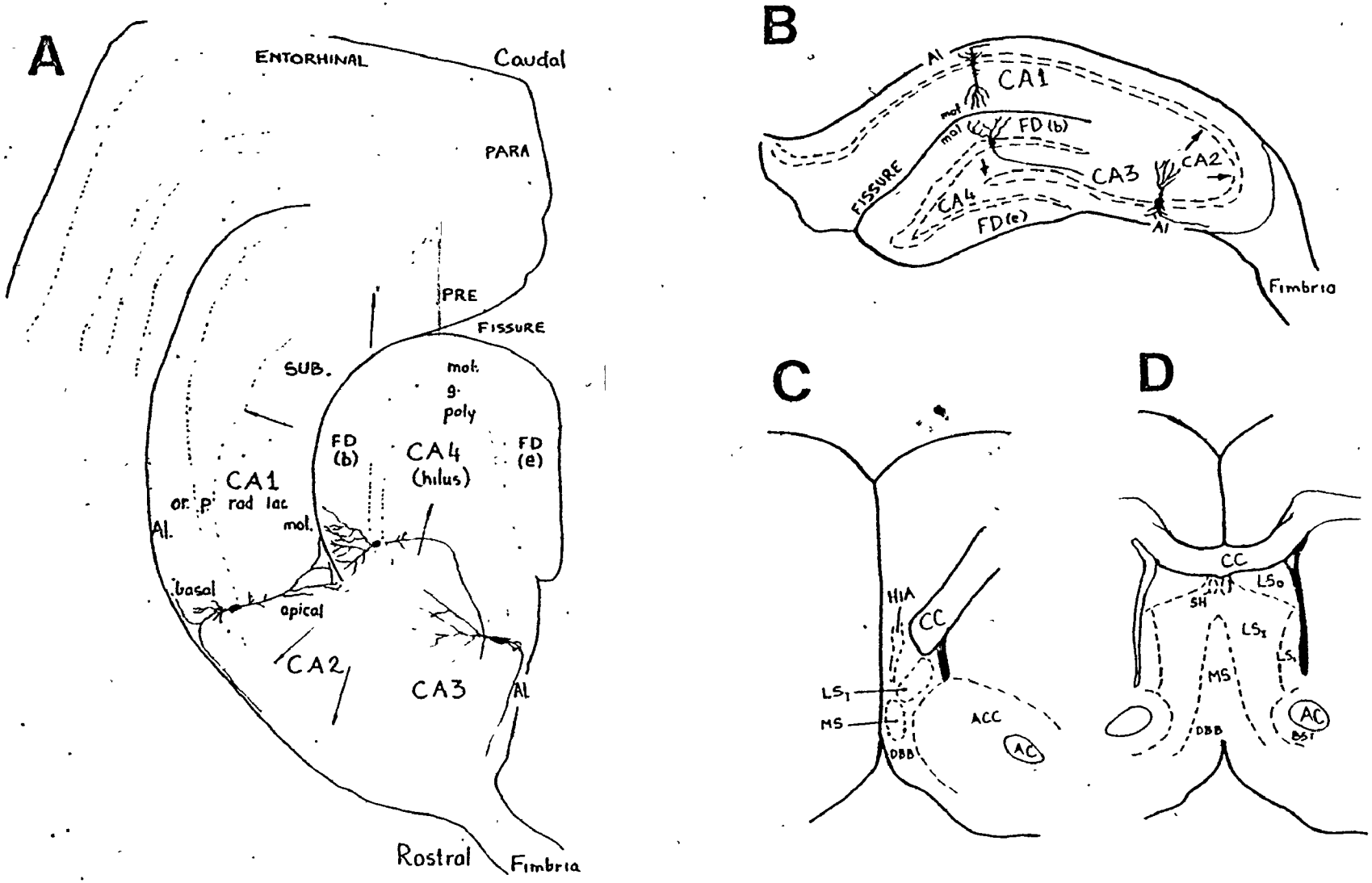

A
ENTORHINAL
Caudal
PARA
PRE
FISSURE
SUB.
mol.
g.
poly
FD
(b)
CA4
(hilus)
FD
(e)
CA1
or. p. rad lac
mol.
Al.
basal
apical
CA2
CA3
Al
Rostral
Fimbria
B
Al
CA1
mol
mol
FD (b)
FISSURE
CA3
CA2
CA4
FD (e)
Al
Fimbria
C
HIA
CC
LS
MS
ACC
AC
DBB
D
CC
LSo
SH
LSI
LS
MS
AC
BST
DBB

Fig. 2

Figure 3. Zones of termination in LSN of different sectors of hippocampus based on four different studies:
A. Raisman et al, 1966;
B. Siegel et al, 1974;
C. Meibach & Siegel, 1977;
D. Swanson & Cowan, 1977.
A and B are based on lesion-degeneration studies, while C and D are based on autoradiographic analysis. The septal sections in B and C are copied directly from summary figures in the reports, while A and D are extrapolations. The section through septum is at a mid rostro-caudal level and the numbers within the section code terminal zones corresponding to estimated sites of origin seen in the hippocampal insert. Only ipsilateral termination is depicted.

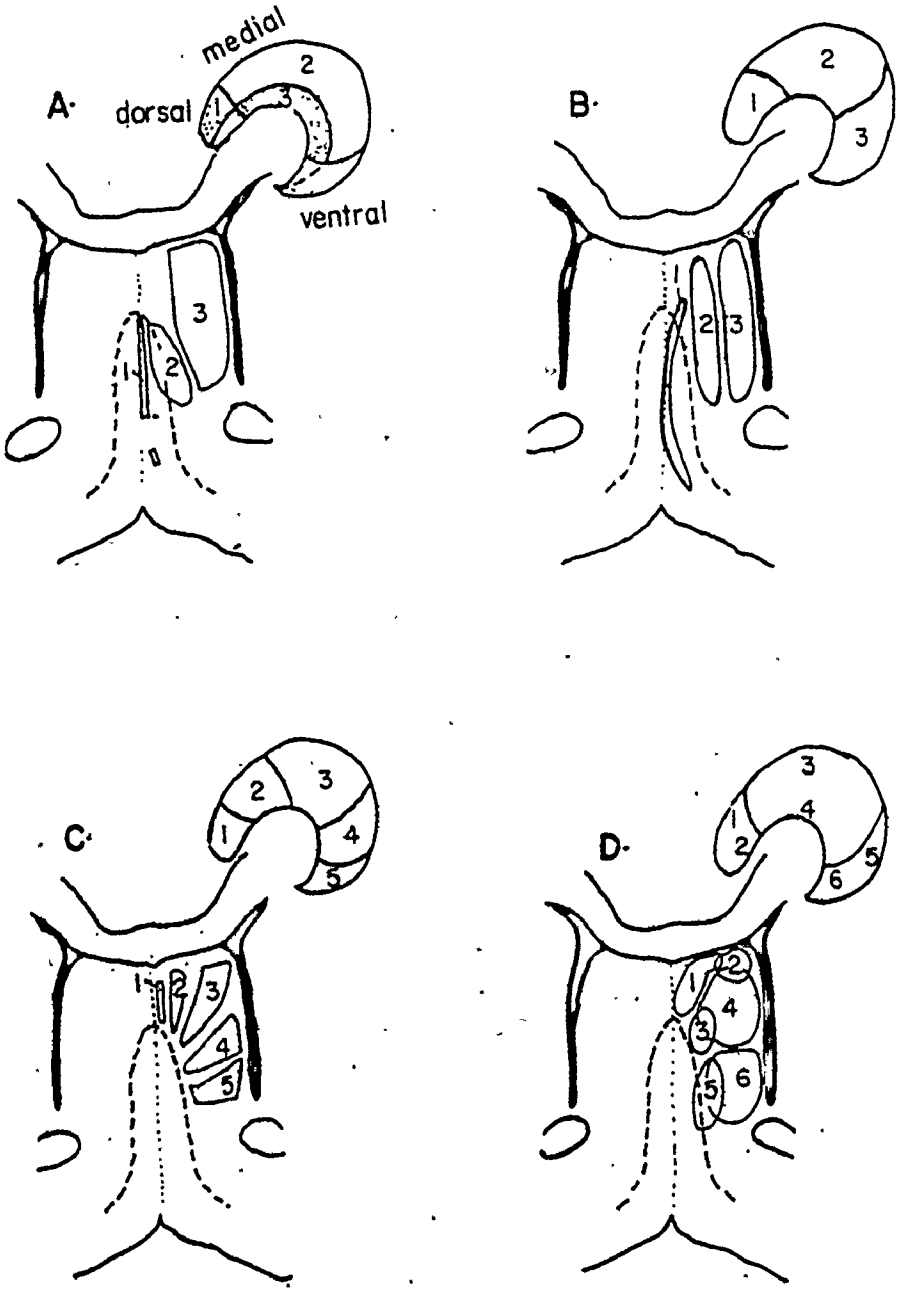


Fig. 3

Figure 4. Location of macroelectrode tips in two coronal sections through dorsal hippocampus are indicated by the squares. Numbers within the squares are rat numbers. Approximate amplitudes and frequencies of theta at each site before and after paralysis are also shown; asterisks indicate high-frequency components in the EEG and question marks indicate that theta frequency could not be determined because of these components.

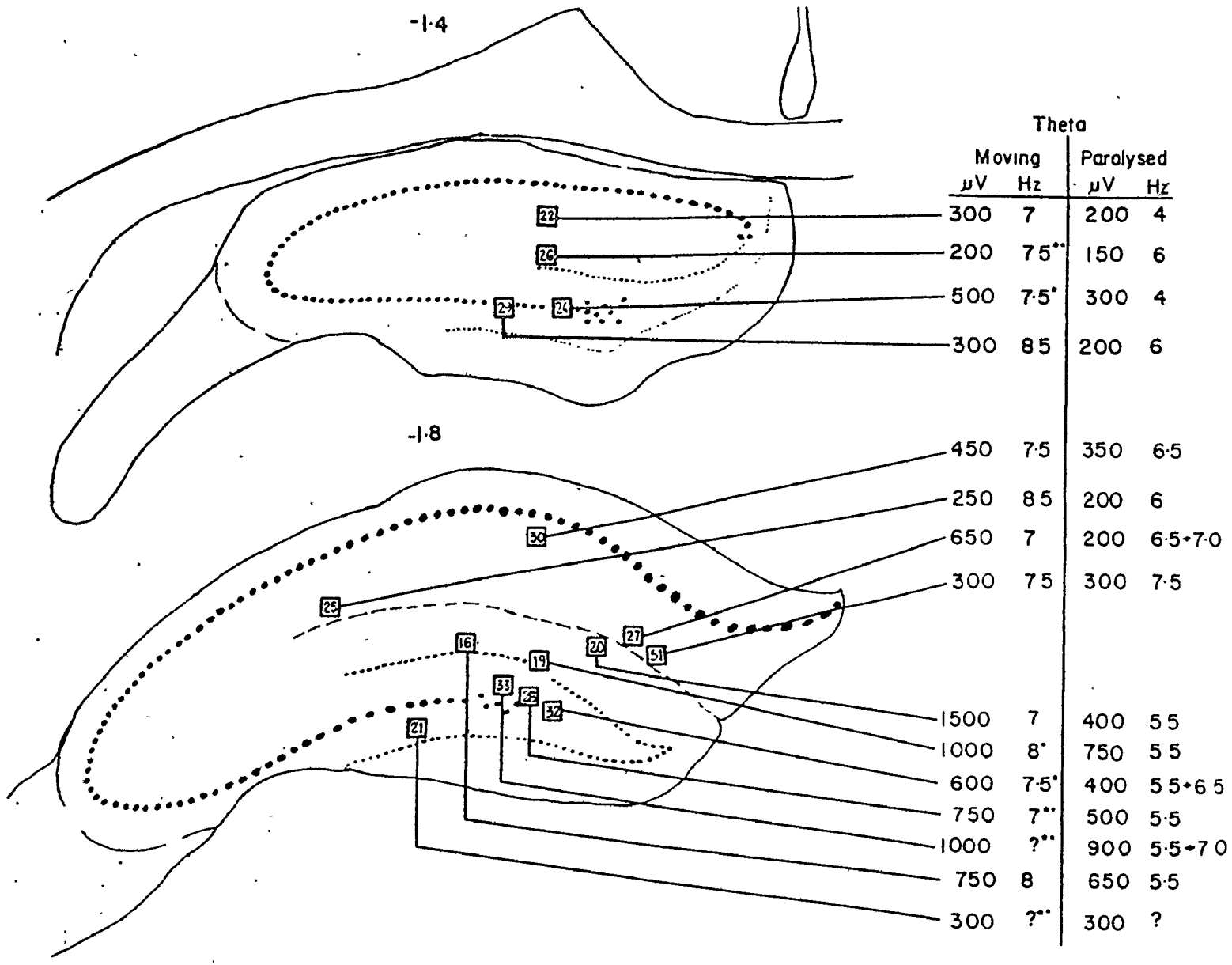

-1·4
-1·8
Theta
Moving
Paralysed
μV Hz μV Hz
300 7 200 4
200 7·5** 150 6
500 7·5* 300 4
300 8·5 200 6
450 7·5 350 6·5
250 8·5 200 6
650 7 200 6·5-7·0
300 7·5 300 7·5
1500 7 400 5·5
1000 8* 750 5·5
600 7·5* 400 5·5-6·5
750 7** 500 5·5
1000 ?** 900 5·5-7·0
750 8 650 5·5
300 ?** 300 ?

Fig. 4

Figure 5. Hippocampal EEG patterns during several different behaviors in two normal rats (12,13). Recordings in Rat 13 are from the hippocampal fissure (top trace) and skull screws in contact with dura (bottom trace). In Rat 12 all tracings are from dorsal CA1; the top two tracings are both labelled A to indicate continuity. Note occurrence of fast (θ_F) and slow (θ_S) theta.

Time marks are 1 sec apart and amplitude marks represent 300 μV except where otherwise indicated. Downward pointing arrowheads mark instants of sensory arousal. Low and high cut-off points on polygraph filters were 0.3 Hz and 3 kHz.

Normal

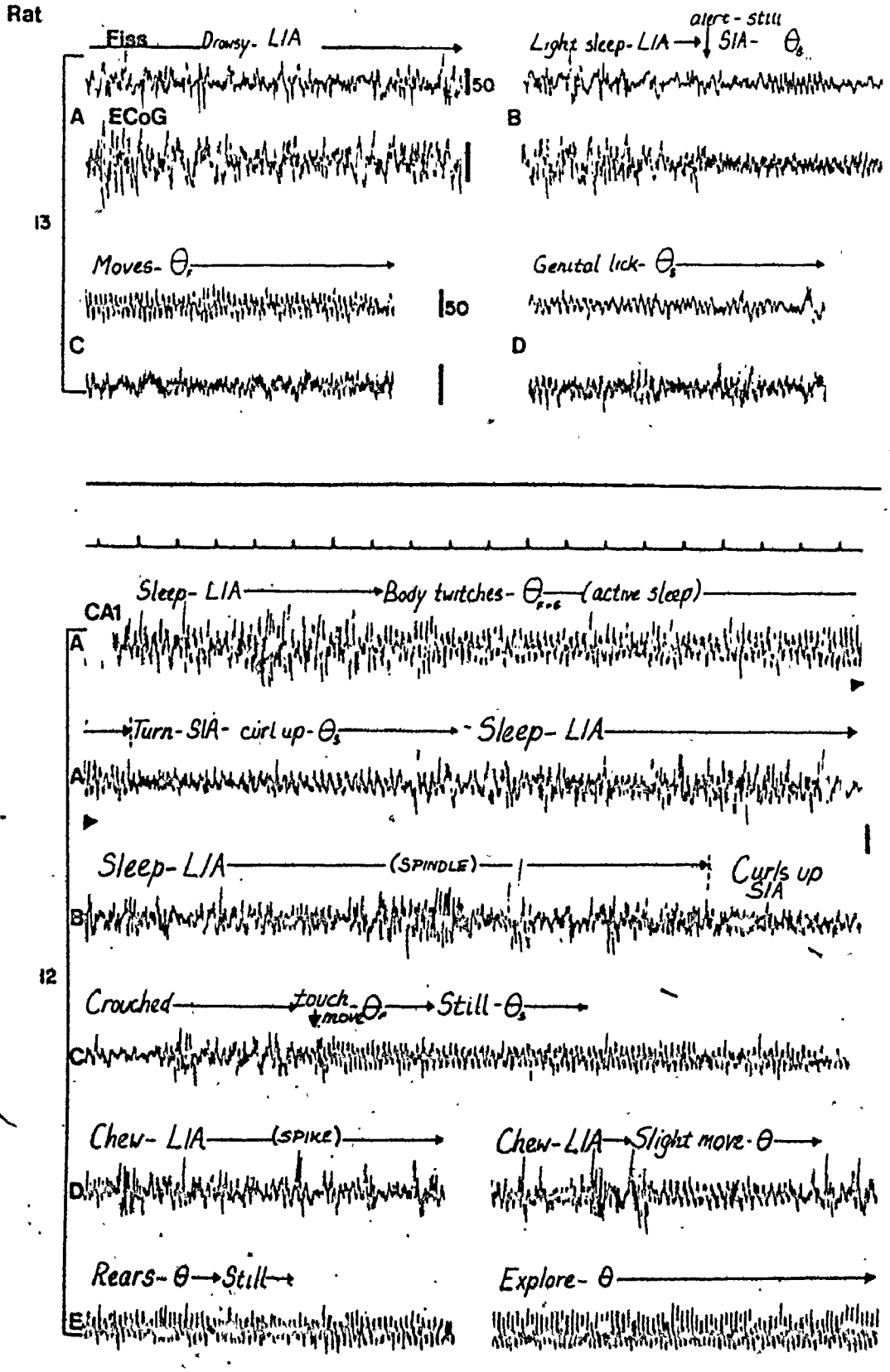

Rat
Fiss
Drowsy- LIA
Light sleep- LIA
alert - still
SIA- θs
50
A ECoG
B
13
Moves- θr
Genital lick- θs
50
C
D
Sleep- LIA
Body twitches- θf.s
(active sleep)
CA1
A
Turn- SIA- curl up- θs
Sleep- LIA
A'
Sleep- LIA
(SPINDLE)
Curls up SIA
B
12
Crouched
touch-move θr
Still- θs
C
Chew- LIA
(SPIKE)
Chew- LIA
Slight move- θ
D
Rears- θ
Still
Explore- θ
E

Fig. 5

Figure 6. ANECTINE. Effects of Anectine on EEG of four rats (22,25,30,20). Pre-Anectine recordings were made during vigorous exploration of the rat. Rat 22 showed the largest reduction in theta frequency and Rat 20 in amplitude. Tracing labelled A for Rat 20 was recorded about 30 min after Anectine, before insertion of the microelectrode. Tracings labelled B were recorded about 11 hours after Anectine from the gross (g) and micro (m) electrodes.

Time marks= 1 sec. Voltage bars= 300 μV. Filters .3 Hz to 3 kHz. Arrowheads mark delivery of peripheral stimulation.

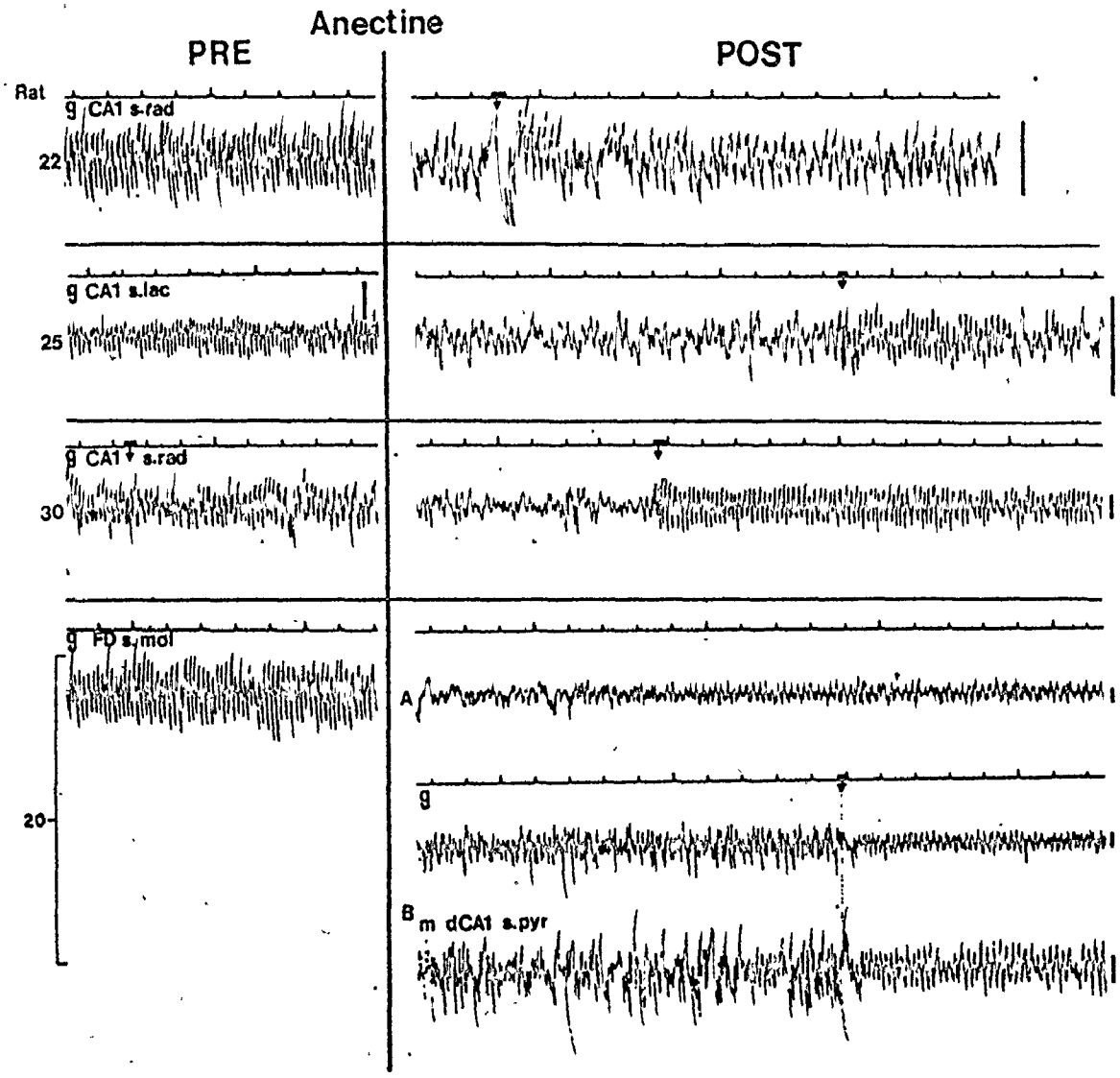

Anectine
PRE
POST
Rat
g CA1 s.rad
22
g CA1 s.lac
25
g CA1 s.rad
30
g FD s.mol
A
20
g
B m dCA1 s.pyr

Fig. 6

Figure 7. Effects of Anectine on high frequency activity recorded from the hilar region of dorsal hippocampus in two rats (28, 33).

Tracings before paralysis (PRE) were recorded during active exploration by the rat. Trace A was recorded whith high frequency filter set to attenuate frequencies above 3 kHz; in trace A1 it was set at 15 Hz.

Tracings during paralysis were recorded at various times following administration of Anectine. Panels labelled D for Rat 28 show continuous tracings from both gross and microelectrodes about 4 hr after Anectine. Note reinstatement of high frequency activity at the gross electrode while the microelectrode shows clear transitions from theta (probably during active sleep) to sleep-associated LIA and to arousal-induced theta.

Time and gain calibration marks same as Figure 6. Filter settings were the same throughout, 0.3 Hz-low and 3kHz-high, except in tracings labelled A1, wherein the high-frequency cut-off is at 15 Hz.

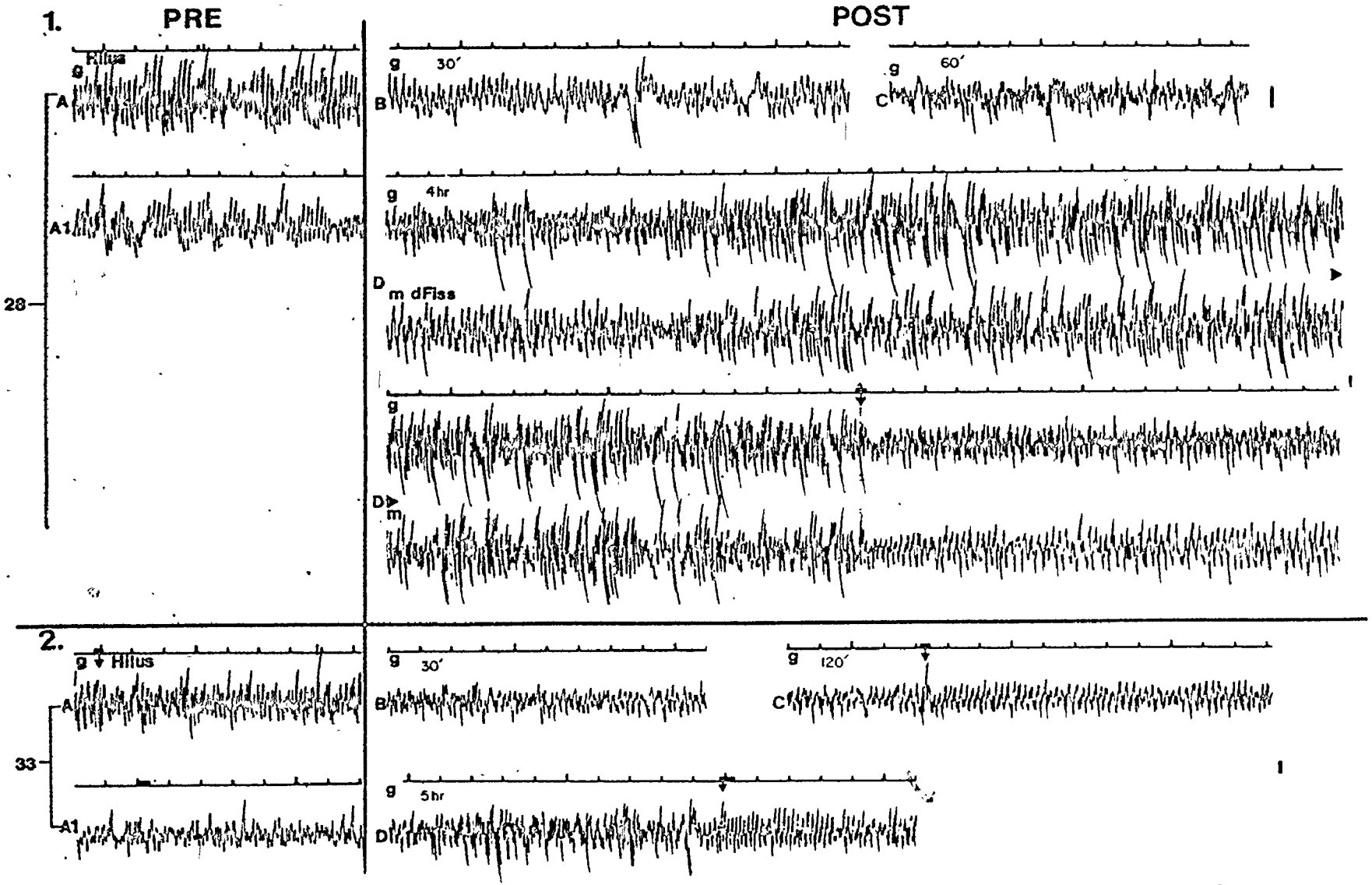

1.
PRE
POST
g
Hilus
A
A1
28
B
g 30'
C
g 60'
g 4hr
D
m dFiss
g
D
m
2.
g Hilus
A
A1
33
B
g 30'
C
g 120'
g 5hr
D

Fig. 7

Figure 8. Effects of atropine sulphate (25 mg/kg) on theta in four paralyzed rats: #'s 1, 4, 33, and 3.

Column 1 is normal EEG during vigorous movement.

Column 2 is EEG at times subsequent to paralysis induction indicated above the trace. In each trace theta is produced by blowing on the rat at times indicated by the arrows.

Column 3 tracings were recorded during paralysis at various times subsequent to atropine sulphate. Blowing is indicated by arrows.

For rat 3, trace a of column 2 represents theta 55 minutes after paralysis with core temperature of 38.6° Centigrade. In trace b, recorded 15 minutes after application of an ice pack, core temperature has dropped to 36.5°C. These times are indicated above the tracing. Again, theta in these tracings was produced by blowing.

In traces b and c of rat 1 anectine infusion has been disconnected for 15 and 40 minutes respectively; artificial respiration was still required. In trace d the rat was mobile again.

The bottom trace, labelled 1a represents EEG activity during normal movement and 30 minutes subsequent to atropine administration. The rat was moving at the times marked by black bars above the trace.

Time marks are one sec apart and voltage bars are 300 μV. Filters set at 1 Hz and 3kHz.

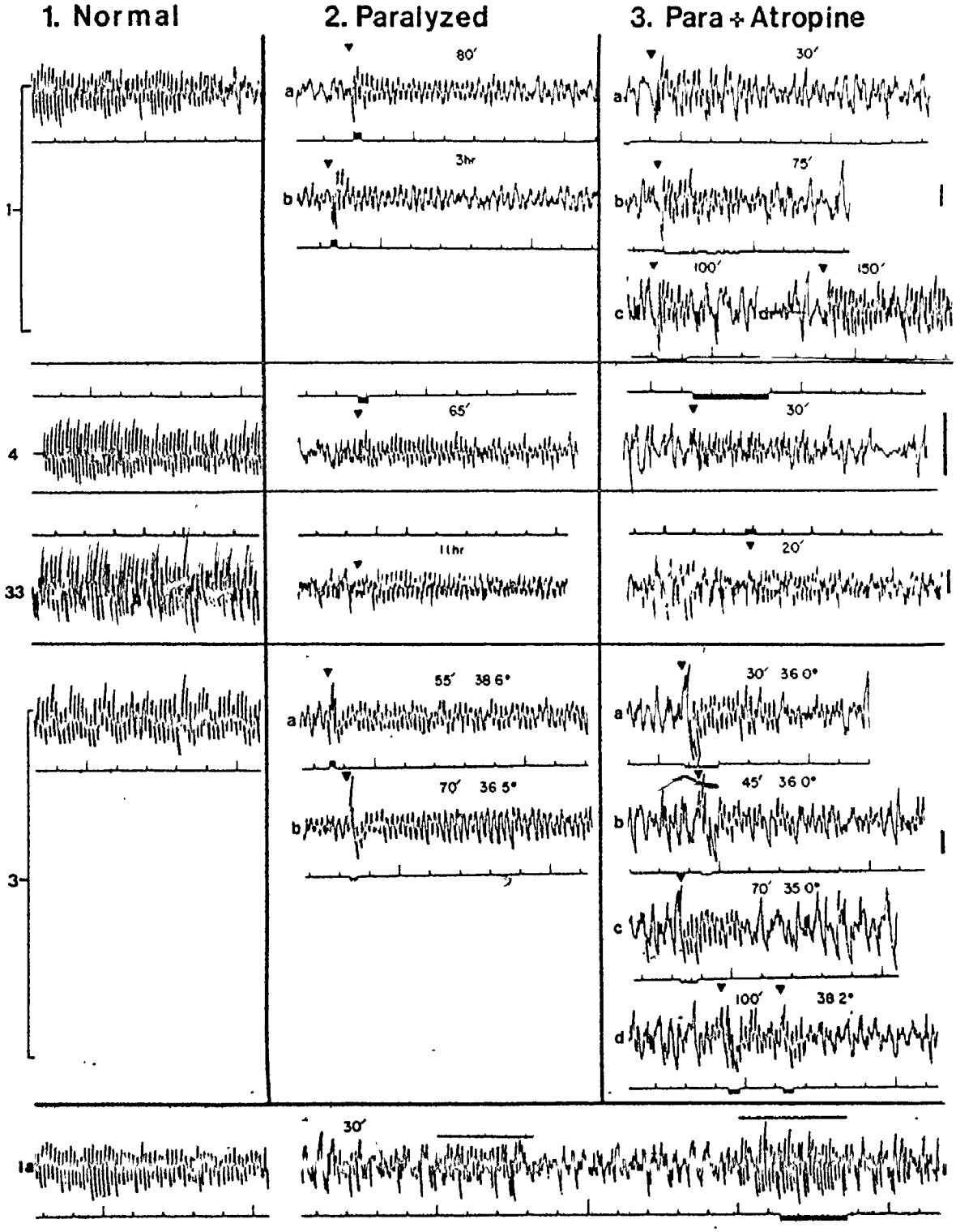

1. Normal
2. Paralyzed
3. Para + Atropine
80′
3hr
30′
75′
100′
150′
65′
30′
11hr
20′
55′ 38 6°
70′ 36 5°
30′ 36 0°
45′ 36 0°
70′ 35 0°
100′ 38 2°
30′
1
4
33
3
1a
a
b
c
d

Fig. 8

Figures 9-10. A cell activity.

Figure 9. TIH's and AUTO's of three different Type A cells found in the pyramidal cell layer of Ammon's horn. The top two histogram pairs are representative of spontaneous activity of A cells. The bottom histograms are associated with slow theta rhythm in the EEG.

In this and following histogram figures each histogram pair represents the activity of a cell during a sample run as defined in the text; m, c, and n are the mean ISI, coefficient of variation, and the number of spikes counted for the particular cell represented. Bin width is 12.8 msec for all histograms, unless otherwise indicated. The last bin of the TIH contains intervals greater than 816 msec; the first bin of the AUTO, which normally holds the total number of spikes counted, has been omitted. Segments of raw discharges giving rise to the histograms are shown in Figure 9 (Strips 1,2, and 5).

Figure 10. Strips 1-3 show selected segments of raw activity of three different Type A cells. The cell in strip 1 was located in dorsal hippocampus; the strip 2 cell was in ventral hippocampus. In strip 3 the largest spike in the mixed burst was processed. Strip 4 provides an example of a "noisier" record in which the largest spikes (marked by dots) were processed. Strip 5 was selected to show regularization of Type A discharges. Strip 6 shows three single high speed sweeps with the oscilloscope beam triggered by the large complex cell of Strip 1. The one second calibration mark applies to strips 1-5. The noise level in this and following photographs represents approximately 50 microvolts. Negative is up.

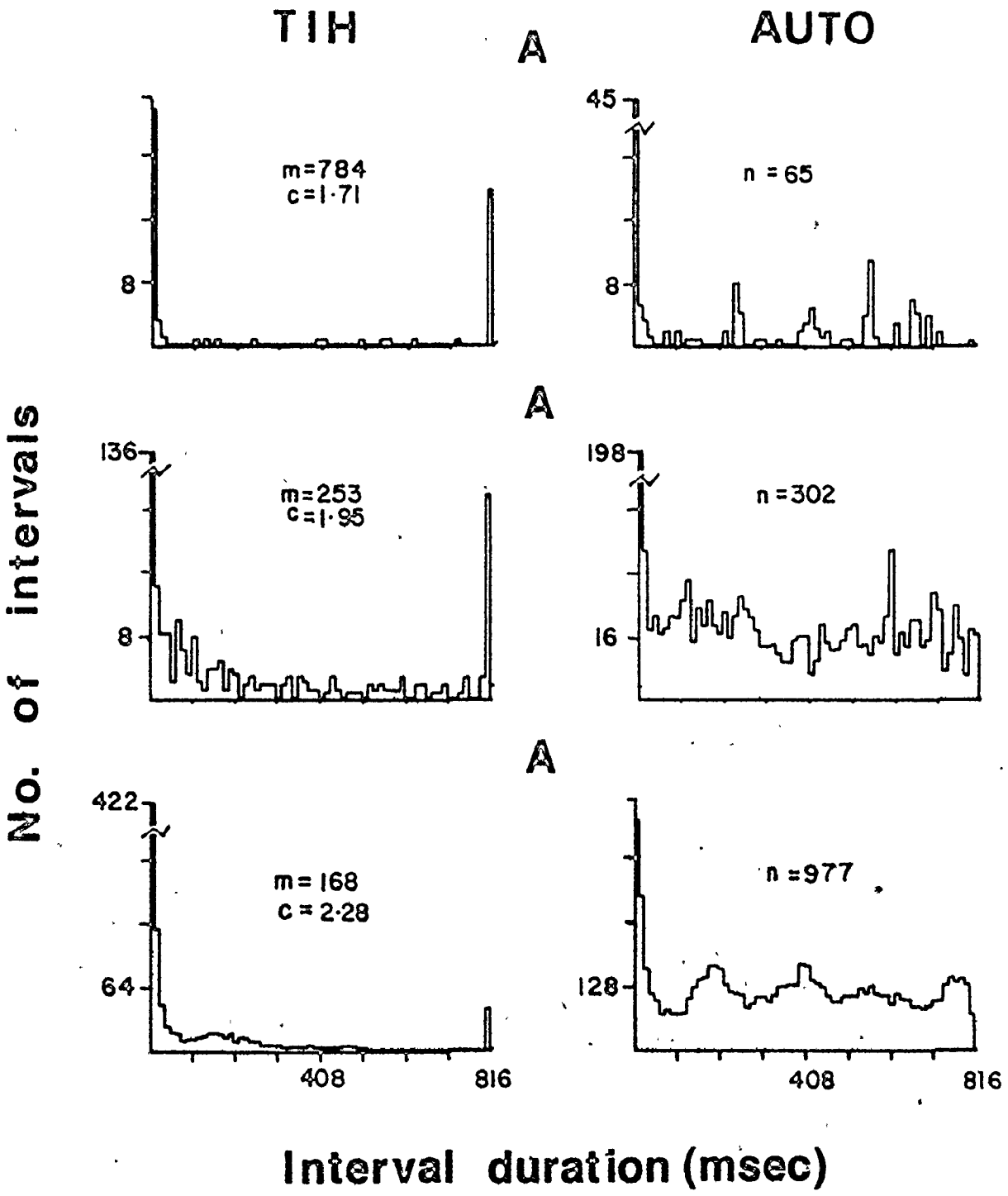

TIH
A
AUTO
m=784
c=1·71
8
45
n = 65
8
A
136
m=253
c=1·95
8
198
n =302
16
A
422
m = 168
c = 2·28
64
n =977
128
408
816
408
816
No. of intervals
Interval duration (msec)

Fig. 9

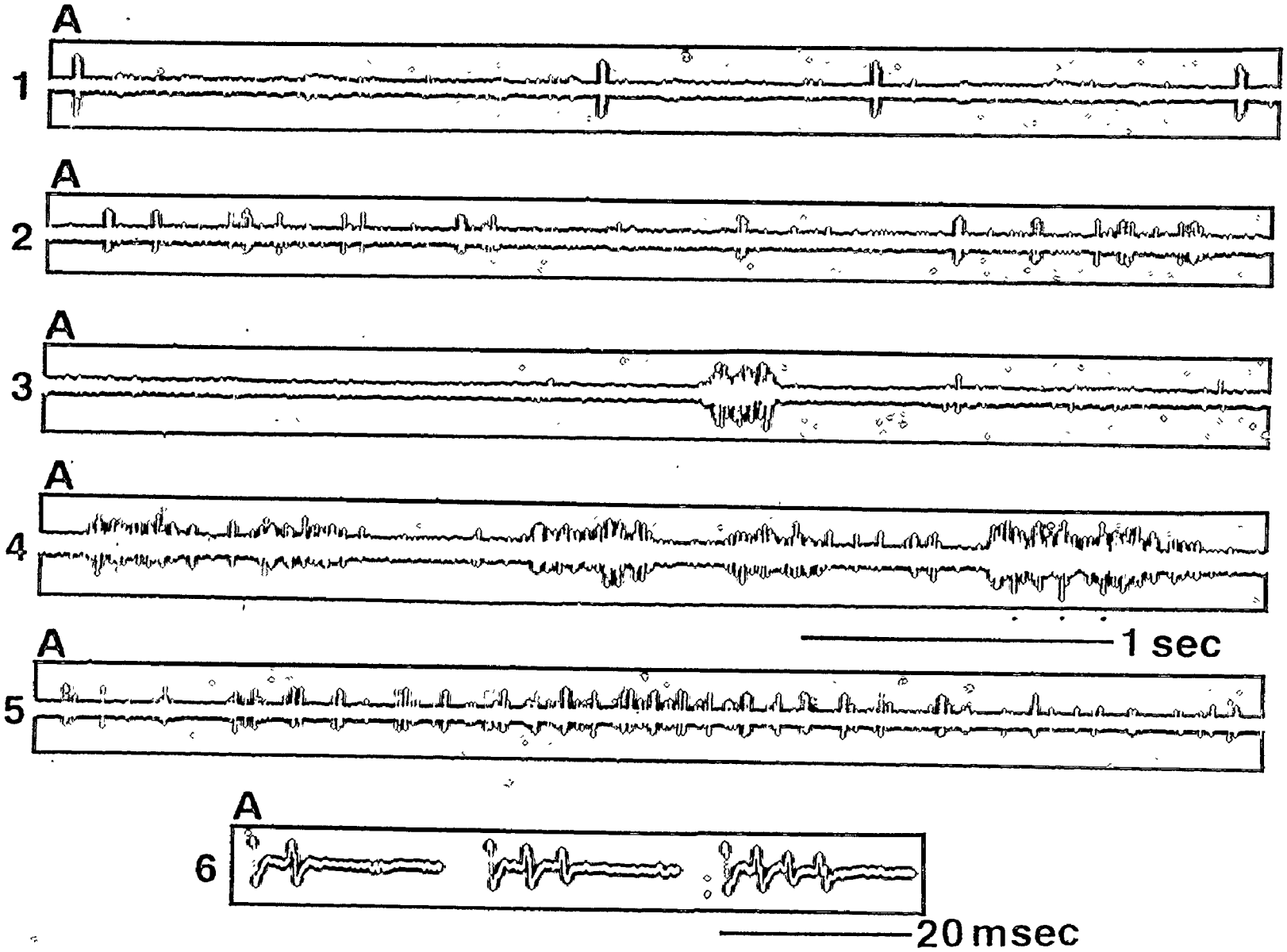


Fig. 10

Figures 11-12. B cell activity.

Figure 11. TIH's and AUTO's for two different B cells. The top histograms are based on the firing patterns of smaller cells in the hippocampal record during arousal while the middle histograms depict the activity in spontaneous states. The bottom histogram pairs are for a cell in the medial septal area.

Figure 12. Raw records of Type B cells. The theta cell of strip 1 is fairly typical of the B type seen in Ammon's horn. Processing of this cell required electronic elimination of the larger cells, not seen in this particular segment. The B cell of strip 2 was located in the dentate area where the best isolation of hippocampal B cells was usually obtained. The B2 cell of strip 5 was in Ammon's horn.

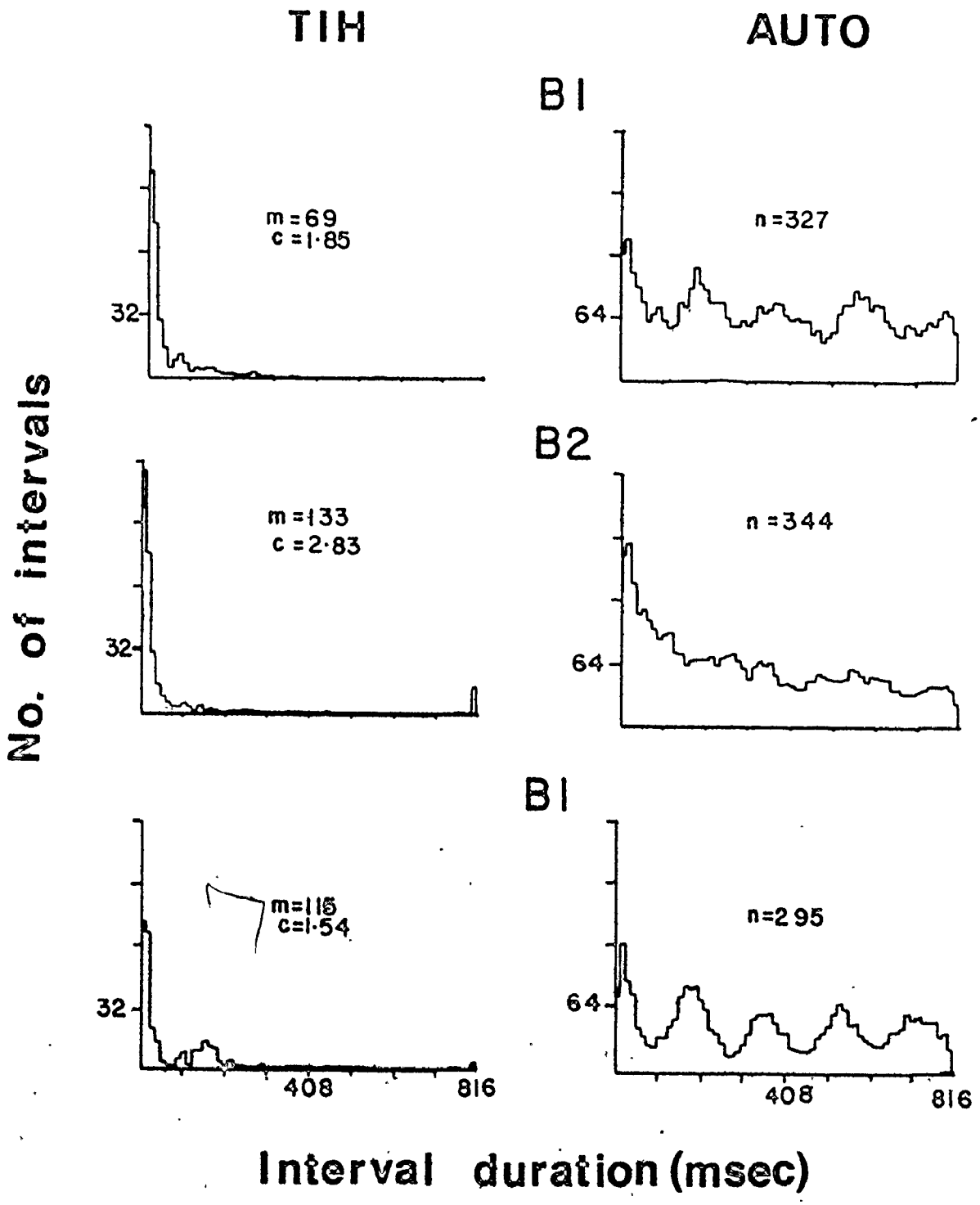

TIH
AUTO
B1
m=69
c=1·85
n=327
32
64
B2
m=133
c=2·83
n=344
32
64
B1
m=116
c=1·54
n=295
32
64
408
816
408
816
No. of intervals
Interval duration (msec)

Fig. 11

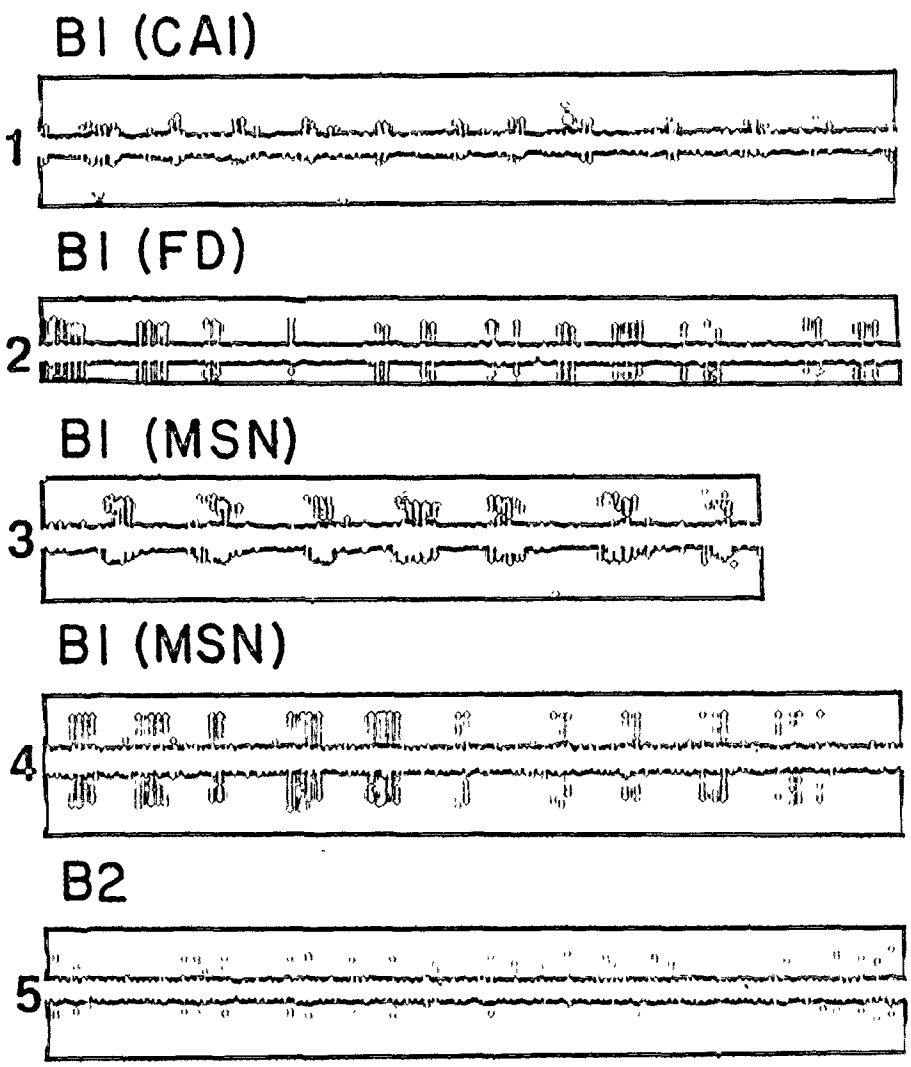


Fig. 12

Figures 13-16. C cell activity.

Figure 13. TIH's and AUTO's of C1, C2, and C3 firing subtypes in septal units.

Figure 14. TIH's and AUTO's representing C4 and C5 discharge patterns.

Figure 15. TIH's and AUTO's based on activity of three different cells of the C6 subtype. The two bottom histogram pairs represent the activity of the same cell; the time resolution of the bottom pair was increased to 8.0 msec. Effective bin width for the bottom TIH is 16 msec; for the AUTO it is 128 msec.

Figure 16. Photographs of discharges of seven different cells in various C modes of firing.

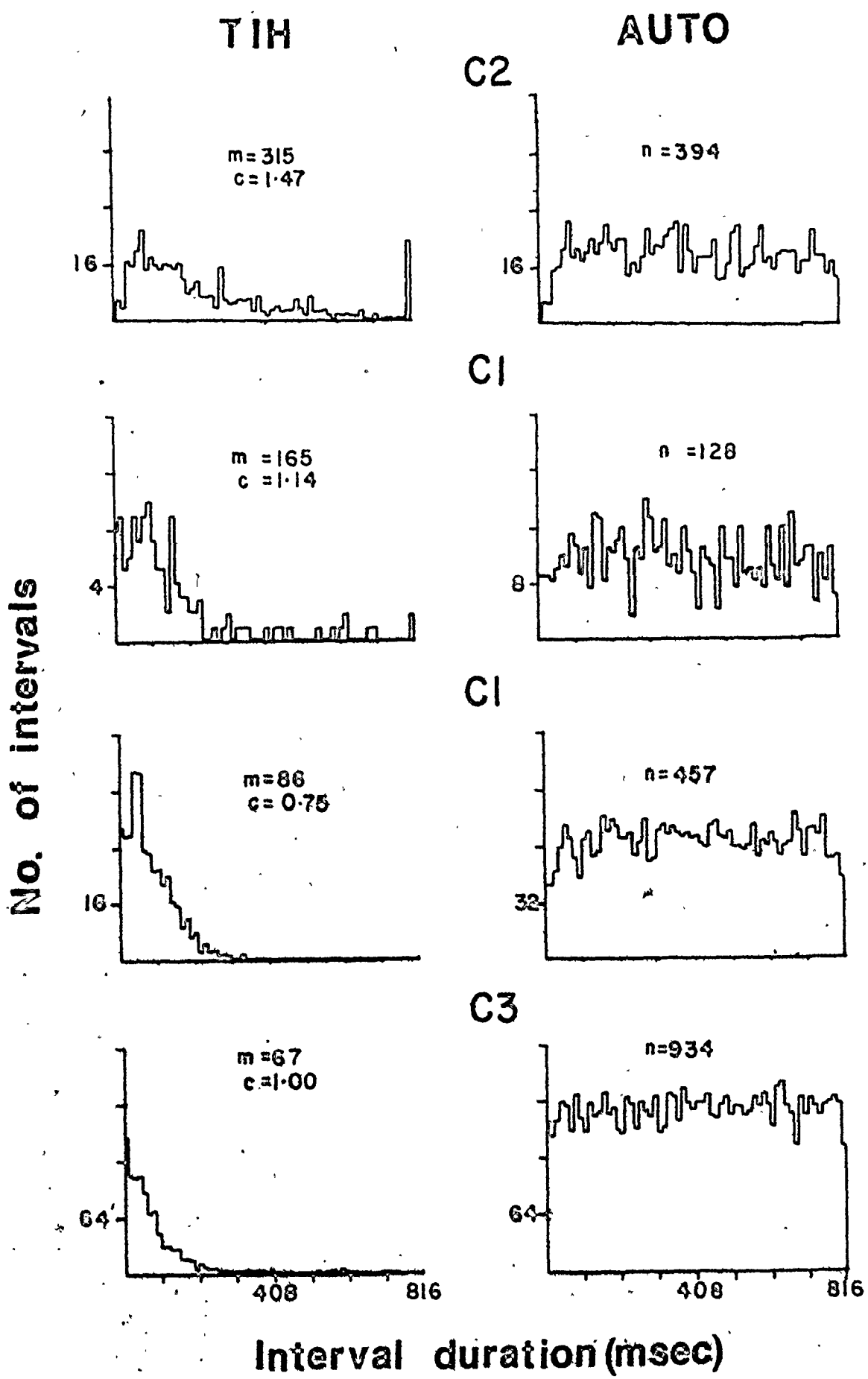

TIH
AUTO
C2
m=315
c=1·47
16
n=394
16
C1
m =165
c =1·14
4
n =128
8
C1
m=86
c= 0·75
16
n=457
32
C3
m =67
c =1·00
64
408
816
n=934
64
408
816
No. of intervals
Interval duration (msec)

Fig. 13

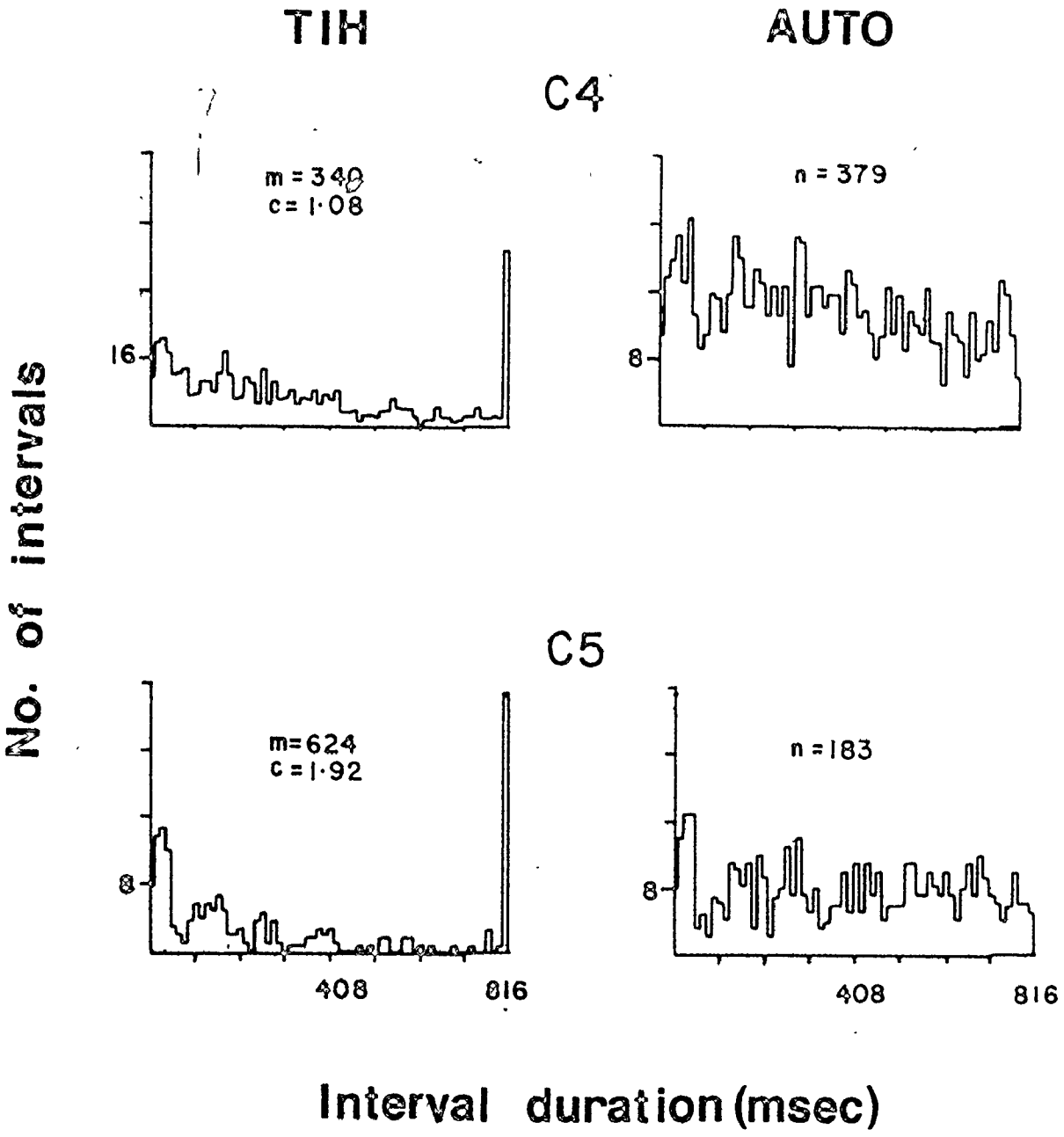

TIH
AUTO
C4
m = 348
c = 1·08
n = 379
16
8
No. of intervals
C5
m=624
c = 1·92
n = 183
8
8
408
816
408
816
Interval duration (msec)

Fig. 14

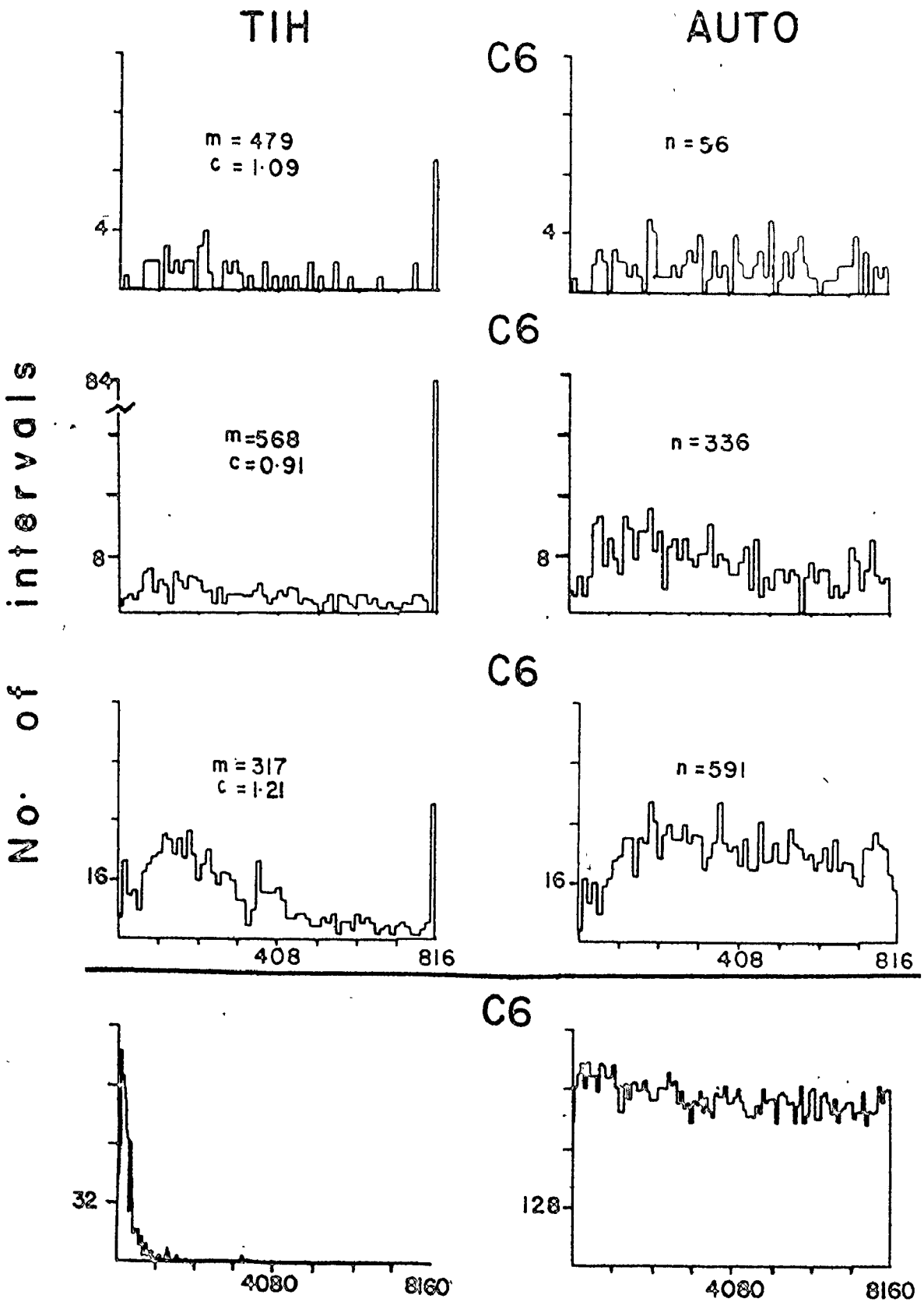

TIH
AUTO
C6
m = 479
c = 1·09
n = 56
4
C6
84
m=568
c=0·91
n = 336
8
No. of intervals
C6
m = 317
c = 1·21
n = 591
16
408
816
C6
32
128
4080
8160
Interval duration (msec)

Fig. 15

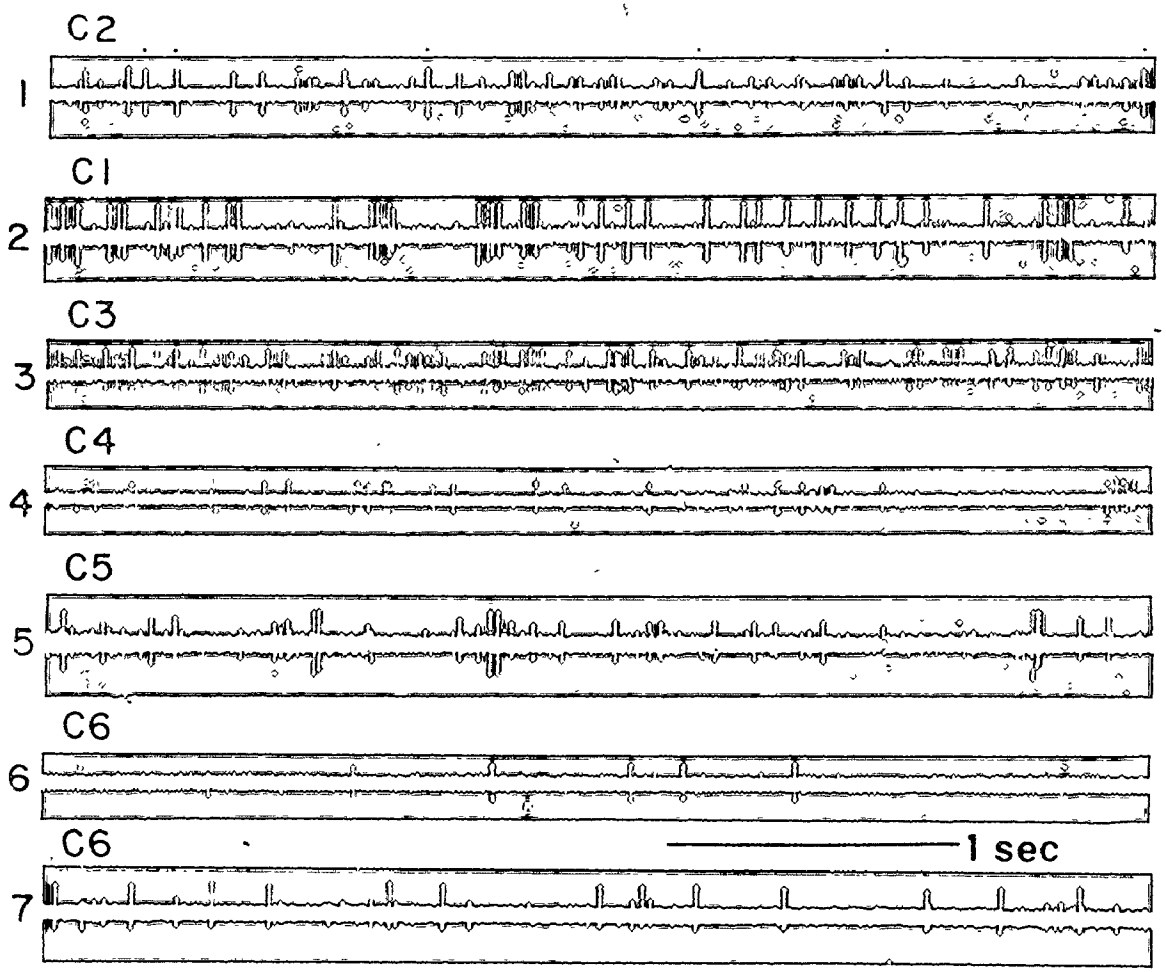


Fig. 1b

Figure 17. Photomicrographs of hippocampal brain sections containing portions of the microelectrode track. Short arrows point to vicinity of track end.

A. Track in medial anterior CA1.

B. Two closely adjacent tracks in lateral anterior CA1.

C. Track passing through CA3 and CA1 of ventral hippocampus.

D. A more difficult to see track passing through ventral hippocampus of a different rat.

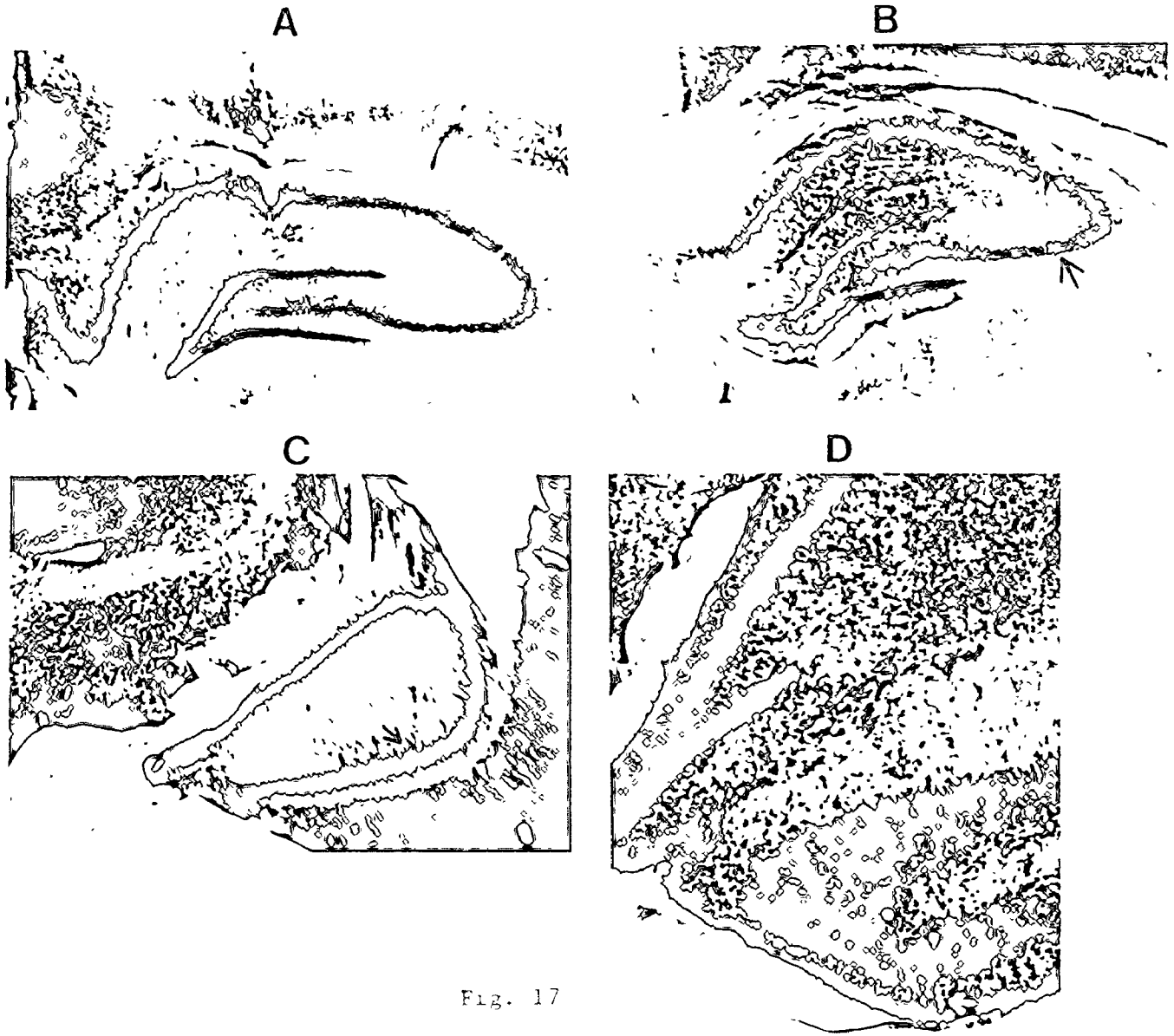


Fig. 17

Figure 18. Estimated anatomical loci of 91 cells sampled during hippocampal penetrations are marked by different geometrical shapes on four representative planes. The numbers in the upper left of each section correspond to the Pellegrino and Cushman (1967) frontal plane co-ordinates. The different shapes correspond to different firing modes or types generated.

△, Type A; ○, Subtype B1; ⊙, Subtype B2; □, Subtype C1; ⊡, Subtype C2; ◪, Subtype C3; ▣, Subtype C4; ◇, Subtype C5; ⌂, Subtype C6; ⊘, Type D; ✣, Type E; ◪, Type F; ⬯, Type G.

Abbreviation: cc = corpus callosum

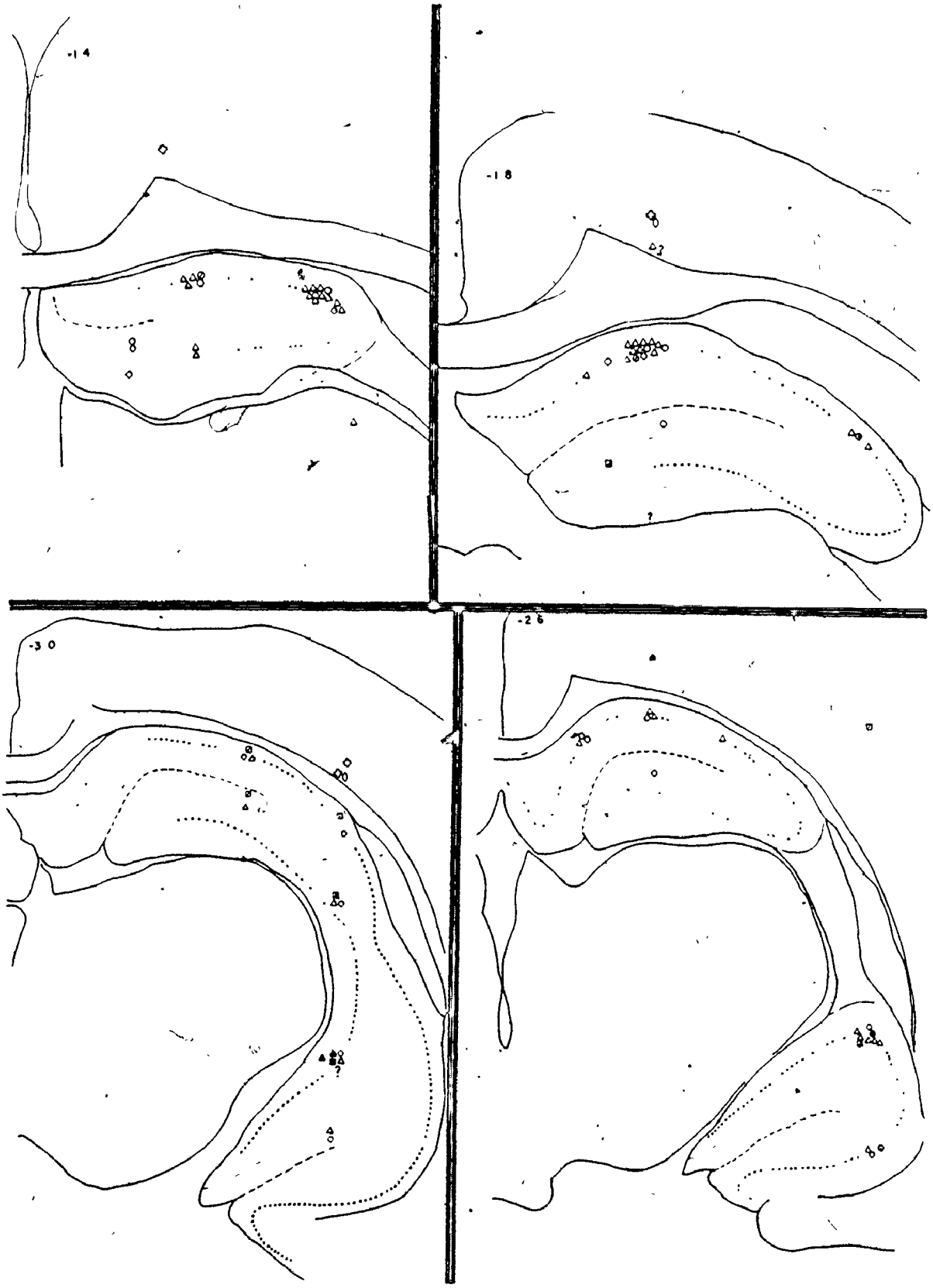


Fig. 18

Figure 19. Photomicrographs showing septal microelectrode tracks in brain sections of four different rats at representative frontal planes. Small arrows point to the vicinity of the track tip.

A. Anterior section. Track passes through anterior continuation of the hippocampus to the medial septal-diagonal band area.

B. Middle section. Track passes through the medial portion of the lateral septum and the medial septum.

C. Middle section. Track indicates that electrode extended through lateral and medial septum beyond the base of the brain.

D. Posterior section. Tip of the track may be in the medial septum.

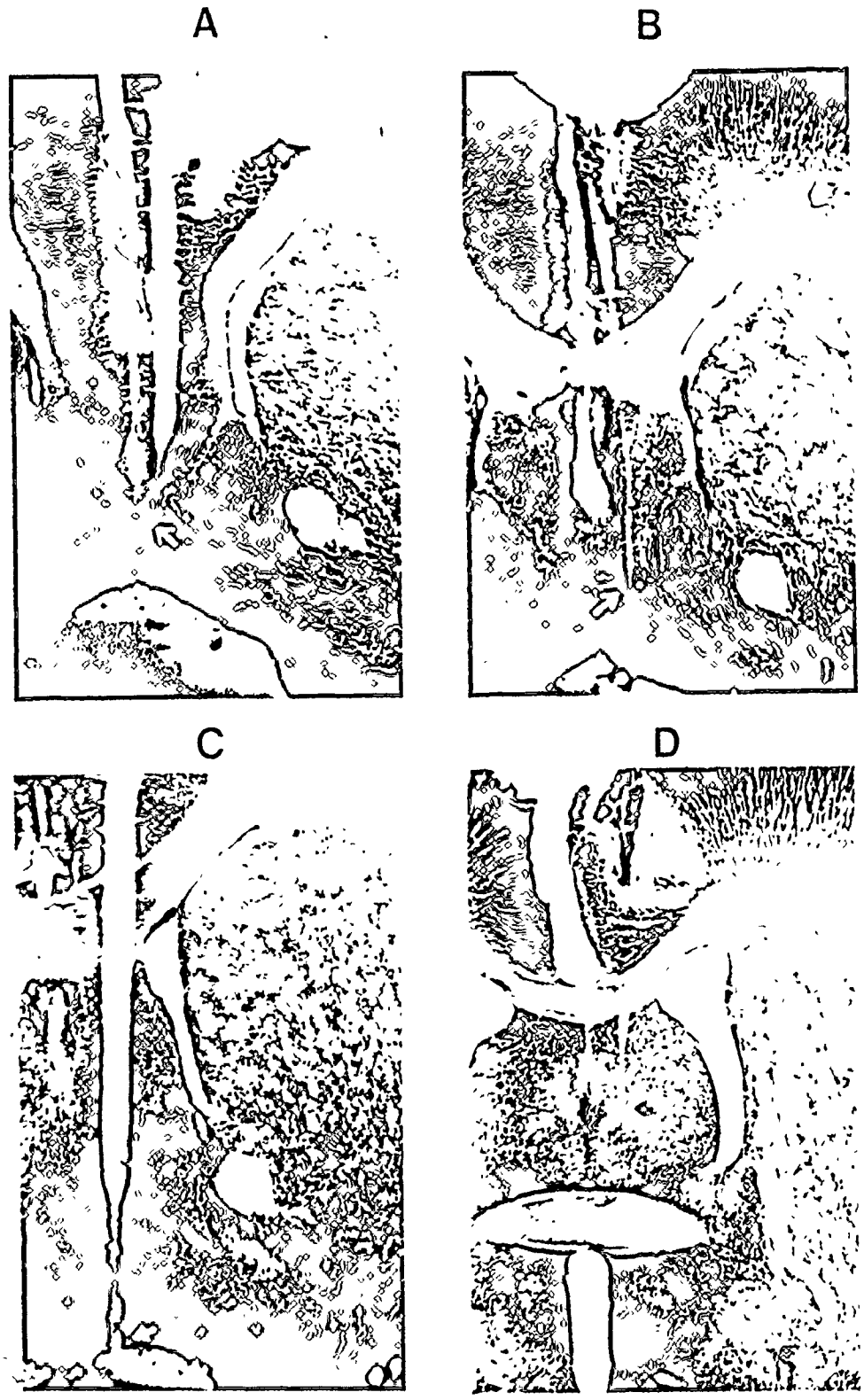


Fig. 19

Figure 20. Estimated anatomical loci of 144 cells detected in septal penetrations are marked on three representative frontal planes. Pellegrino and Cushman (1967) co-ordinates are at the upper left. As in Figure 18 the different shapes represent different discharge types. Key is given in Figure 18.

Abbreviations: ac, antérior commissure; acc, nucleus accumbens; cc, corpus callosum; lsn, lateral septal nucleus; msn, medial septal nucleus.

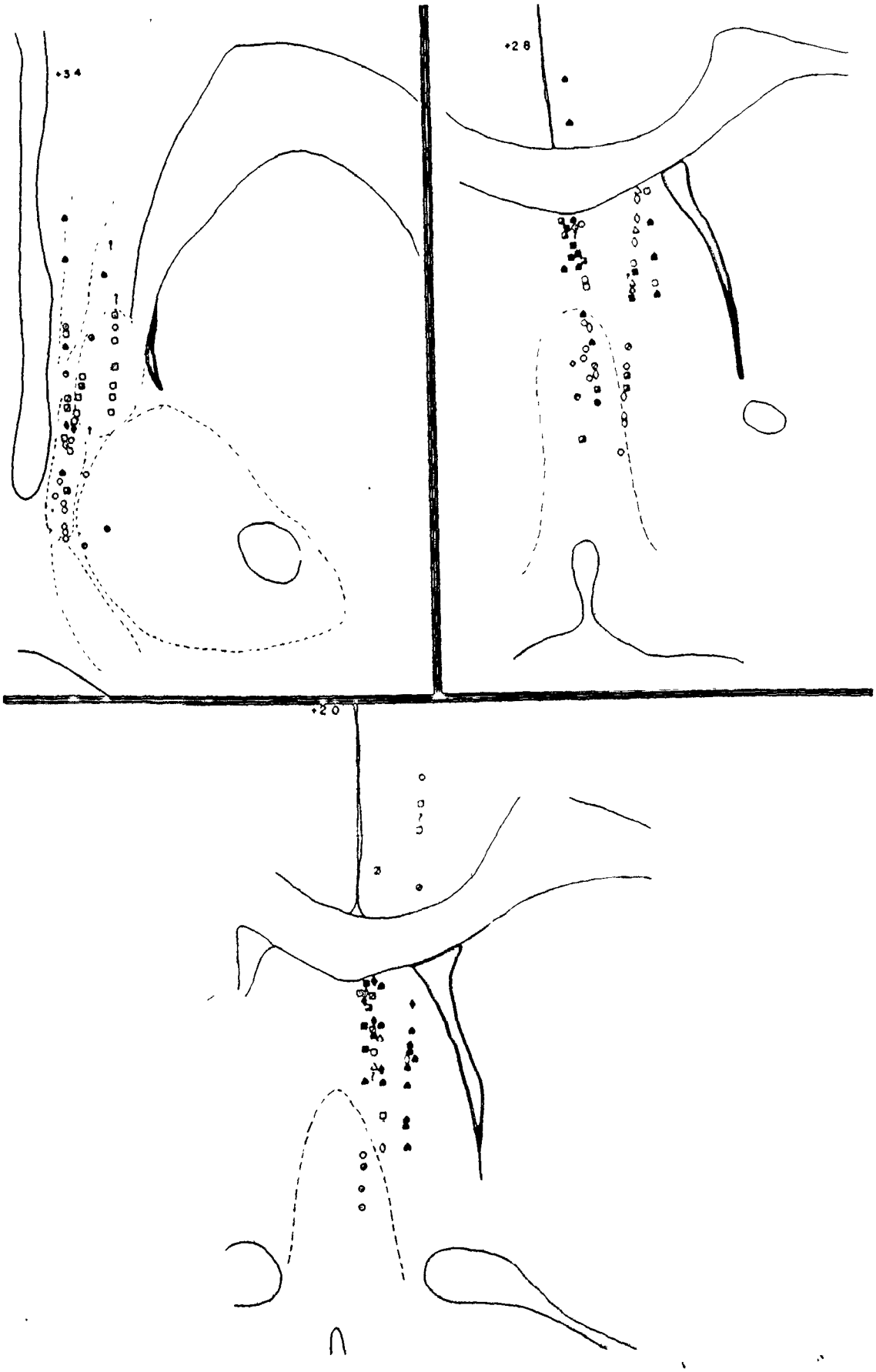


Fig. 20

Figure 21. Ratio of cells in anterior (2.6 to 3.4) and posterior (1.6 to 2.6) septum giving rise to C1 through C6 discharge patterns. The co-ordinates refer to sections in Pellegrino and Cushman (1967). The anterior portion contains 87 cells recorded in 8 rats; the posterior section was based on 50 cells in 6 rats. To arrive at the ordinate ratios computations such as the following were made for each subtype at both anterior and posterior sites:

$$\text{Subtype C1} = \frac{\text{no. of C1 cells (anterior)/total no. of C1 cells}}{\text{no. of cells (anterior)/total no. of cells}}$$

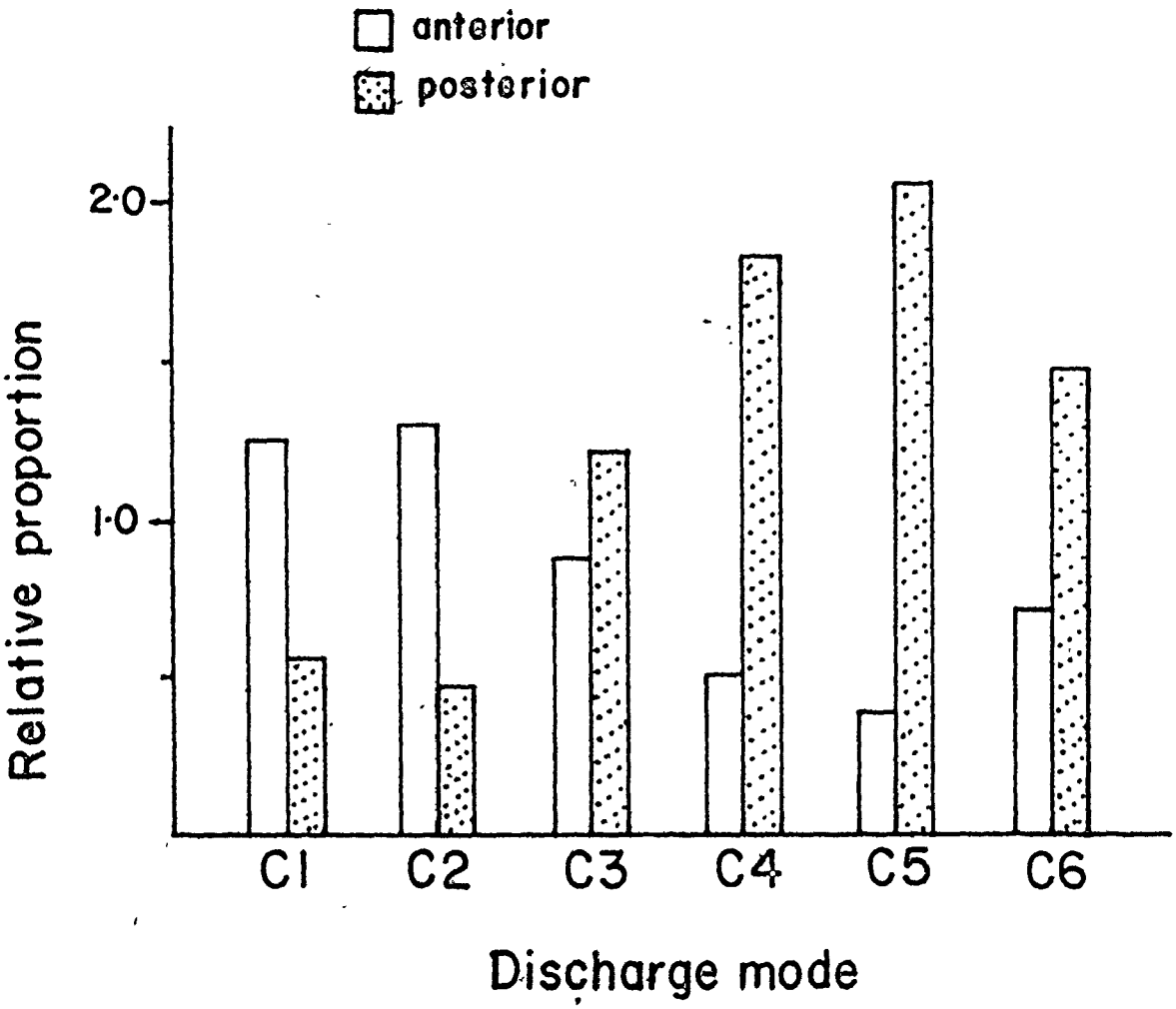

anterior
posterior
2·0
1·0
Relative proportion
C1
C2
C3
C4
C5
C6
Discharge mode

Fig. 21

Figure 22. 1. Cell 20-12A. Transition from LIA to theta produced by blowing at vertical arrow . Note sustained rhythmic discharge of B cell during theta (in phase) and suppression of large cell. Black bars above units mark periods of strong B cell firing which appear to be correlated with strong theta.
2. Cell 20-12. A cell discharging during theta elicited by blowing at arrow. Note: 1. phase relation, 2. presence of smaller amplitude cell (marked by X), 3. lack of firing during some of the theta waves (as with B cell in 1.)

In this and subsequent figures temporally successive strips are indicated by labelling with the same letter. Time calibration marks are one second unless otherwise specified.

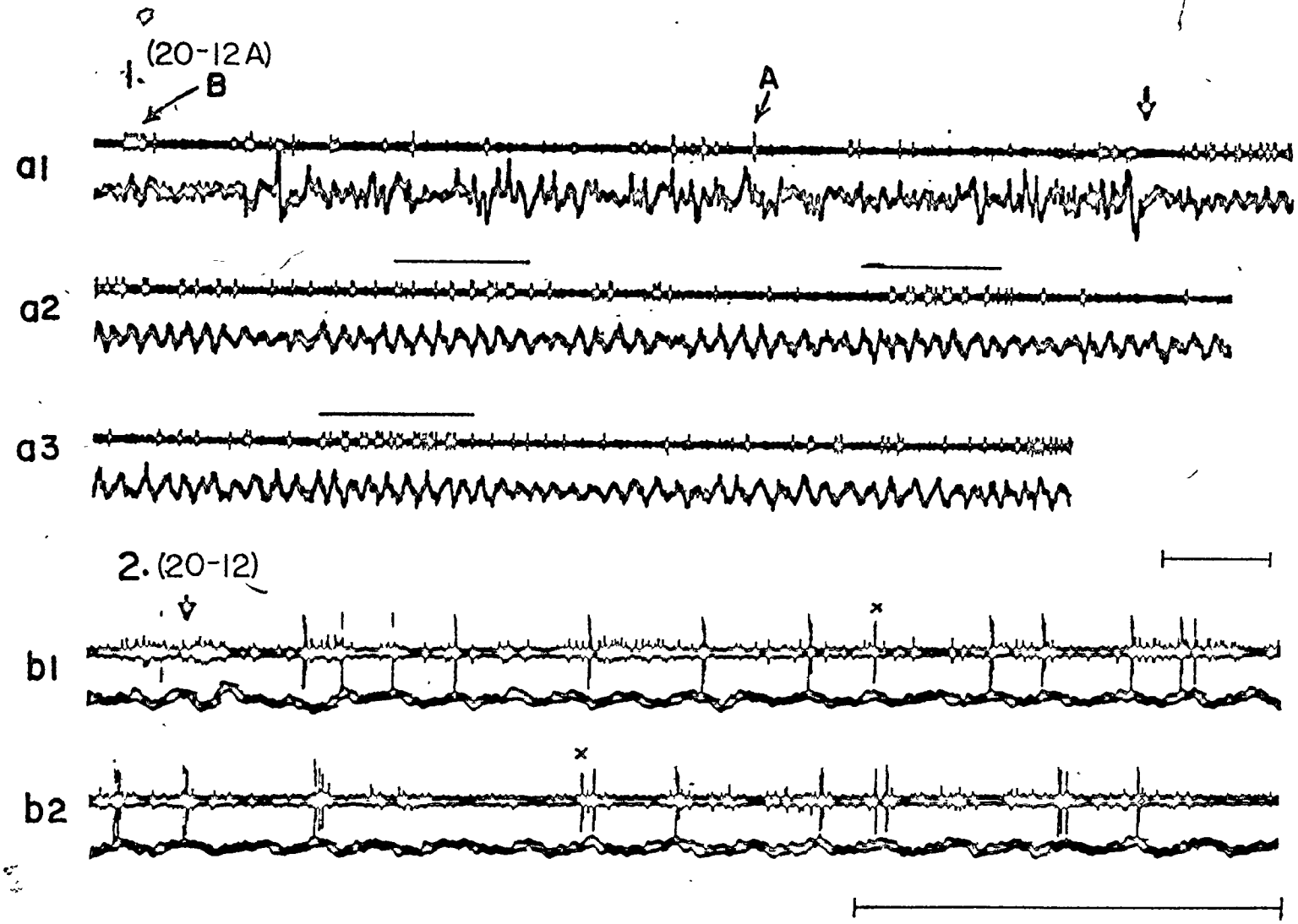


Fig. 22

Figure 23. 1. Cell 26-1. Mixed burst (MB) is shown coinciding with LIA spike in a1. Blowing at arrow (a2) accelerates B cell while MB is eliminated until recovery of LIA spike. 2. Another MB eliminated by stimulation at arrow with acceleration and rhythmic bursting in B cell.

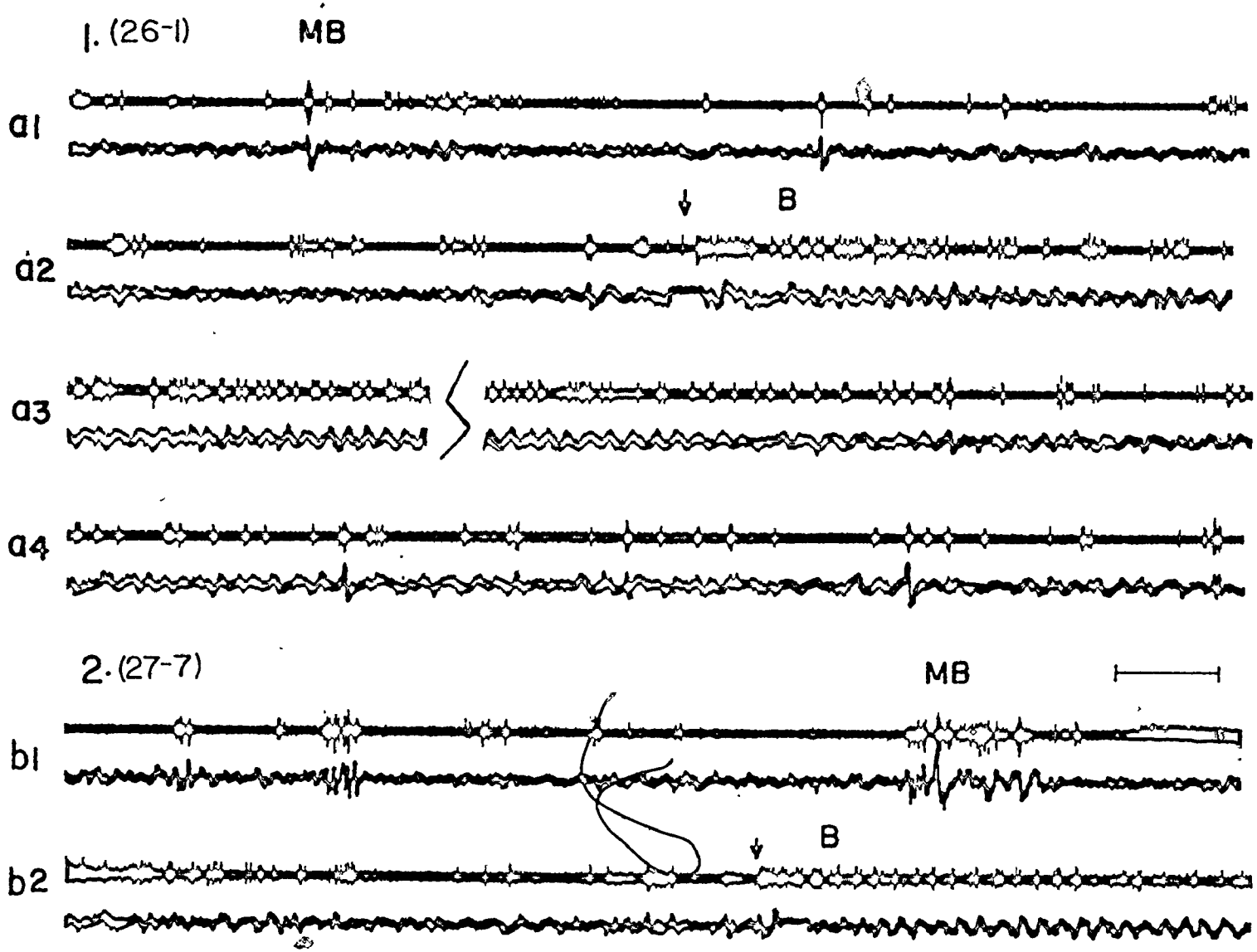


Fig. 23

Figure 24. Cell 31-10, A cell in ventral hippocampus. Spontaneous theta precedes blow at arrow. Activity of A cell subsequent to blow is similar to prestimulus activity.

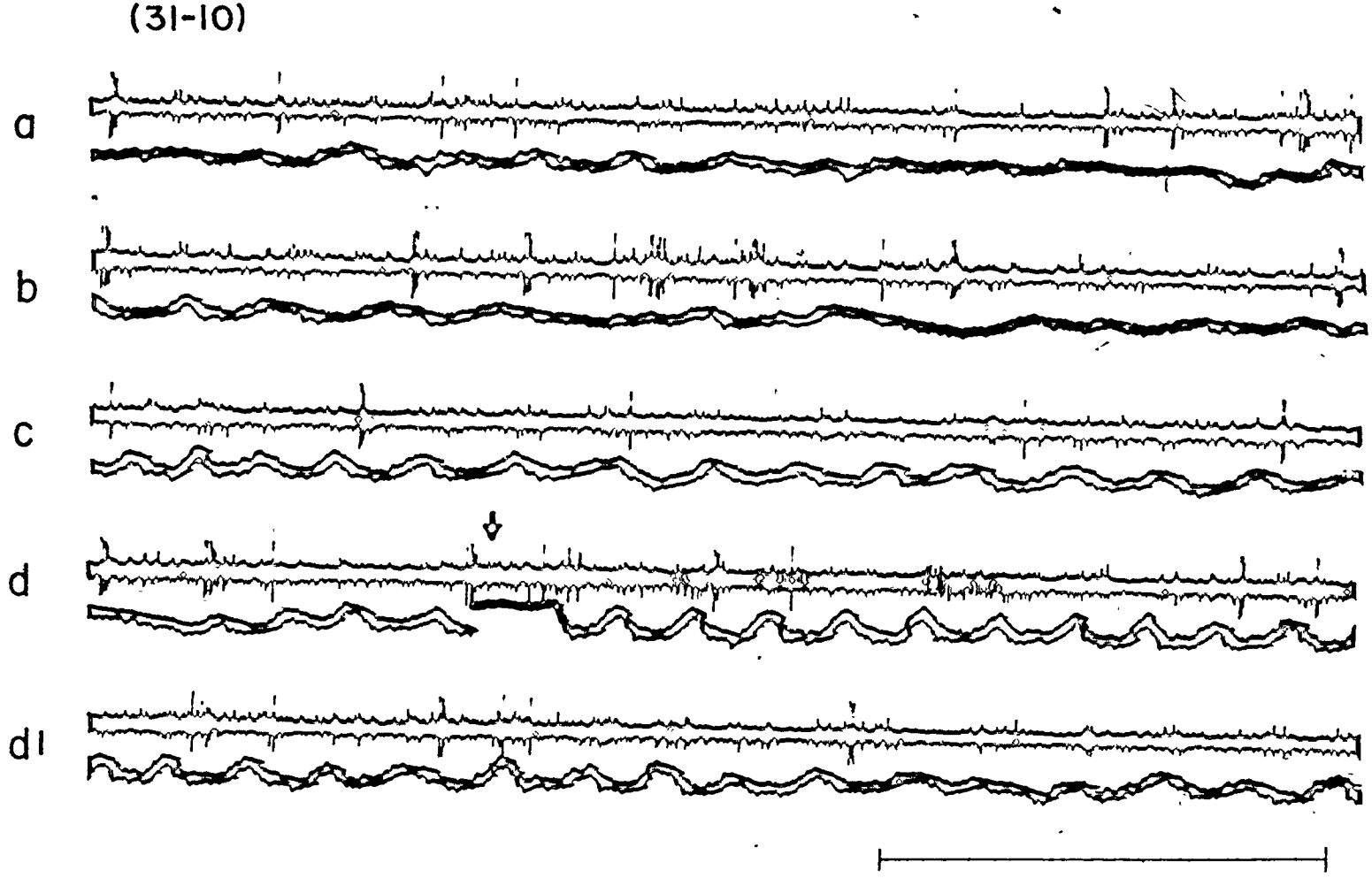


Fig. 24

Figure 25. Cell 20-3. LLA period in a1 interrupted by spontaneous transition to theta at X with slowing of cells, but rapid recovery accompanied by rhythmic bursting in a3. Strip b represents activity immediately post-blow; c1 is a different run showing post-blow activity with recovery in c2.

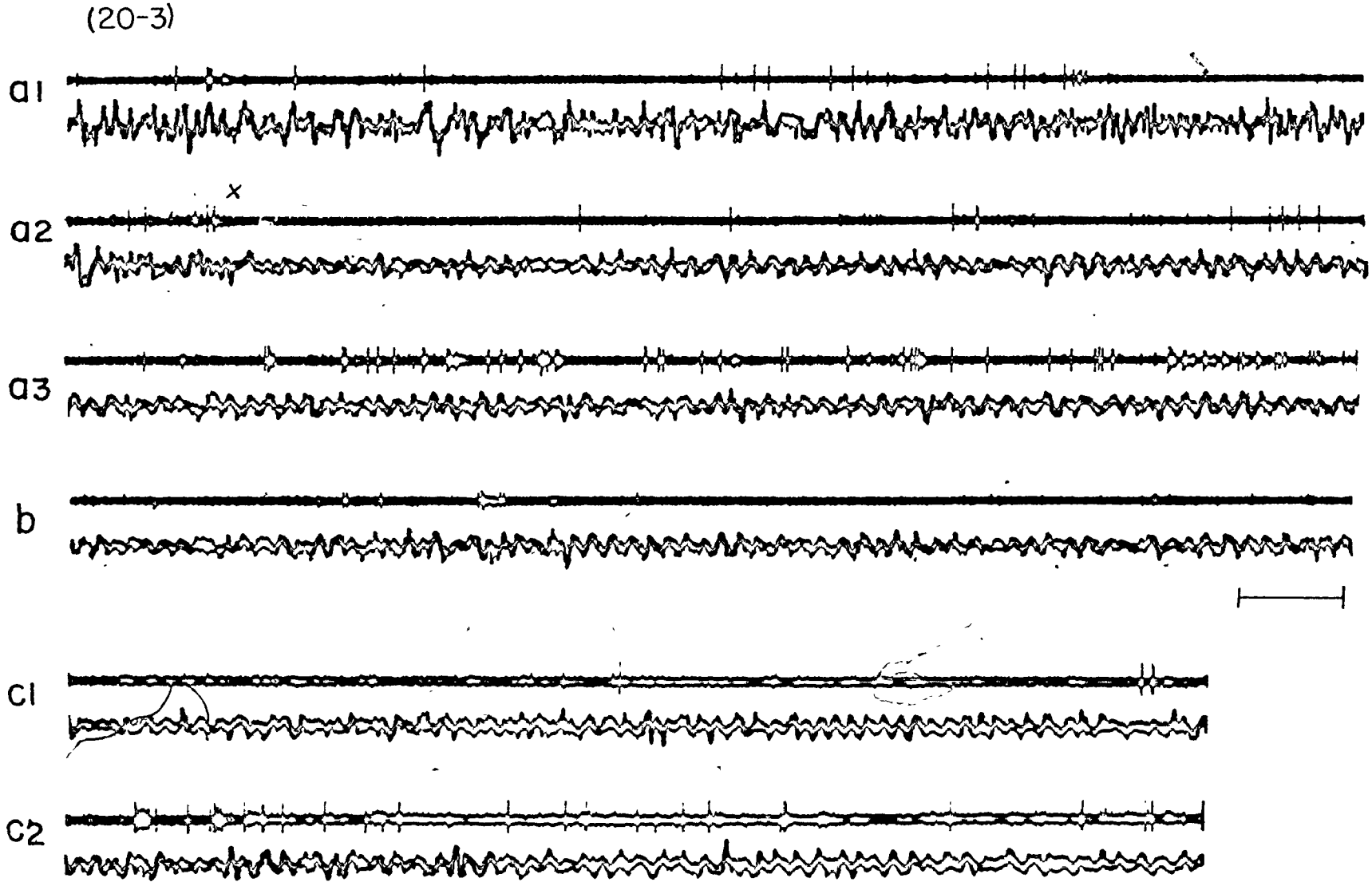


Fig. 25

Figure 26. Cell 26-2. Segment starts with LIA spikes; inset shows detail of MB coinciding with LIA spike. Blowing at arrow in a1 initiates slow-wave theta accompanied by quiet period (a2, a3). In a4 the A cell begins to discharge rhythmically. Detail of A cell firing occurring between asterisks is shown in b. The time calibration mark in the inset is 100 msec.

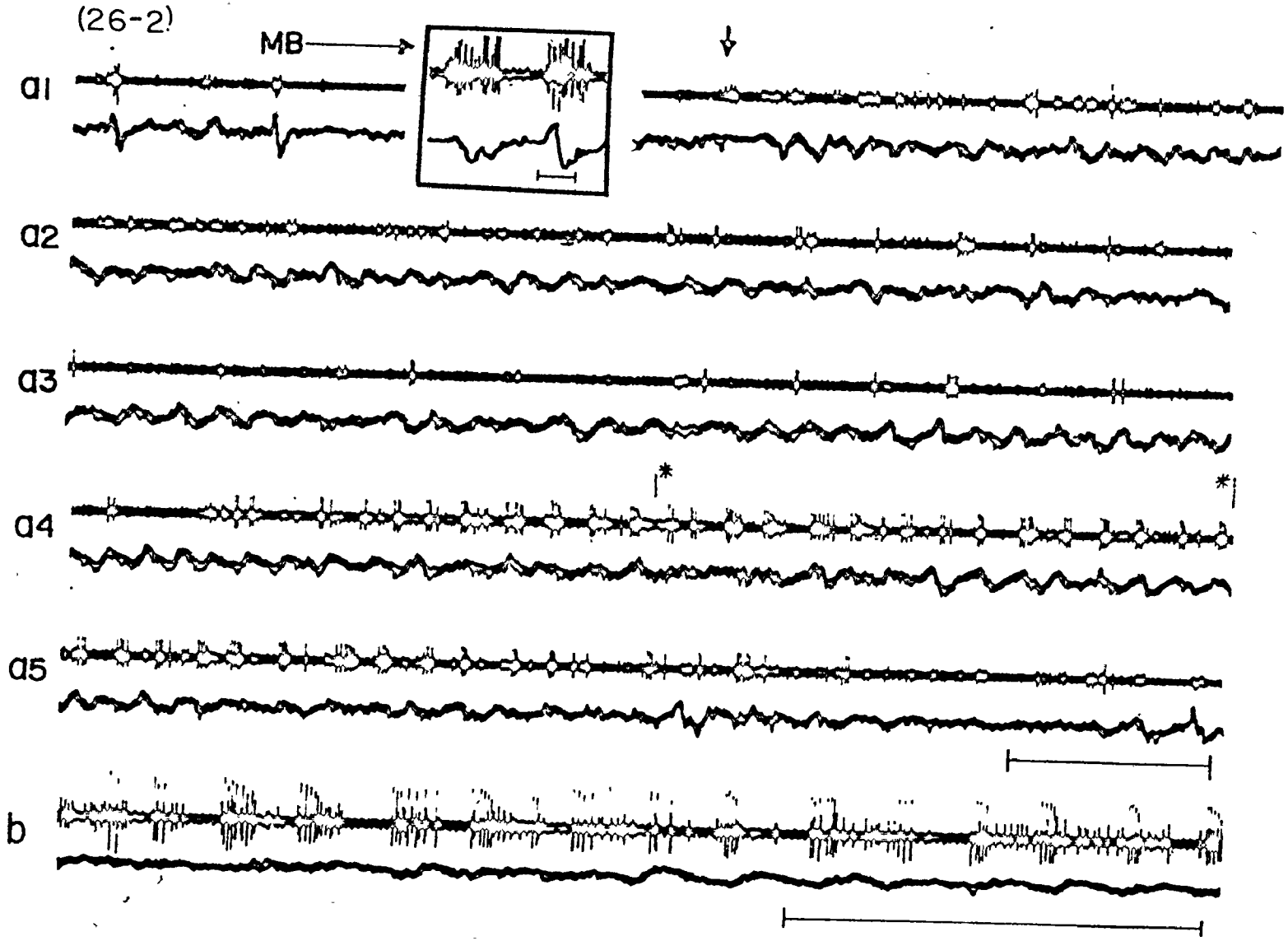


Fig. 26

Figure 27. 1. Cell 27-7. A cell firing during recovery post-blow. Note different amplitudes as well as decrease in amplitude of noise level as A cell recovers.
2. Cell 26-2. Spontaneous theta is seen in b. Note different amplitudes. c1, c2 provide detail of segment marked by asterisks in b.

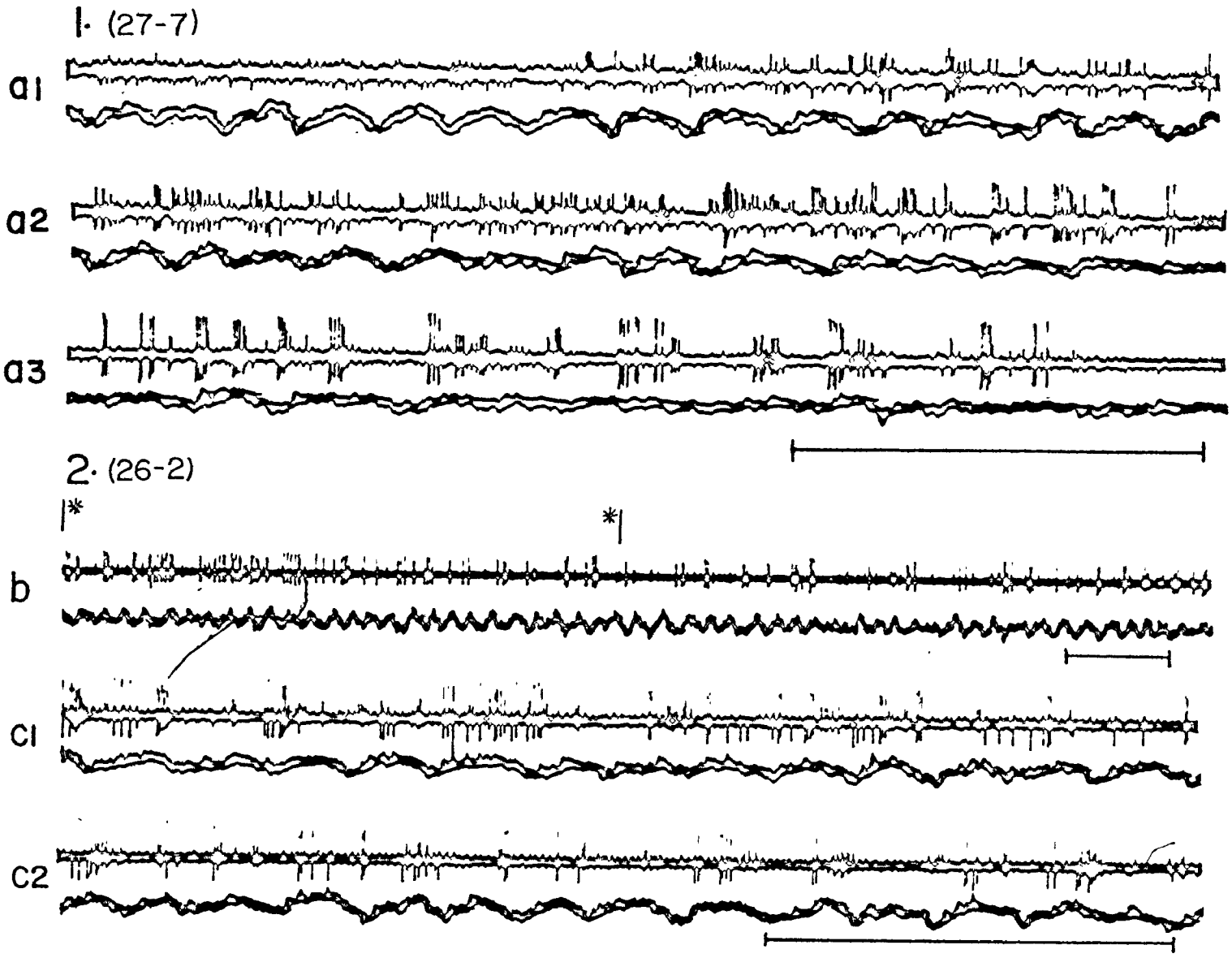


Fig. 27

Figure 28. Effects of electrical stimulation of antero-dorsal hippocampus on cells in three different antero-posterior levels of contralateral septum, A, B, and C. The numbers in upper left are co-ordinates from Pellegrino and Cushman (1967). The heavy black lines demarcate areas giving rise to excitation-inhibition sequences (see A). The dashed vertical lines represent electrode tracks.

D. (following page) Response of cell in dorsomedial LSN to stimulation of ipsilateral CA1 in pilot animal. a, top sweep is fast activity; bottom is slow activity. Time mark is 10 msec. b, same as a only time mark is 100 msec.

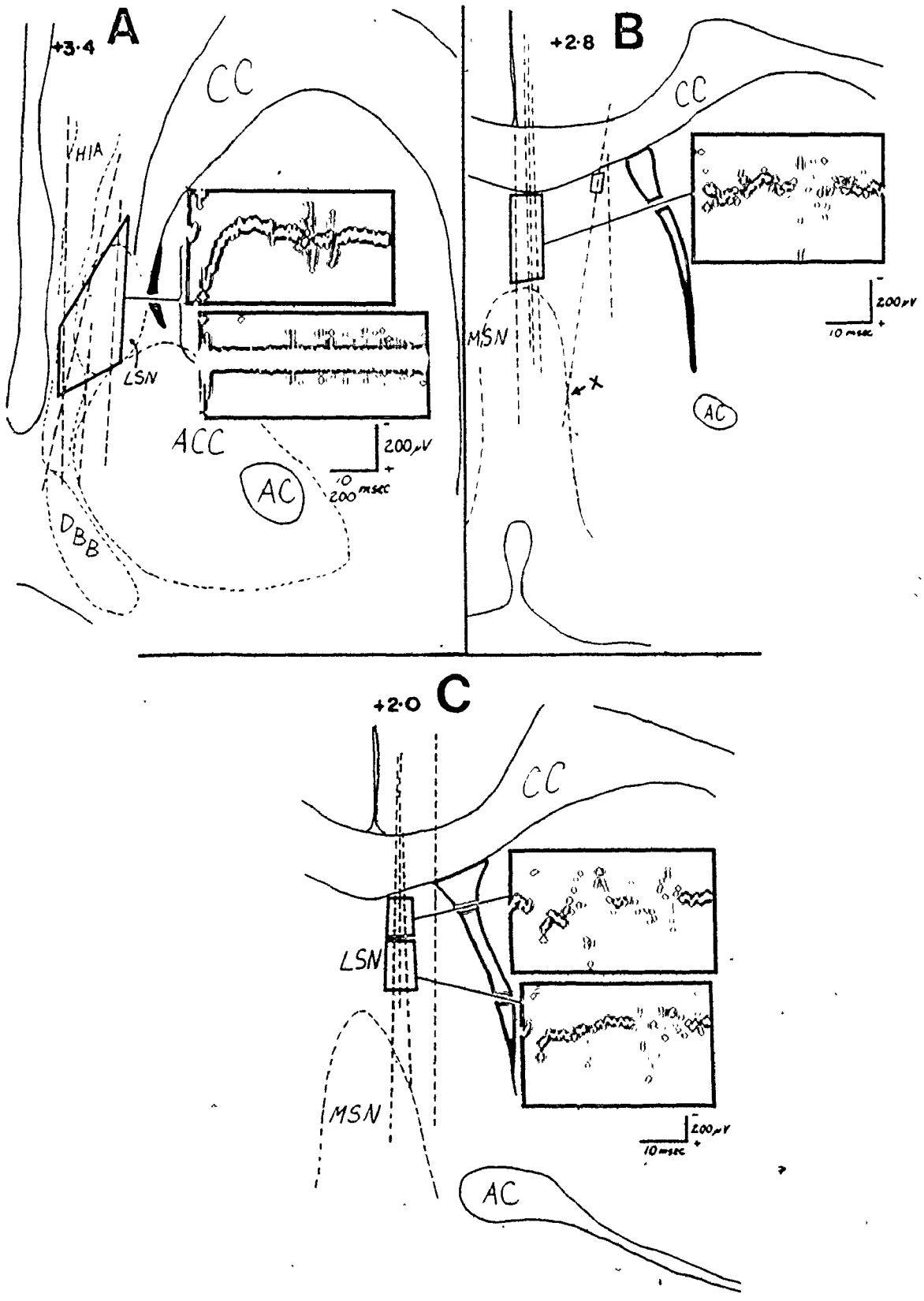
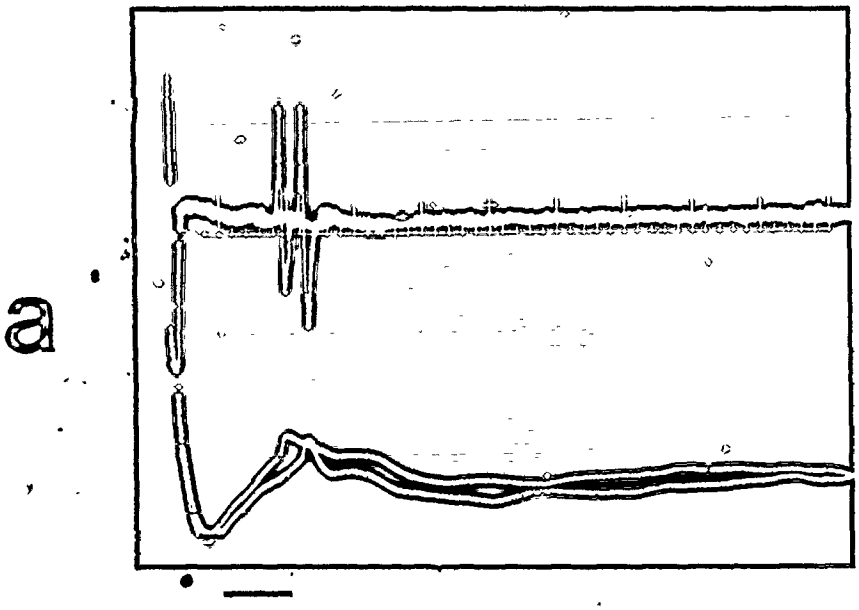

+3·4
A
CC
VHIA
LSN
ACC
AC
DBB
200 μV
10 msec
200 msec
+2·8
B
CC
MSN
AC
200 μV
10 msec
+2·0
C
CC
LSN
MSN
AC
200 μV
10 msec

Fig. 28

D.



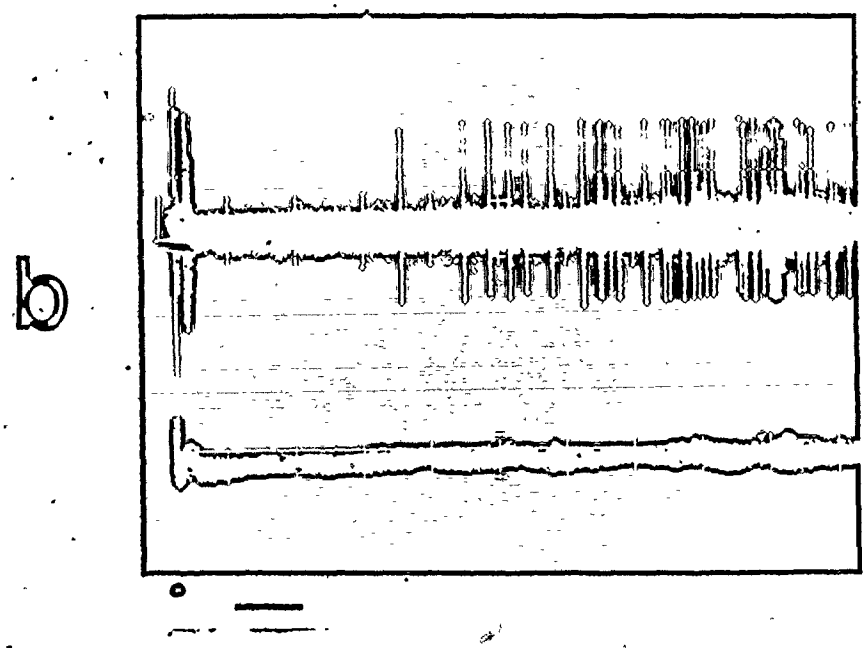


Fig. 28D

Figure 29. Cell 20-12. A. Anatomical loci of cells giving rise to histograms in B.

B. Auto- and cross-correlograms during LIA and theta. The left half of the CC (0 to -418 msec) is the distribution of cross-intervals from septal to hippocampal spikes. The right half (0 to +418 msec) is the hippocampo-septal frequency distribution. It is primarily with the right half of the CC that this thesis is concerned. Bin width is 12.8 msec. Note initial peak during LIA; during theta peak is attenuated. There is also a suggestion of symmetry in the theta CC. Note also that, although individual AUTO's were not strikingly different between states, the CC's during LIA and theta are different. Hippocampal cell is type A; septal cell is type G. The activity of the A cell during a portion of theta used to construct the CC can be seen in Fig. 22-2. This A cell is seen interacting with a different septal cell in Fig. 23.

C. 1. Photograph of raw spikes during LIA with the hippocampal A spike (upper trace) triggering the sweep. Thirteen sweeps are shown and the time mark in this and other similar photos is 50 msec. 2. "Post-stimulus histogram" during LIA, 15 sweeps. The histogram is constructed by using the hippocampal event as the stimulus and the septal spikes as the response. 3. "Post-stimulus histogram" during theta, rapid firing of A spike, 15 sweeps.

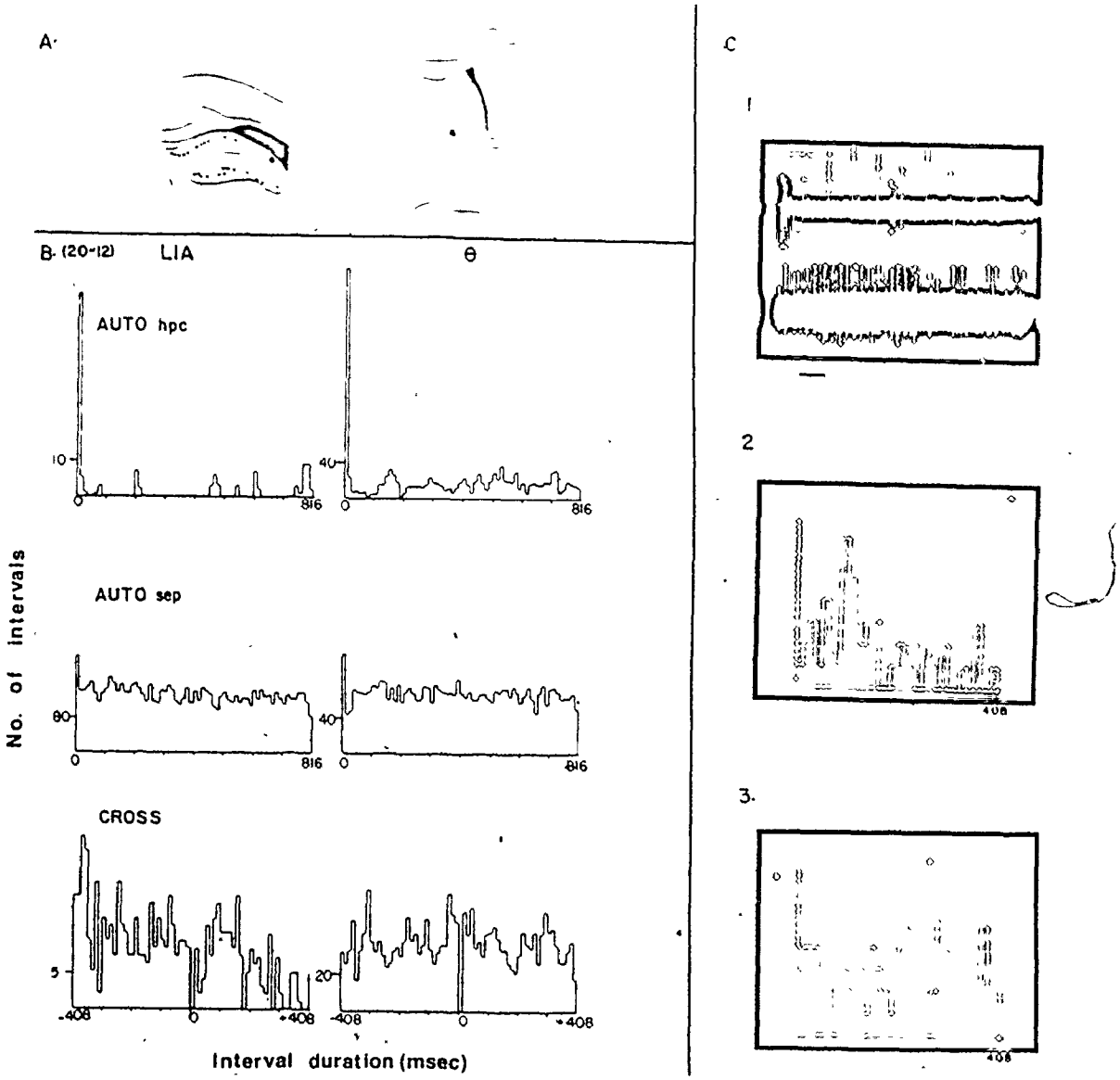

A.
B. (20-12) LIA θ
AUTO hpc
10
40
0
816
AUTO sep
80
40
CROSS
5
20
-408
+408
No. of intervals
Interval duration (msec)
C
1
2
3.
408

Fig. 29

Figure 30. Cell 26-1. A. Raw data film strip during LIA. Time mark=one second.

B. Auto- and cross- correlograms during LIA and theta. Note peak during LIA extending slightly to negative side of zero; symmetrical peaks during theta. Hippocampal cell is type A; septal cell is C4. The spontaneous activity of the hippocampal cell involved in the cross-correlation is also seen in Figures 23-1 and 26.

C. Anatomical loci.

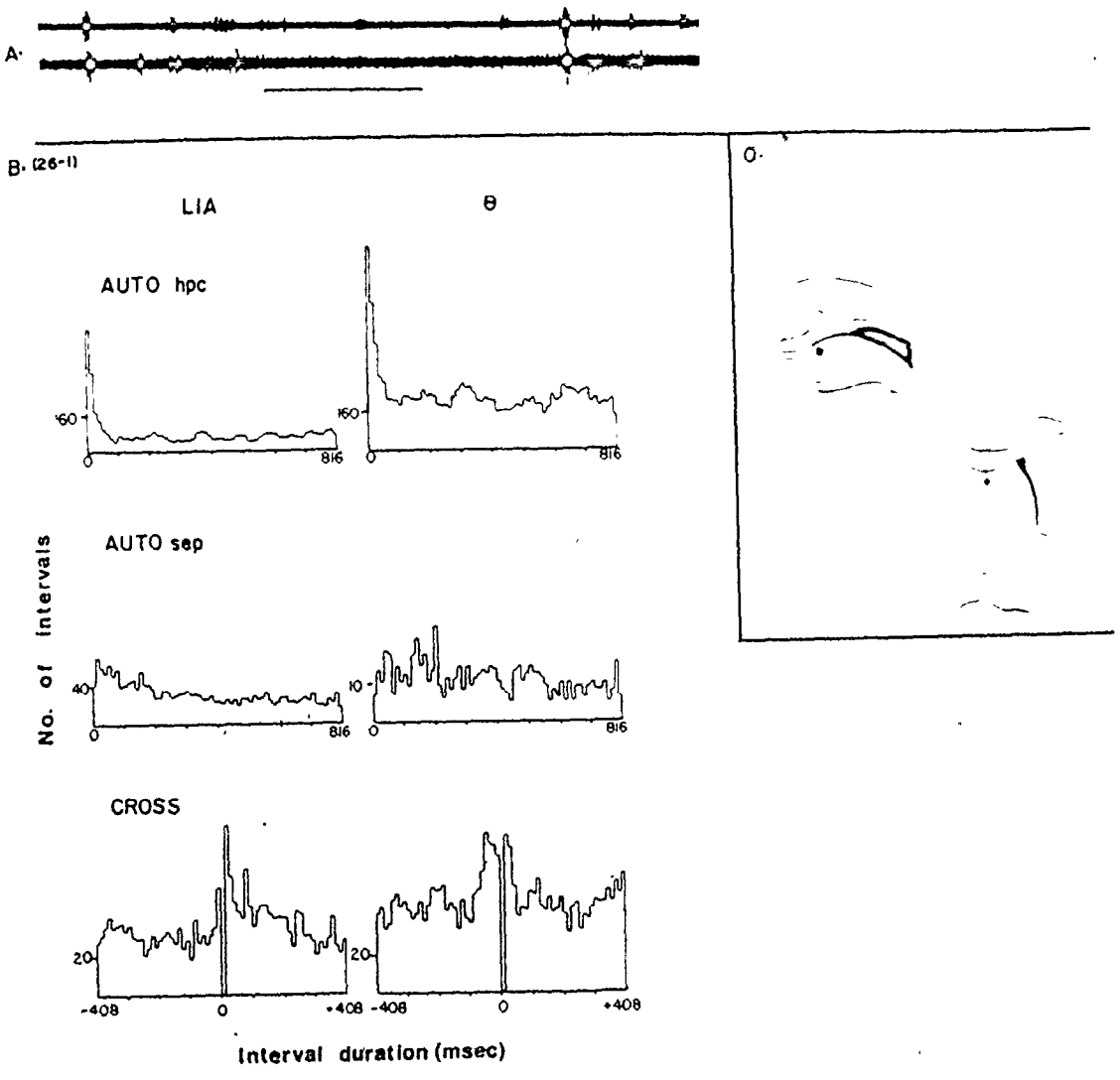

A.
B. (26-1)
C.
LIA
θ
AUTO hpc
160
0
816
AUTO sep
40
10
No. of intervals
CROSS
20
-408
0
+408
Interval duration (msec)

Fig. 30

Figure 31. Cell 27-15. A. Auto- and cross-correlograms during LIA and theta. Peak during LIA extending slightly to left of zero. During theta time-locking disappears. Hippocampal cell is type A (same cell as cell 27-8 of Fig. 32); septal cell is type B.

B. Raw data strips during LIA; time mark is one second.

C. Anatomical loci.

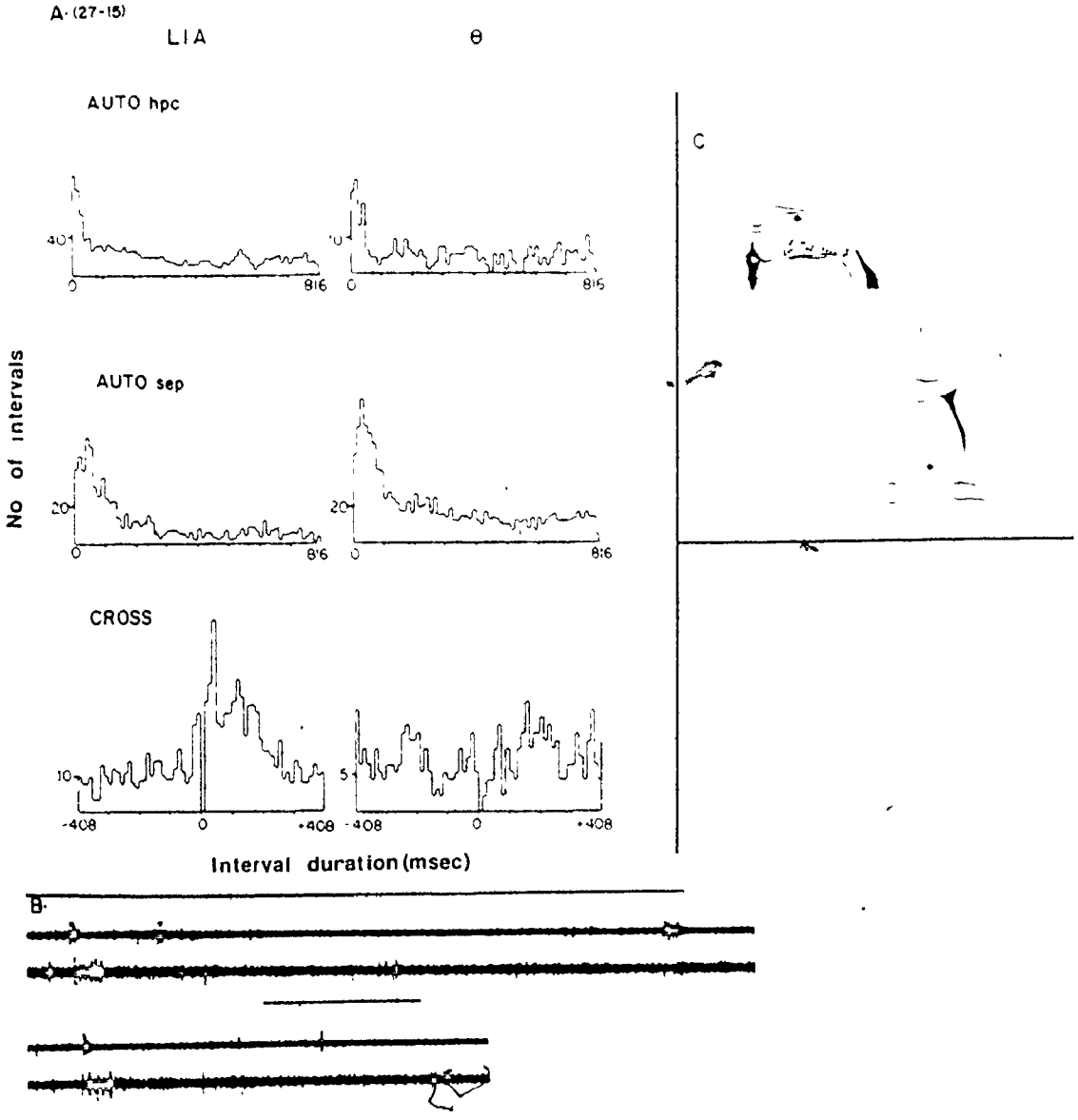

A·(27-15)
LIA
θ
AUTO hpc
AUTO sep
CROSS
No of intervals
Interval duration (msec)
B·
C

Fig. 31

Figure 32. Cell 27-8. A. Auto- and cross-correlograms during LIA and theta. Brief short-latency peak during LIA; still present during theta with latency shift. Hippocampal cell is type A (same cell as 27-15 of Fig. 31); septal cell is type C3.

B. Anatomical loci.

C. Thirty sweeps during theta.

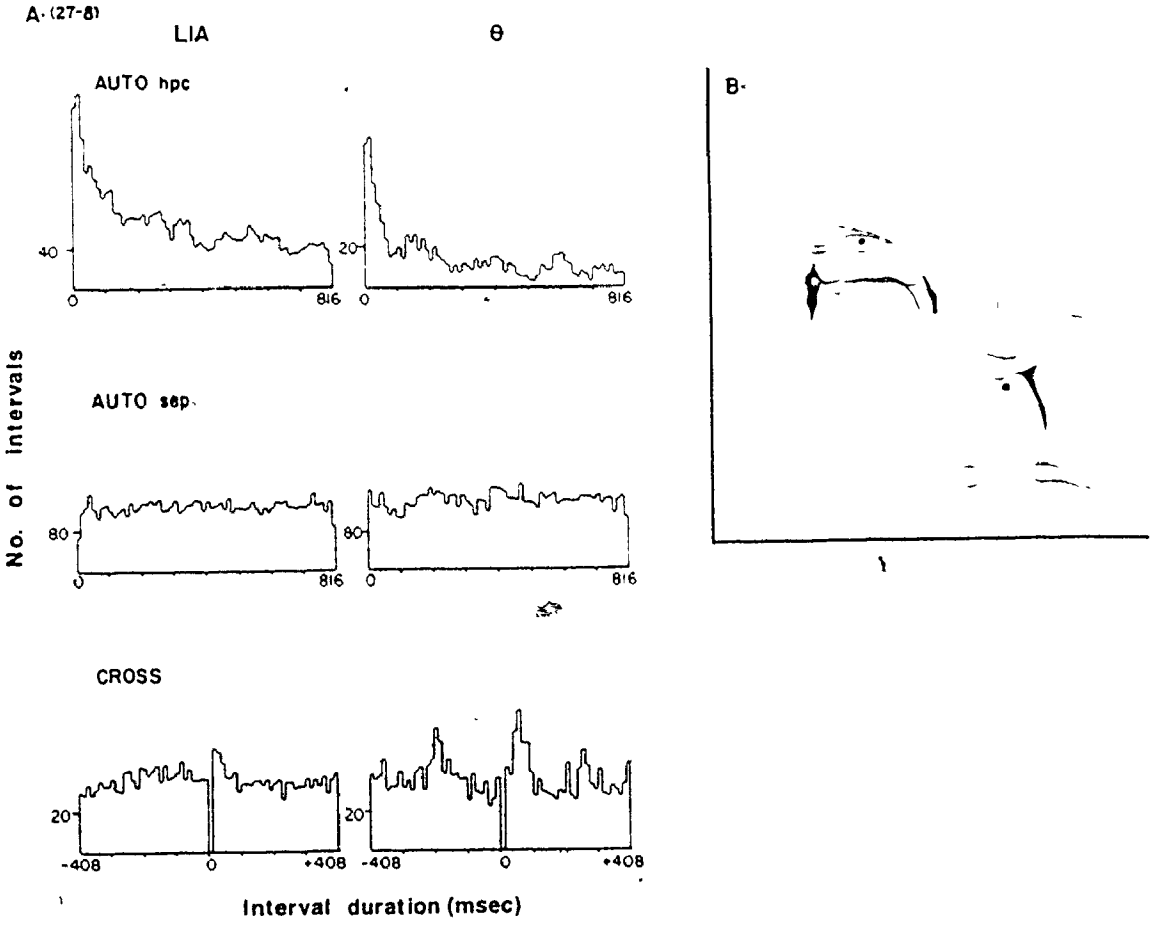

A·(27-8)
LIA
θ
AUTO hpc
40
0
816
20
0
816
AUTO sep
80
0
816
80
0
816
No. of Intervals
CROSS
20
-408
0
+408
20
-408
0
+408
Interval duration (msec)
B·

Fig. 32

Figure 33. Cell 20-3. A. Auto- (AUTO) and cross-correlogram (CROSS) during LIA and theta. Note initial trough during LIA; delayed trough during theta. Hippocampal cell type is A; its spontaneous activity is seen in Fig. 25; septal is G. The A cell is seen interacting with a different septal cell in Fig. 29.

B. Anatomical loci.

C. Fifteen sweeps during LIA.

D. (following page) Dotgram for cell 20-3 incorporating spontaneous transition from LIA to theta. The top is the time line with each large division representing 40 msec. Each single dot on the left line is produced by successive hippocampal events (burst or single), while the row of dots following the hippocampal dots represents the septal spikes. This figure corroborates the CC's seen in A. During LIA there is an initial inhibition which is weakened during the spontaneous transition to theta.

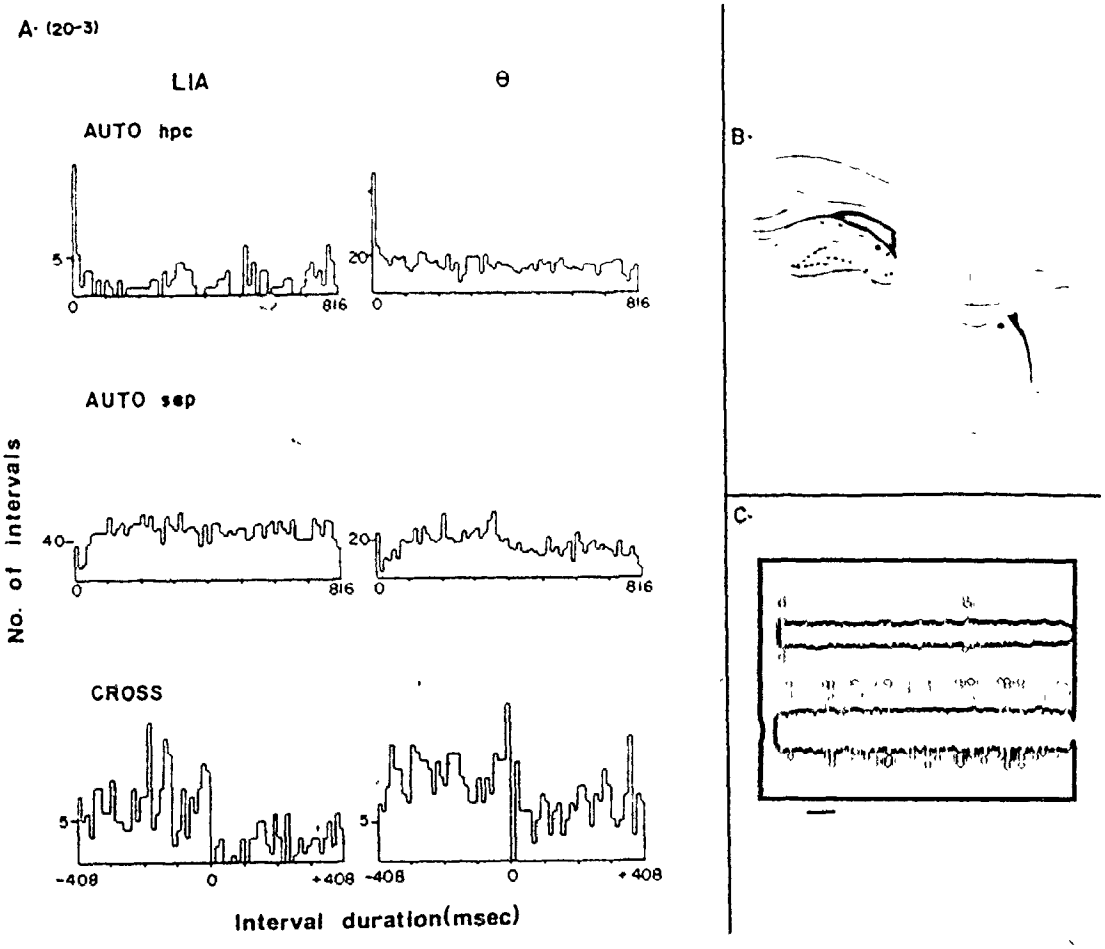

A· (20-3)
LIA
θ
AUTO hpc
5
0
816
20
0
816
AUTO sep
40
0
816
20
0
816
No. of intervals
CROSS
5
-408
0
+408
5
-408
0
+408
Interval duration(msec)
B·
C·
a
b

Fig. 33

D-20-3

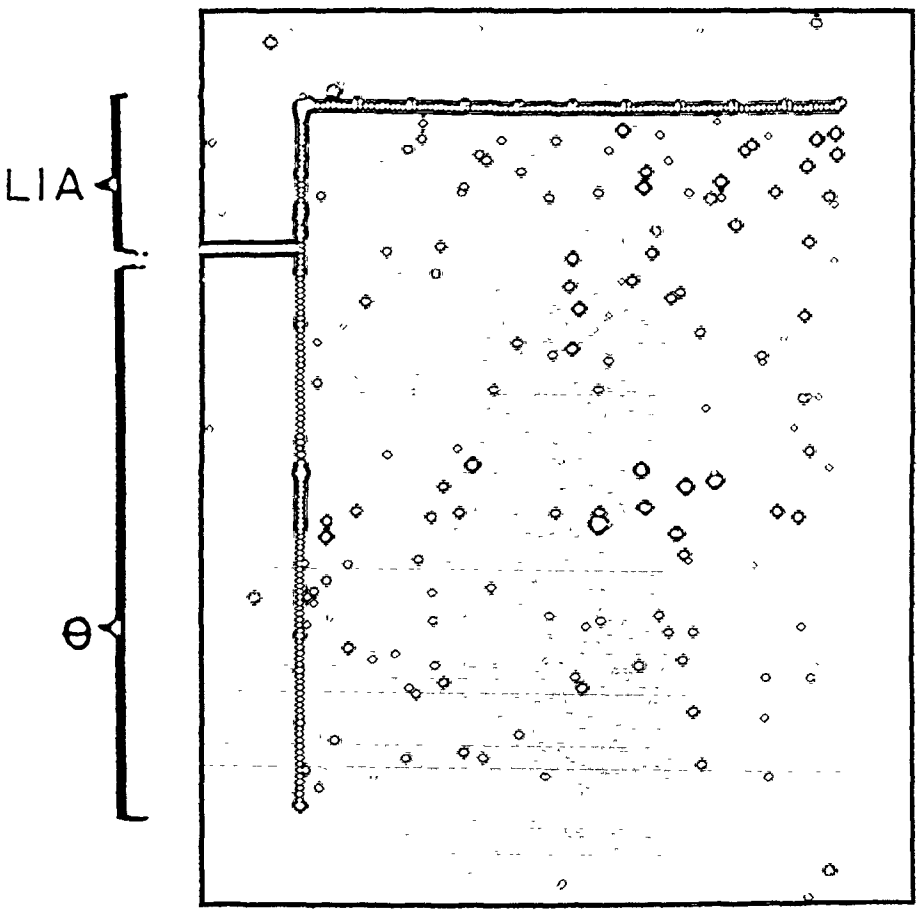


Figure 34. A. Cell 22-3. Top: Anatomical loci. Bottom: Auto- and cross-correlograms during LIA. Note initial trough; n too small during theta. Hippocampal cell is A cell; Septal cell is a C6 type.

B. Cell 28-10. Top: Anatomical loci. Bottom: Auto- and cross-correlogram during LIA, n too small during theta. Hippocampal and septal cells are both A cell types.

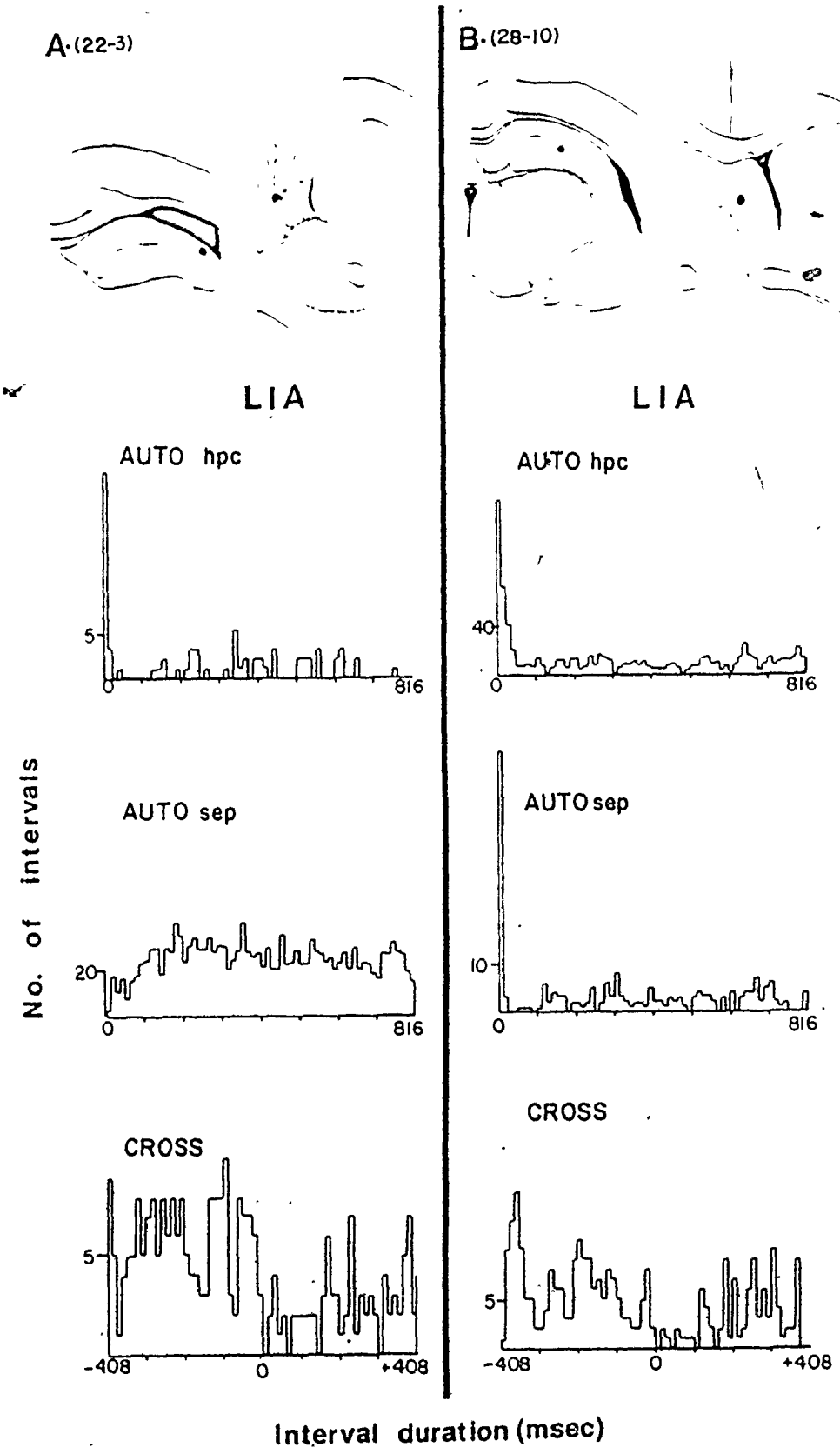

A·(22-3)
B·(28-10)
LIA
LIA
AUTO hpc
AUTO hpc
5
0
816
40
0
816
No. of intervals
AUTO sep
AUTO sep
20
0
816
10
0
816
CROSS
CROSS
5
-408
0
+408
5
-408
0
+408
Interval duration (msec)

Fig. 34

Figure 35. Cell 22-7. A. Anatomical loci.

B. Auto- and cross-correlograms during LIA and theta. Note delayed trough during LIA; during theta, effect was classified as peak or peak-trough, but may represent symmetry.

C. 1. Post-stimulus histogram constructed with hippocampal spike acting as stimulus; septal spike as response. 50 sweeps during LIA. Note delayed trough and rebound. 2. Post-stimulus histogram; 30 sweeps during theta. 3. 60 sweeps during LIA.

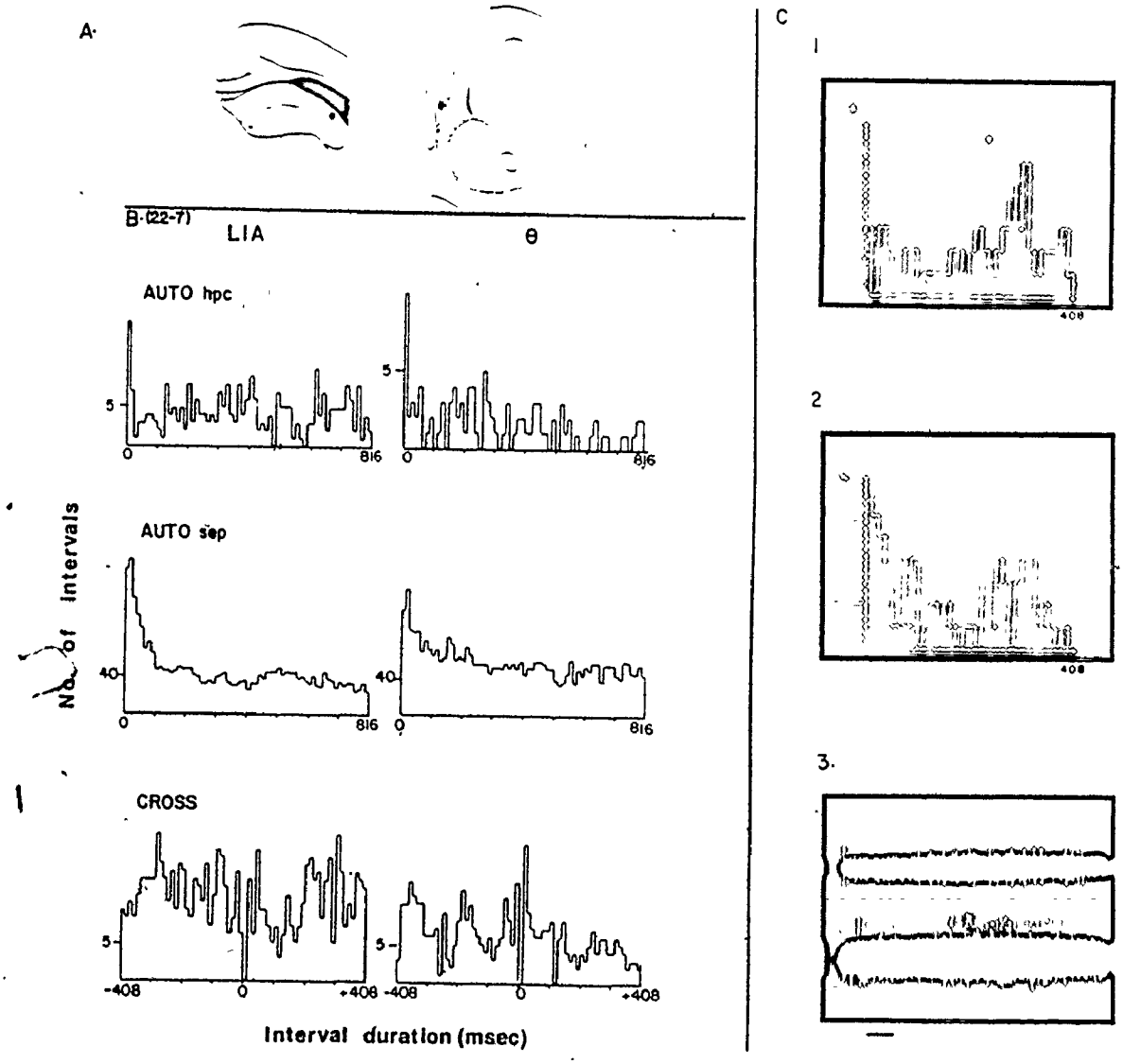

A.
B (22-7)
LIA
θ
AUTO hpc
AUTO sep
CROSS
No. of intervals
Interval duration (msec)
C
1
2
3.

Fig. 35

Figure 36. Schematic depiction of some synaptic interactions that may induce correlations in spike activity of cells in area CA1 and the lateral septal nucleus (LSN). Solid lines represent pathways of transsynaptic output from area CA1 to the LSN. Pathway 1 is a monosynaptic connection, while pathway 2 and 3 represent CA1 influences on LSN via a relay in subiculum (SUB). The broken lines represent pathways entering CA1 and the LSN from a common source. Pathways 4 and 5 originate in CA3 while 6 and 7 arise in brainstem sites, possibly the raphe nuclei and the ventral tegmentum.

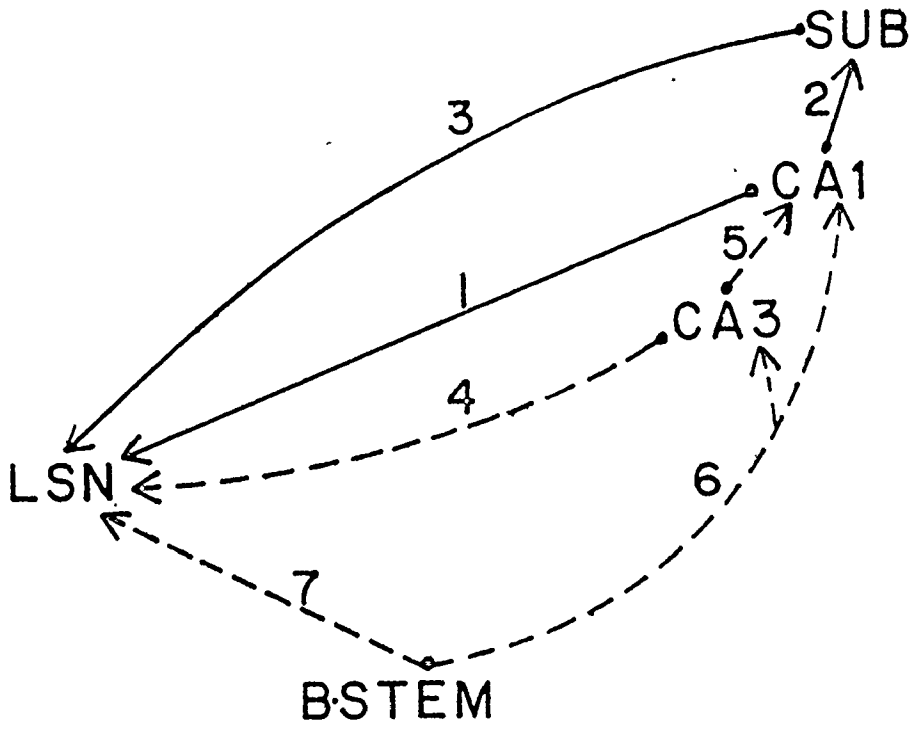


Fig. 36

Table 1

Differences between theta cells and complex-spike cells in the hippocampus

		Theta cells	Complex-spike cells
1(a)	Complex spikes	Never	All have some
(b)	Simple action potentials	Always	All have some
2	Duration of extracellular negative spike (distorted)	All 0.15-0.25 ms	All 0.3-0.5 ms in single spikes and spikes of complex spikes
3	Rate of firing most of the time awake and SWS	Almost all >8/s	All <12/s, most <2/s, many off*
4	Maximum rate of firing	29-147/s, sustained for many seconds	All <40/s, most <20/s, sustained for less than 2 seconds*
5	Patterns of firing	Comparatively regular	Irregular
6	During theta rhythm in slow waves in paradoxical sleep or awake		
	(a) Rate	At maximum rate if and only if theta rhythm is present	No simple relation usually less than 1/s*
	(b) Phase relations	Most have clear phase relation	Most have clear phase relation
7	Relation to LIA spike	Almost all fire with bursts	Sometimes fire
8	Spike heights	Usually <200 μV ($\bar{x}$=164 μV)	Larger than theta units ($\bar{x}$=267 μV)
9	Anatomical location		
	in CA1	Stratum pyramidale Stratum oriens	Stratum pyramidale
	in CA3	Stratum pyramidale Apical dendritic layers	Stratum pyramidale
	in F.D.	Stratum granulosum Hilus of F.D.	Stratum granulosum
10	Antidromically driven by stimulation	Never	Yes

*A complex spike is counted as a single potential.
From Fox and Ranck (1975, 1977) and Ranck (1973).

Table 2

Firing modes in individual rats: hippocampal penetrations

	A	B1	B2	C2	C3	C6	D	E	F	G	U*	Sum
16			2		1					1		4
19	2	2										4
20	3	2	1					1		1	1	9
21	5		1									6
22	9	3					1					13
23									2		1	3
24	1	3						1				5
25	9	2					1				1	13
26	1											1
27	2	2				1						5
28	2	1							2			5
29	2			1	1			1		1		6
30			1									1
31	2	3	1		1	1		1			1	10
32	2								1			3
33	4	2		1			1					8
	44	20	6	2	3	2	3	4	5	3	4	96
	.46	.27			.07		.03	.04	.06	.03	.04	1.00

*U=Unknown

Table 3

Firing modes in individual rats: septal penetrations

	A	B1	B2	C1	C2	C3	C4	C5	C6	D	E	F	G	U*	Sum
19						3			1	1	1				6
20	4	2		1		2	2			1			6	1	19
21		3		2	3	1		1	2	1				2	15
22		5	3		2			1	3			3			17
23				1	1		1		1						4
24		1								1				1	3
25			1	2				1	9	1			1	1	16
26		1					3								4
27		1	2		1	1	3	2	1	1		1			13
28	1			1				2	1			2		1	8
29	1			1	1			1	3				1		8
30		1		4	2					1				1	9
31		2	1				1		4					1	9
32				1	1				3						5
33			1		2	2			3	2			2		12
	6	16	8	13	13	9	10	8	31	9	1	6	10	8	148
	.04	.16				.57				.06	.01	.04	.07	.05	1.00

*U=Unknown

Table 4

Anatomical distribution of different discharge patterns

	A	B1	B2	C1	C2	C3	C4	C5	C6	D	E	F	G	U	Sum
Ammon's horn	40	15	4		1	2			1	3		2		1	69
Dentate	2	5	1			1						1		1	11
Subiculum			1										1		2
Lateral septum	6	1	1	9	9	5	9	6	21	1		2	4	5	79
Medial septum		15	4	1	2	4		2	3	5	1	3	6	1	47
Ant. hippocampus			2		1				4					1	8
Accumbens										2					2
Cingulate			1	2					2	1		1		1	8
Neocortex	1				1				1		4		2	1	10
Thalamus	1														1
Unknown				1	1		1		1			2		1	7
	50	36	14	13	15	12	10	8	33	12	5	11	13	12	244

Table 5

Effect in cross-correlograms during LIA and theta.

	LIA		Theta	
Primary effects:				
Initial peaks	13		8	
Initial peaks and troughs	4		4	
Initial troughs	8		2	
Late troughs	5	30 (.81)	4	18 (.49)
No effect:				
Flat or lack of outstanding feature	7		12	
Symmetry	0		3	
Reduced n	0	7 (.19)	4	19 (.51)
		37		37

REFERENCES

Adey, W.R. Neurophysiological correlates of information transaction and storage of brain tissue. In: E. Stellar and J.M. Sprague (Eds.), Progress in Physiological Psychology. Vol.1. NY: Academic, 1966, pp. 1-43.

Altman, J., Brunner, R.L., and Bayer, S.L. The hippocampus and behavioral maturation. Behavioral Biology, 1973, 8, 557-596.

Amaral, D.G. A Golgi study of cell types in the hilar region of the hippocampus in the rat. Journal of Comparative Neurology, 1978, 182, 851-914.

Andersen, P., Bland, B.H., and Dudar, J.D. Organization of the hippocampal output. Experimental Brain Research, 1973, 17, 152-168.

Andersen, P., Bland, B.H., Ganes, T. and Sveen, O. Analysis of rhythmicity of identified hippocampal neurons during theta activity. Submitted to Experimental Brain Research, 1978.

Andersen, P., Bliss, T.V.P., and Skrede, K.K. Lamellar organization of hippocampal excitatory pathways. Experimental Brain Research, 1971, 13, 222-238.

Andersen, P. and Eccles, J.C. Inhibitory phasing of neuronal discharge. Nature, 1962, 196, 645-647.

Andersen, P., Eccles, J.C., and Loyning, T. Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses. Nature, 1963, 198, 540-542.

Andersen, P., Eccles, J.C., and Loyning, T. Pathways of post-synaptic inhibition in the hippocampus. Journal of Neurophysiology, 1964, 27, 608-619. (a)

Andersen, P., Eccles, J.C., and Loyning, T. Location of post-inhibitory synapses on hippocampal pyramids. Journal of Neurophysiology, 1964, 27, 592-607. (b)

Andersen, P. and Sears, T.A. The role of inhibition in the phasing of spontaneous thalamo-cortical discharge. Journal of Physiology (London), 1964, 173, 459-480.

Arnett, D.W. Correlation analysis of units recorded in the cat dorsal lateral geniculate nucleus. Experimental Brain Research, 1975, 24, 111-130.

Arnett, D.W. Statistical dependence between neighboring retinal cells in the goldfish. Experimental Brain Research, 1978, 32, 49-53.

Bennett, T.L. The electrical activity of the hippocampus and processes of attention. In: R.L. Isaacson and K.H. Pribram (Eds.), The hippocampus: a comprehensive treatise. Vol. 2, Neurophysiology and behavior. NY: Plenum, 1975, pp. 71-99.

Black, A.H. Hippocampal electrical activity and behavior. In: R.L. Isaacson and K.H. Pribram (Eds.), The hippocampus: a comprehensive treatise. N.Y.: Plenum Press, 1975.

Black, A.H. and Young, G.A. The electrical activity of the hippocampus and cortex in dogs operantly trained to move and to hold still. Journal of Comparative and Physiological Psychology, 1972, 79, 128-141.

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

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

Bland, B.H., Andersen, P., and Ganes, T. Participation of identified hippocampal neurons in sponaneous and induced theta activity. Manuscript submitted to Experimental Brain Research, 1978.

Brodal, A. The hippocampus and the sense of smell. Brain, 1947-48, 70-71, 179-222.

Bryant, Jr., H.L., Marcos, A.R., and Segundo, J.P. Correlations of neuronal spike discharges produced by monosynaptic connections and by common inputs. Journal of Neurophysiology, 1973, 36, 205-255.

Chronister, R.B. and DeFrance, J.F. Organization of projection neurons of the hippocampus. Experimental Neurology, 1979, 66, 509-523.

Chronister, R.B., Sikes, R.W., and White, L.E. The septo-hippocampal system: significance of the subiculum. In: J.F. DeFrance (Ed.), The Septal Nuclei. New York: Plenum Press, 1976, pp. 115-132.

Chronister, R.B., Zornetzer, S.F., Bernstein, J.J., and White, L.E., Jr. Hippocampal theta rhythm: intra-hippocampal formation contributions. Brain Research, 1974, 65, 13-28.

CIBA Foundation Symposium 58 (new series). Functions of the Septo-hippocampal System. Amsterdam: Elsevier, 1978.

Creutzfeldt, O. and Jung, R. Neuronal discharges in the cat's motor cortex during sleep and arousal. In: G.E.W. Wolstenholme and M. O'Connor (Eds.) The Nature of Sleep. London: Churchill, 1961, pp. 131-170.

DeFrance, J.F., Kitai, S.T., Hatada, K., and Christensen, C. Contrasting effects of fimbria stimulation in the septum of the cat. Brain Research, 1973, 58, 240-244. (a)

DeFrance, J.F., Kitai, S.T., and Shimono, T. Electrophysiological analysis of the hippocampal-septal projections: I. Response and topographical characteristics. Experimental Brain Research, 1973, 17, 447-462. (b)

DeFrance, J.F., Kitai, S.T., and Shimono, T. Electrophysiological analysis of the hippocampal-septal projections: II. Functional characteristics. Experimental Brain Research, 1973, 17, 463-476. (c)

DeFrance, J.F., Shimono, T., and Kitai, S.T. Anatomical distribution of the hippocampal fibres afferent to the lateral septal nucleus. Brain Research, 1971, 34, 176-180.

DeFrance, J.F., Shimono, T., and Kitai, S.T. Hippocampal inputs to the lateral septal nucleus: patterns of facilitation and inhibition. Brain Research, 1972, 37, 333-339.

DeFrance, J.F., Yoshihara, H., and Chronister, R.B. Electrophysiological studies of the septal nuclei: II. The medial septal region. Experimental Neurology, 1978, 58, 14-31.

Dickson, J.W. and Gerstein, G.L. Interactions between neurons in auditory cortex of the cat. Journal of Neurophysiology, 1974, 37, 1239-1261.

Douglas, R.J. The hippocampus and behavior. Psychological Bulletin, 1967, 67, 416-442.

Eckhorn, R. and Popel, B. A contribution to neuronal network analysis in cat lateral geniculate body. Simultaneous recording of maintained activity. International Journal of Neurosciences, 1972, 4, 137-142.

Edinger, H., Siegel, A., and Troiano, R. Single unit analysis of the hippocampal projections to the septum in the cat. Experimental Neurology, 1973, 41, 569-583.

Euler, C.V. and Green, J.D. Excitation, inhibition, and rhythmical activity in hippocampal pyramidal cells in rabbit. Acta Physiologica Scandinavica, 1960, 48, 110-125.

Ferguson, G.A. Statistical Analysis in Psychology and Education. NY: McGraw-Hill, 1971.

Finch, D. M. and Babb, T.L. Response decrement in a hippocampal basket cell. Brain Research, 1977, 130, 354-359.

Fox, C.A. Certain basal telencephalic centers in the cat. Journal of Comparative Neurology, 1940, 72, 1-62.

Fox, S.E. and Ranck, Jr., J.B. Localization and anatomical identification of theta and complex-spike cells in dorsal hippocampal formation of rat. Experimental Neurology, 1975, 49, 299-313.

Fox, S.E. and Ranck, Jr., J.B. Hippocampal complex-spike and theta cell activity evoked by stimulation of limbic structures in unrestrained rats. Neurosciences Abstracts, 1977, Abstract 613.

Franzini, C., Calasso, M., and Parmeggiani, P.L. Hippocampal unit activity in active sleep with synchronized or desynchronized hippocampogram. Sleep Research, 1975, 4, Absract 78.

Fujita, Y. Two types of depolarizing after-potentials in hippocampal pyramidal cells of rabbits. Brain Research, 1975, 94, 435-446.

Fujita, Y. and Iwasa, H. Electrophysiological properties of so-called inactivation response and their relationship to dendritic activity in hippocampal pyramidal cells of rabbits. Brain Research, 1977, 130, 89-99.

Fujita, Y. and Sato, T. Intracellular records from hippocampal pyramidal cells in rabbit during theta rhythm activity. Journal of Neurophysiology, 1964, 27, 1011-1025.

Goddard, G.V. Component properties of the memory machine: Hebb revisited. In P.W. Jusczyk and R.M. Klein (Eds.), The Nature of Thought: Essays in Honour of D.O. Hebb. New Jersey: Lawrence Erlbaum Assoc., 1979-80.

Grastyán, E. The hippocampus and higher nervous activity. In: Second Conference on the Central Nervous System and Behavior, Transactions. NY: Josiah Macy, Jr. Foundation, 1959, pp. 119-205.

Gray, J.A. Medial septal lesions, hippocampal theta rhythm and the control of vibrissal movement in the freely-moving rat. Electroencephalography and Clinical Neurophysiology, 1971, 30, 189-197.

Green, J.D. The Hippocampus. Physiological Reviews, 1964, 44, 561-608.

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

Green, J.D. and Machne, X. Unit activity of rabbit hippocampus. American Journal of Physiology, 1955, 181, 219-224.

Green, J.D. and Petsche, H. Hippocampal electrical activity. II. Virtual generators. Electroencephalography and Clinical Neurophysiology, 1961, 13, 847-853.

Green, J.D., Maxwell, D.S., and Petsche, H. Hippocampal electrical activity. III. Unitary events and genesis of slow waves. Electroencephalography and Clinical Neurophysiology, 1961, 13, 854-867.

Green, J.D., Maxwell, D.S., Schindler, W.J., and Stumpf, Ch. Rabbit EEG "theta" rhythm: its anatomical source and relation to activity in single neurons. Journal of Neurophysiology, 1960, 23, 403-420.

Gutnick, M.J. and Feldman, S. Effects of hippocampal and hypothalamic afferents on neuronal activities in the rat septum. Experimental Neurology, 1977, 57, 212-230.

Hartse, K.M., Eisenhart, S.F., Bergmann, B.M., and Rechtschaffen, A. Hippocampal spikes during sleep, wakefulness, and arousal in the cat. Sleep Research, 1975, 4, Abstract 30.

Hill, A.J. First occurrence of hippocampal spatial firing in a new environment. Experimental Neurology, 1978, 62, 282-297.

Hjorth-Simonsen, A. Hippocampal efferents to the ipsilateral entorhinal area: An experimental study in the rat. Journal of Comparative Neurology, 1971, 142, 417-438.

Hjorth-Simonsen, A. Some intrinsic connections of the hippocampus in the rat: An experimental analysis. Journal of Comparative Neurology, 1973, 147, 145-162.

Holmes, O. and Houchin, J. Units in the cerebral cortex of the anaesthetized rat and the correlations between their discharges. Journal of Physiology, 1966, 187, 651-671.

Isaacson, R.L. and Pribram, K.H. (Eds.) The hippocampus: a comprehensive treatise. Vol. 1: Structure and Development. Vol. 2: Neurophysiology and Behavior. NY: Plenum, 1975.

Izquierdo, I. The hippocampus and learning. Progress in Neurobiology, 1975, 5, 37-75.

Jung, R. and Kornmüller, A.E. Eine methodik der ableitung lokalisierter Potential schwankungen aus subcorticalen Hirngebieten. Archives of Psychiatry, 1938, 109, 1-30.

Kandel, E.R. and Spencer, W.A. Electrophysiology of hippocampal neurons. II. After potentials and repetitive firing. Journal of Neurophysiology, 1961, 24, 243-259.

Kandel, E.R., Spencer, W.A., and Brinley, F.J. Electrophysiology of hippocampal neurons. I. Sequential invasion and synaptic organization. Journal of Neurophysiology, 1961, 24, 225-242.

Kimble, D.P. Hippocampus and internal inhibition. Psychological Bulletin, 1968, 70, 285-295.

Klemm, W.R. Effects of electrical stimulation of brainstem reticular formation on hippocampal theta rhythm and muscle activity in unanaesthetized, cervical and midbrain-transected rats. Brain Research, 1972, 41, 331-344.

König, J.F.R. and Klippel, R.A. The Rat Brain. Baltimore: Williams & Wilkins, 1963.

Kramis, R.C., Vanderwolf, C.H., and Bland, B.H. Two types of hippocampal rhythmic slow activity in both the rabbit and the rat: Relations to behavior and effects of atropine, diethyl-ether, urethane, and pentobarbital. Experimental Neurology, 1975, 49, 58-85.

Kristan, W.B., Jr. Characterization of connectivity among invertebrate motor neurons by cross-correlation of spike trains. In F.O. Schmitt and F.G. Worden (Eds-in-chief), The Neurosciences. Third Study Program. Cambridge, Mass.: MIT Press, 1970.

Lasek, R.J. Axonal transport and the use of intracellular markers in neuroanatomical investigations. Federation Proceedings, 1975, 34, 1603-1624.

Li, C.H. Synchronization of unit activity in the cerebral cortex. Science, 1959, 129, 783-784.

Lidsky, T.I., Levine, M.S., and MacGregor, Jr., S. Hippocampal units during orienting and arousal in rabbits. Experimental Neurology, 1974, 44, 171-186.

Lorente de Nó, R. Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. Journal of Psychological Neurology, 1934, 46, 113-177.

Lynch, G., Rose, G., and Gall, C. Anatomical and functional aspects of the septo-hippocampal projections. In: CIBA Foundation Symposium 58 (new series), Functions of the Septo-Hippocampal System. Amsterdam: Elsevier, 1978, pp. 5-24.

MacLean, P.D. Psychosomatic disease and the "visceral brain". Recent developments bearing on the Papez theory of emotion. Psychosomatic Medicine, 1949, 11, 338-353.

Mays, L.E. and Best, P.J. Hippocampal unit activity to tonal stimuli during arousal from sleep and in awake rats. Experimental Neurology, 1975, 47, 268-279.

McCarley, R.W. and Hobson, J.A. Simple spike firing patterns of cat cerebellar Purkinje cells in sleep and waking. Electroencephalography and Clinical Neurophysiology, 1972, 333, 471-483.

McCleary, R.A. Response-modulation functions of the limbic system: Initiation and suppression. In E. Stellar and J.N. Sprague (Eds.), Progress in Physiological Psychology. NY: Academic, 1966.

McLennan, H. and Miller, J.J. The hippocampal control of neuronal discharges in the septum of the rat. Journal of Physiology, 1974, 237, 607-624.

Meibach, R.C. and Siegel, A. Efferent connections of the hippocampal formation in the rat. Brain Research, 1977, 124, 197-224.

Miller, S.W. and Groves, P.M. The release of cellular inhibition in the rat hippocampus following medial septal lesions. Neurosciences Abstracts, 1977, Abstract 560.

Milner, P.M. Physiological Psychology. New York: Holt, Rinehart, and Winston, 1970.

Moore, G.P., Perkel, D.H., and Segundo, J.P. Statistical analysis and functional interpretation of neuronal spike data. Annual Review of Physiology, 1966, 28, 493-522.

Moore, G.P., Segundo, J.P., Perkel, D.H., and Levitan, H. Statistical signs of synaptic interaction in neurons. Biophysics Journal, 1970, 10, 876-900.

Mosko, S., Lynch, G., and Cotman, C.W. Distribution of the septal projection to the hippocampal formation of the rat. Journal of Comparative Neurology, 1973, 152, 163-174.

Nadel, L. and O'Keefe, J. The hippocampus in pieces and patches: an essay on modes of explanation in physiological psychology. In R. Bellairs and E.G. Gray, Essays on the Nervous System. A Festschrift for Prof. J.Z. Young. Oxford: Clarendon Press, 1974, pp. 367-390.

Noda, H. and Adey, W.R. Firing of neuron pairs in cat association cortex during sleep and wakefulness. Journal of Neurophysiology, 1970, 33, 672-684.

Noda, H., Manohar, S., and Adey, W.R. Correlated firing of hippocampal neuron pairs in sleep and wakefulness. Experimental Neurology, 1969, 24, 232-247.

O'Keefe, J. Place units in the hippocampus of the freely moving rat. Experimental Neurology, 1976, 51, 89-109.

O'Keefe, J. and Nadel, L. The Hippocampus as a Cognitive Map. Oxford: Oxford University Press, 1978.

Oomura, Y., Ooyama, H., Yamamoto, T., Naka, F., Kobayashi, N. and Ono, T. Neuronal mechanisms of feeding. Progress in Brain Research, 1967, 27, 1-33.

Papez, J.W. A proposed mechanism of emotion. Archives of Neurology and Psychiatry, 1937, 38, 725-743.

Passouant, P. (Ed.) Physiologie de l'Hippocampe. Paris: Editions du Centre Nationale de la Recherche Scientifique, 1962.

Pellegrino, L.J. and Cushman, A.J. A Stereotaxic Atlas of the Rat Brain. New York: Appleton-Century-Crofts, 1967.

Penfield, W. and Milner, B. Memory deficit produced by bilateral lesions in the hippocampal zone. Archives of Neurological Psychiatry, 1958, 79, 475-497.

Perkel, D.H., Gerstein, G.L., and Moore, G.P. Neuronal spike trains and stochastic point processes. I. The single spike train. Biophysics Journal, 1967, 7, 391-418. (a)

Perkel, D.H., Gerstein, G.L., and Moore, G.P. Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. Biophysics Journal, 1967, 7, 419-440. (b)

Petsche, H. and Stumpf, Ch. Hippocampal arousal and seizure activity in rabbits: toposcopical and microelectrode aspects. In: P. Passouant, (Ed.) Physiologie de l'Hippocampe. Paris: Editions du Centre Nationale de la Recherche Scientifique, 1962.

Petsche, H., Stumpf, Ch., and Gogolak, G. The siginificance of the rabbit's septum as a relay station between the midbrain and hippocampus. I. The control of hippocampal arousal activity by the septum cells. Electroencephalography and Clinical Neurophysiology, 1962, 14, 202-224.

Purpura, D. Discussion in Grastyan (1959). In: Second Conference on the Central Nervous System and Behavior, Transactions. NY: Josiah Macy, Jr. Foundation, 1959, pp 171-189.

Raisman, G. A comparison of the mode of termination of the hippocampal and hypothalamic afferents to the septal nuclei as revealed by electron microscopy of degeneration. Experimental Brain Research, 1969, 7, 317-343.

Raisman, G., Cowan, W.M., and Powell, T.P.S. An experimental analysis of the efferent projections of the hippocampus. Brain, 1966, 89, 83-108.

Ramón y Cajal, S. Studies on the cerebral cortex (limbic structures). Translated by Lisbeth M. Kraft. Chicago: Year Book Publishers, 1955.

Ranck, Jr., J.B. Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. Experimental Neurology, 1973, 41, 461-555.

Ranck, Jr., J.B. Behavioral correlates and firing repertoires of neurons in septal nuclei in unrestrained rats. In: J.F. DeFrance (Ed.), The Septal Nuclei. NY: Plenum Press, 1976.

Rawlins, J.N.P. and Green, K.F. Lamellar organization in the rat hippocampus. Experimental Brain Research, 1977, 28, 335-344.

Renshaw, B., Forbes, A., and Morison, B.R. Activity of isocortex and hippocampus: electrical studies with microelectrodes. Journal of Neurophysiology, 1940, 3, 74-105.

Roberts, L.E. McMaster University, Technical Report No. 52, January 1973.

Robinson, T.E., Kramis, K.C., and Vanderwolf, C.H. Two types of cerebral activation during active sleep: relations to behavior. Brain Research, 1977, 124, 544-549.

Rodieck, R.W. Maintained activity of cat retinal ganglion cells. Journal of Neurophysiology, 1967, 30, 1043-1071.

Routtenberg, A. Hippocampal activity and brainstem reward-aversion loci. Journal of Comparative and Physiological Psychology, 1970, 72, 161-170.

Scoville, W. and Milner. B. Loss of recent memory after bilateral hippocampal lesion. Journal of Neurological and Neurosurgical Psychiatry, 1957, 20, 11-21.

Segal, M. A correlation between hippocampal responses to interhemispheric stimulation, hippocampal slow rhythmical activity and behavior. Electroencephalography and Clinical Neurophysiology, 1977, 45, 409-411.

Segal, M. The acetylcholine receptor in the rat hippocampus; nictinic, muscarinic, or both? Neuropharmacology, 1978, 17, 619-623.

Siegel, A., Edinger, H., and Ohgami, S. The topographical organization of the hippocampal projection to the septal area: A comparative neuroanatomical analysis in the gerbil, rat, rabbit and cat. Journal of Comparative Neurology, 1974, 157, 359-378.

Stevens, J.K. and Gerstein, G.L. Interactions between cat lateral geniculate neurons. Journal of Neurophysiology, 1976, 39, 239-256.

Stumpf, Ch. Drug action on the electrical activity of the hippocampus. International Review of Neurobiology, 1965, 8, 77-138. (a)

Stumpf, Ch. The fast component in the electrical activity of the rabbit hippocampus. Electroencephalography and Clinical Neurophysiology, 1965, 18, 477-486. (b)

Swanson, L.W. The anatomical organization of the septo-hippocampal projections. In: CIBA Foundation Symposium 58 (new series) Functions of the Septo-Hippocampal System. Amsterdam: Elsevier, 1978, pp. 25-48.

Swanson, L.W. and Cowan, W.M. Hippocampal-hypothalamic connections: Origin in subicular cortex, not Ammon's horn. Science, 1975, 189, 303-304.

Swanson, L.W. and Cowan, W.M. Autoradiographic studies of the development and connections of the septal area in the rat. In: J. DeFrance (Ed.), The Septal Nuclei. New York: Plenum Press, 1976.

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

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

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

Vanderwolf, C.H. Limbic-diencephalic mechanisms of voluntary movement. Psychological Review, 1971, 78, 83-113.

Vanderwolf, C.H. Neocortical and hippocampal activation in relation in to amphetamine. Journal of Comparative and Physiological Psychology, 1975, 88, 300-323.

Vinogradova, O. Registration of information and the limbic system. In: G. Horn and R.A. Hinde (Eds.) Short-term changes in neural activity and behavior. Cambridge, Cambridge University Press, 1970.

Vinogradova, O.S. Discussion In: CIBA Foundation Symposium 58 (new series), Functions of the Septo-Hippocampal System. Amsterdam: Elsevier, 1978, p. 197.

Whishaw, I.Q. and Vanderwolf, C.H. Hippocampal EEG and behavior: changes in amplitude and frequency of RSA (Theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. Behavioral Biology, 1973, 8, 461-484.

Whishaw, I.Q., Bland, B.H., Robinson, T.E., and Vanderwolf, C.H. Neuromuscular blockade: the effects on two hippocampal RSA (theta) systems and neocortical desynchronization. Brain Research Bulletin, 1976, 1, 573-581.

Wilson, C.J., Young, S.J., and Groves, P.M. Statistical properties of neuronal spike trains in the substantia nigra: cell types and their interactions. Brain Research, 1977, 136, 243-260.

Wilson, C.L., Motter, B.C., and Lindsley, D.B. Influences of hypothalamic stimulation upon septal and hippocampal electrical activity in the cat. Brain Research, 1976, 107, 55-68.

Winson, J. Interspecies differences in the occurrence of theta. Behavioral Biology, 1972, 36, 291-301.

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

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

Winson, J. Hippocampal theta rhythm. II. Depth profiles in the freely moving rabbit. Brain Research, 1976, 103, 71-80. (b).

Winson, J. and Abzug, C. Neuronal transmission through hippocampal pathway dependent on behavior. Journal of Neurophysiology, 1978, 41, 716-732.

Wolfson, S., Fox, S.E., and Ranck, Jr., J.B. Theta cells in dorsal CA1, CA3, and dentate fire maximally on the positive phase of dentate theta rhythm in walking rat. Neurosciences Abstracts, 1979, Abstract 950.