ELECTRICAL ACTIVITY IN THE HIPPOCAMPAL FORMATION OF THE RAT: ROLE OF ASCENDING MONOAMINE-CONTAINING SYSTEMS.

by

SOUHILE Y. ASSAF

B.Sc., University of Western Ontario, 1974

M.Sc., University of Western Ontario, 1975

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Department of Physiology

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date Oct, 1978.

ABSTRACT

The effects of electrical stimulation of the monoamine-containing nuclei, the median raphe (MR) and the locus coeruleus (LC), on extracellularly recorded hippocampal electrical activty were studied in urethane anaesthetized rats. The following observations suggested that serotonergic and noradrenergic systems originating in MR and LC, respectively, modulate rhythmical slow activity (RSA) recorded in the dentate gyrus and/or the population response of granule cells to stimulation of the perforant path (PP) input.

1. RSA (3-7 Hz.) recorded in the dentate gyrus (DG) was related to the bursting discharge pattern of medial septal (MS) neurones. Electrolytic or kainate lesions of the MS abolished RSA thereby supporting the conclusion that rhythmical hippocampal activity was initiated by MS neurones.

2. Stimulation of MR resulted in disruption of the bursting discharge of MS neurones and desynchronization of RSA. Depletion of forebrain serotonin following pretreatment with para-chlorophenylalanine (p-CPA) eliminated these responses while quipazine (1 mg/kg), a serotonin agonist, mimicked the effects of MR stimulation. These data confirmed that a serotonin-containing system mediates the disruption of bursting discharge in MS and desynchronization of hippocampal electrical activity.

3. LC stimulation evoked RSA. Intracerebral injections of 6-hydroxydopamine (6-OHDA) which depleted hippocampal noradrenaline (NA) did not eliminate this response. In addition,

electrolytic lesions of the LC did not eliminate RSA. These data suggested that NA-containing afferents to the hippocampal formation are not essential for the generation of rhythmical activity.

4. Stimulation of the PP resulted in synaptic potentials in the dendritic layer of DG and population spikes recorded in the granule cell body layer. Conditioning stimulation of MR or LC increased the amplitude of the population spike evoked by a test PP pulse without altering the amplitude or rate of rise of the synaptic potential. The effects of MR and LC stimulation were blocked by p-CPA and 6-OHDA, respectively, suggesting that these monoamine-containing nuclei specifically influenced the responsiveness of dentate granule cells.

5. The observations that changes in the amplitude of the population spike, such as those observed following stimulation of MR and LC, could occur in the absence of changes in the synaptic potential were confirmed using an additional afferent to the dentate via the commissural projection. These data indicated that extrinsic afferents to DG may potentiate the discharge of a population of granule cells without enhancing synaptic potentials.

On the basis of the above data, it was concluded that pharmacologically distinct neuronal systems originating in MR and LC modulate electrical activity in the hippocampal formation.

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DECLARATION

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TABLE OF EQUIVALENTS AND ABBREVIATIONS

Eqivalents

hippocampus = ammons horn = hippocampal fields CA1-4

dentate gyrus = fascia dentata = dentate

hippocampal formation = hippocampus + dentate gyrus

potentiation = facilitation = enhancement of test responses

Frequently Used Abbreviations

AP	anterior-posterior stereotaxic co-ordinate
B-neurone	septal neurone which discharges in bursts
CNS	central nervous system
COMM	hippocampal commissural pathway
DA	dopamine
DG	dentate gyrus
EC	entorhinal cortex
EPSP	excitatory postsynaptic potential
G-cell	dentate granule cell
HRP	horseradish peroxidase
I-neurone	septal neurone displaying irregular discharge
L	medial-lateral stereotaxic co-ordinate
LC	locus coeruleus
MF	mossy fibre pathway
MS	medial septal nucleus
MR	median raphe nucleus
NA	noradrenaline (norepinephrine)
PP	perforant path
RSA	rhythmical slow activity

p-CPA	p-chlorophenylalanine
5-HT	5-hydroxytryptamine (serotonin)
6-OHDA	6-hydroxydopamine

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1.0 INTRODUCTION

The foundations of contemporary neuroscience are based on the assumption that the nervous system is composed of individual nerve cells that generate electrical activity which, by chemical processes, is transmitted to adjacent neurones at specialized regions called 'synapses' (Langley, 1905; Sherrington, 1906; Cajal, 1911; Dale, Feldberg and Vogt, 1936; Eccles, Katz and Kuffler, 1941). Although these concepts are now firmly established, new modes of synaptic organization including chemical and electrical junctions between dendrites, somata and axons have extended this view of the nervous system. The mechanisms by which information is transmitted from one region of the CNS to another and the intrinsic synaptic organization of the various nuclei are the concerns of systems neurophysiology.

The methodology of the neurosciences has parallelled these developments resulting in a combination of electrophysiological, pharmacological and anatomical techniques. The use of microelectrodes for recording intracellular and extracellular electrical activity of single cells combined with analysis of slow waves generated by populations of neurones has provided information about the synaptic organization of major neuronal systems. In order to understand the role of these systems in the overall

pattern of brain function electrophysiological data must be related to both anatomical and biochemical data.

This integrative approach is possible in the study of the hippocampal formation which has a relatively simple and well defined synaptic organization. Its afferents terminate in discrete layers or subfields allowing correlation of neuroanatomical data with the characteristic features of its electrical activity (Adey and Meyer, 1952; Green and Arduini, 1954; Gloor, Vera and Sperti, 1964; Andersen, Eccles and Loyning, 1964; Lomo, 1971a).

The spontaneous slow wave patterns of the hippocampal formation, particularly rhythmical slow activity (RSA; theta rhythm), have been studied most extensively and related to the activity of single units in the hippocampus and the septal area (Jung and Kornmuller, 1938; Green and Arduini, 1954; von Euler and Green, 1960; Kandel and Spencer, 1961; Petsche, Stumpf and Gogolak, 1962). In addition to the spontaneous patterns of hippocampal activity, characteristic extracellular field potentials are evoked by stimulation of the entorhinal cortex (Gloor et al, 1964; Lomo, 1971a). These field responses are potentiated by repeated stimulation and have thus been used to study processes associated with neuronal transmission and storage of information (Lomo, 1971b;

Bliss and Lomo, 1973; Douglas and Goddard, 1975; Steward, White, Cotman and Lynch, 1976).

Early studies have proposed that hippocampal electrical activity is controlled by neuronal systems originating in diffuse regions of the brainstem (Green and Arduini, 1954). While the precise neuroanatomical and neurochemical origins of these systems are not known, the recent development of histochemical techniques for mapping pharmacologically distinct systems has revealed that the hippocampal formation receives direct serotonin- and noradrenaline-containing projections from the median raphe nucleus and the locus coeruleus, respectively (Falck, Hillarp, Thieme and Torp, 1962; Fuxe, 1965; Ungerstedt, 1971; Moore, 1975). Since these monoamine systems are within the diffuse brainstem regions which modulate cortical and hippocampal 'arousal' (Moruzzi and Magoun, 1949; Green and Arduini, 1954; Lindsley, 1960), they may be involved in determining the characteristic features of hippocampal electrical activity.

The present study examines the role of the ascending monoamine systems in the control of slow electrical activity and evoked field potentials recorded in the hippocampal formation of the rat. The aim of the experiments has been to delineate the mechanisms by which these extrinsic inputs influence the spontaneous patterns of rhythmical hippocampal

activity and neuronal transmission between the entorhinal cortex and the dentate gyrus.

1.1. REVIEW OF THE LITERATURE

1.1.1 The Hippocampal Formation

The hippocampal formation is a horn shaped cortical area lying adjacent to the wall of the lateral ventricles. This region is subdivided into the hippocampus proper, the dentate gyrus (Fascia Dentata) and the subicular complex. The hippocampus proper can be further subdivided into 4 fields (CA1, CA2, CA3 and CA4) in which the major cell type is the pyramidal cell (Lorente de No, 1934; Blackstad, 1956). The CA4 cell layer enters the hilus of the dentate gyrus which is formed by a curved sheet of dentate granule cells.

Both the hippocampal pyramidal cells and dentate granule cells form well defined layers. As shown in Fig. 1-1, these layers contain almost exclusively either pyramidal cell bodies (Stratum Pyramidale), the dendrites of pyramidal cells (Stratum Oriens, Radiatum and Lacunosum), dentate granule cell bodies or the dendrites of granule cells (Stratum Moleculare). In addition to the long axoned pyramidal and granule cells many cell types having short axons can be found in the hippocampal formation. The latter neurones, which may be intrinsic to the hippocampal formation, include Golgi type II (basket) cells which appear to contact numerous pyramidal and granule cells (Cajal, 1968). In addition to basket cells there are several layers of

polymorphic cells (Strata Polymorphe) or modified pyramidal cells in the dentate hilus (Lorente de No, 1934).

As shown in Fig.1-1, the axons of granule cells form the mossy fibre system which contacts the dendrites of CA3 cells (Cajal, 1968; Barber, Vaughan and Wimer, 1974). The axons of CA3 cells project into the fimbria giving off Schaeffer collaterals which reach the basal dendrites of CA1 and CA2 pyramidal cells whose axons, in turn, form the alveus. This synaptic organization suggests that any afferents which influence dentate granule cells would sequentially affect CA3 and CA1 cells along this tri-synaptic loop. The precise laminar organization of this neuronal loop will become more apparent following an examination of the afferents to the hippocampal formation.

Major Afferents

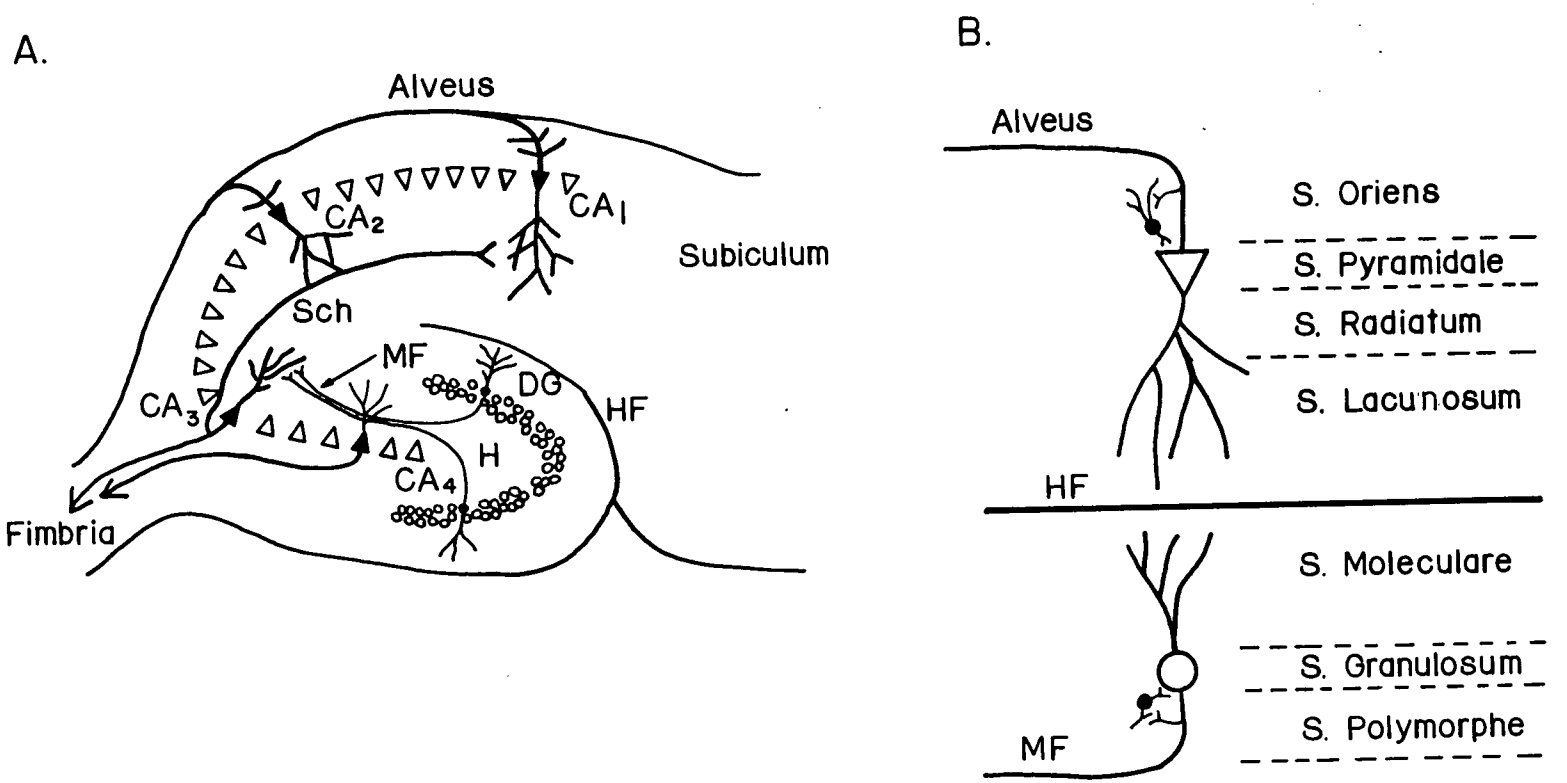
A. The Perforant Path

The major afferent projection to the hippocampal formation is from the entorhinal cortex by way of the perforant path (PP) fibre system (Lorente de No, 1934). The anatomical studies of Hjorth-Simonsen (1973) demonstrate that the lateral entorhinal region is the source of PP fibres which terminate in the outer portion of the dentate molecular layer while the medial

FIG. 1- 1: Drawing Of The Intrinsic Synaptic Organization Of The Hippocampal Formation.

A: transverse section showing pyramidal cell layers CA1-CA4 and granule cells in the dentate gyrus (DG). Axons of granule cells form mossy fibres (MF) which project to CA3. Projections from CA3 are via the fimbria to the septal area and by Schaeffer collaterals (Sch) onto dendrites of CA2 and CA1.

B: detail of the various layers from dorsal surface of CA1 pyramidal cell to the lower blade of the dentate gyrus. The hippocampal fissure (HF) is the demarcation zone. This figure is modified from Green (1964) and Cajal (1911).


A.
Alveus
CA2
CA1
Subiculum
Sch
MF
DG
HF
CA3
H
CA4
Fimbria
B.
Alveus
S. Oriens
S. Pyramidale
S. Radiatum
S. Lacunosum
HF
S. Moleculare
S. Granulosum
S. Polymorphe
MF

entorhinal region gives rise to PP fibres that terminate in the middle molecular layer of the dentate. Electron microscopic investigations suggest that the mode of termination is primarily en passage onto dendritic spines of dentate granule cells (Nafstad, 1967).

These anatomical data have been confirmed by electrophysiological analysis of field potentials recorded in the dentate following stimulation of the PP or the entorhinal cortex itself (Gloor, Vera and Sperti, 1964; Lomo, 1971a; Steward, White, Cotman, and Lynch, 1976). The relationships between these extracellular potentials and intracellular recordings of single unit activity indicated a monosynaptic excitatory PP input onto the dendrites of granule cells. The potency of this input is reflected by the synchronous activation of a population of granule cells by single pulse stimulation of the PP (Andersen, Bliss and Skrede, 1971).

Furthermore, the input from the PP to the dentate is organized in parallel strips or lamellae such that stimulation of a discrete part of PP excites granule cells only in a thin band of tissue roughly transverse to the longitudinal axis of the hippocampus (Lomo,1971a). From recordings of single unit activity it appears that an afferent volley excites a population of granule cels within a lamella (Andersen et al,

1971;). Andersen, 1975). Following the single spike activation of granule cells produced by PP stimulation, a long period of inhibition is recorded (Lomo, 1971a). This inhibition is similar to that reported by Spencer and Kandel (1961) in CA1 cells in that nearly all cells are inhibited independent of whether they were previously activated by the stimulus. As will be described in a later section, this inhibition is postulated to result from a recurrent inhibitory mechanism (Gloor, 1963; Andersen et al, 1964).

In addition to the massive PP projection to the dentate a lesser number of PP fibres are known to terminate in CA3 (Hjorth-Simonsen and Jeune, 1972; Hjorth-Simonsen, 1973) and possibly in CA1 (Raisman, Cowan and Powell, 1965) subfields of the hippocampus. However, the sparse degeneration in CA1 following lesions of the entorhinal cortex may be due to degeneration of fibres en route to CA3 (Hjorth-Simonsen, 1973). Electrophysiological evidence tends to support these anatomical data since monosynaptic potentials are recorded in CA3 following stimulation of the PP (Gloor et al, 1964). In contrast, the fields recorded in CA1 have substantially smaller amplitudes and longer latencies than those recorded in the dentate or CA3 and may therefore be mediated through th trisynaptic circuitry described previously (Andersen, 1975; Winson and Abzug, 1978).

B. Commissural/Associational System

The commissural (COMM) connections of the hippocampus have been mapped out using degeneration (Blackstad, 1956), autoradiographic (Gottlieb and Cowan, 1973) and electrophysiological techniques (Andersen et al, 1961; Deadwyler, White, Cotman and Lynch, 1975). Whereas all fields of the hippocampus and dentate gyrus receive COMM inputs, the origin of COMM fibres is restricted to CA3-CA4 of the contralateral hippocampus (Gottlieb and Cowan, 1973; Hjorth-Simonsen and Laurberg, 1978). The large myelinated COMM fibres leave the contralateral hippocampus via the alveus proximal to the inferior blade of the dentate and project through the ventral psalterium, re-entering the hippocampus near CA3.

In the dentate, the COMM input is restricted to the inner molecular layer below the PP synapses. Remarkably, there is no overlap between the entorhinal and COMM system. However, the same zone that is innervated by the COMM input is also the zone of termination of the associational system originating in ipsilateral CA3c (Zimmer, 1970). The significance of the overlap between the termination of COMM and associational fibres arising from contralateral and ipsilateral CA3, respectively, is not yet clear. There are few detailed electrophysiological studies of these projection systems and those available are discussed in

more detail in Chapter 7.

The COMM input to CA1 terminates on apical and basal dendrites of pyramidal cells (Andersen, 1960; Gottlieb and Cowan, 1973). The input onto apical dendrites is more massive as indicated by the dense degeneration following COMM transections (Blackstad, 1958) and a larger negative wave evoked by stimulation of the COMM pathway (Cragg and Hamlyn, 1957). The similarities between the synaptic wave evoked by stimulation of Schaeffer collaterals and COMM stimulation suggests that both of these inputs terminate primarily on apical dendrites (Andersen, 1960; see Fig. 4 in Andersen, 1975)

C. Septal-Hippocampal Projection

Although the projections from the septal area to the hippocampus have been studied in a number of species using various techniques, the precise organization of this system is far from clear. Powell (1963), on the basis of a Nauta-Gygax degeneration study in the rat, concluded that fibres from midline septal regions enter the body of the fornix reaching the hippocampus over the alveus to terminate throughout the hippocampus. Raisman (1966) using the same experimental preparation found that only the caudal aspect of the medial septal nucleus and the diagonal band send fibres through the ventral portion of the

fimbria which terminate in all hippocampal regions except CA1. More recently, Siegel and Tassoni (1971) disagreed with Raisman (1966) since they reported that lesions of the medial septal area result in degeneration only in the dorsal hippocampus including CA1. There is no simple explanation for the topographical differences found in the above studies. In any case the interpretations of the pattern of degeneration following septal lesions have to be tentative in light of possibilities that lesions also disrupt ascending fibres coursing through the septal area.

Another technique that has been used to map the septal-hippocampal projections is the orthograde transport of tritiated amino acids to the hippocampal formation. The advantage of this technique is that, with proper precautions, it minimizes the contribution of fibres of passage (Cowan, Gottlieb, Hendrickson and Price, 1972). Furthermore, autoradiography often reveals terminal areas which cannot be easily demonstrated using degeneration techniques (Swanson and Cowan, 1977). Following 3H-proline injections into the medial septal-diagonal band area, substantial labelling is observed in several regions of the hippocampus including CA2, CA3, CA4 of the hippocampus and the hilar region of the dentate gyrus. In contrast to the degeneration experiments and those based on histochemical localization of acetylcholinesterase

(reviewed below) Swanson and Cowan (1974) were unable to find evidence for a significant projection from the medial-septal diagonal band complex to CA1 or the inner molecular layer of the dentate gyrus. More recently, Rose, Hattori and Fibiger (1976) supported these findings since they reported that injections of 3H-leucine into the medial septal nucleus results in substantial labelling of CA3 and CA4 zones of the hippocampus but only sparse labelling in the molecular layer of the dentate. However, injections of 3H-adenosine into the medial septal nucleus result in substantial trans-synaptic labelling of the inner molecular layer as well as in the hilar region (Rose and Schubert, 1977).

In addition to the anatomical techniques described above, histochemical staining for acetylcholinesterase (AchE) has been used to map out the termination of the septal afferents. This enzyme is located in discrete layers in the hippocampus and dentate gyrus (Shute and Lewis, 1967; Fonnum, 1970; Storm-Mathisen, 1970; Mellgren and Srebo, 1973). The dentate region stains more heavily for AchE than the hippocampus proper and the heaviest staining consists of a band in the innermost portion of the molecular layer adjacent to the granule cell bodies and the dentate hilus (Mosko et al, 1973; Lynch and Cotman, 1975).

Following electrolytic lesions of the medial

septal-diagonal band area the above pattern of AchE staining is abolished (Lewis et al, 1967; Srebro and Mellgren, 1974) and the overall activity of AchE and cholineacetyltransferase (CAT) is greatly reduced (McGeer, Wada, Tergo and Jung, 1969; Storm-Mathisen, 1970, 1972). In contrast, lesions of the lateral septum or lateral hypothalamus did not influence hippocampal AchE (McGeer et al, 1969; Mellgren and Srebro, 1973) suggesting that a cholinergic input to the hippocampal formation originates in or passes through the medial septal area.

Under normal conditions the middle molecular region of the dentate which contains associational, commissural and entorhinal terminals does not stain for AchE, but destruction of these systems results in a marked intensification of AchE staining in the deafferented zones (Lynch, Mathews, Mosko, Parks and Cotman, 1972; Lynch and Cotman, 1975). A subsequent lesion in the medial septal area abolishes this new pattern of AchE staining as well as the normal layered pattern of AchE in the hilus (Lynch, 1972). On the basis of these data, it has been suggested that septal afferents may undergo collateral sprouting and take up positions in the outer molecular layer vacated by degenerating entorhinal afferents (See Lynch and Cotman, 1975 for review).

Studies of the uptake of the precursor choline and

the subsequent release of acetylcholine (Ach) also support the suggestion that Ach is probably released in the hippocampal formation from terminals originating in the septal area. Hippocampal synaptasomes have a high affinity uptake system for choline which is greatly reduced following medial septal lesions (Kuhar, Roth and Aghajanian, 1972; Kuhar, 1975). This decrease is only in the hippocampus and cortex suggesting that the uptake of choline may be a reasonably specific indicator of Ach turnover (Yamamura and Snyder, 1972).

Studies which measure Ach release from the hippocampus, utilizing a modification of the cortical cup technique, suggest that the resting release of Ach is markedly increased by stimulation of the medial septum (Smith, 1972, 1974; Dudar, 1975, 1977). These effects are specific to medial septal stimulation since stimulation of lateral septum or contralateral hippocampus does not influence Ach release in the hippocampus (Smith, 1972, 1974; Dudar, 1975). Administration of hemicholinium-3 (which blocks choline uptake) causes a gradual, dose-dependent depletion of Ach following medial septal stimulation (Rommelspacker and Kuhar, 1974).

The action of Ach on hippocampal neurones has been studied using micro-iontophoretic techniques (See Straughan, 1975 for review). Many hippocampal neurones are excited by Ach (Herz and Nacimiento, 1965;

Salmoiraghi and Stefanis, 1965; Biscoe and Straughan, 1966). The receptors of these cholinoceptive neurones may be muscarinic since the excitation produced by Ach, but not that produced by glutamate, is blocked by atropine (Biscoe and Straughan, 1965; Wheal and Miller, 1977). Unfortunately, few of these cholinoceptive cells were electrophysiologically identified but it appears that both pyramidal and granule cells are cholinoceptive. More recently, Wheal and Miller (1977) have reported that electrical stimulation of the medial septal area and the PP activate the same granule cells but only the septal evoked activation is blocked by atropine. This further supports the suggestion that a cholinergic system originating in the medial septal region excites dentate granule cells. However, it is extremely difficult to be sure that stimulation selectively excites septal cells and their axons but not fibres of passage coursing through this area. Thus any conclusions based on electrical stimulation of the septal area can only be tentative.

In conclusion, the neuroanatomical, histochemical and pharmacological studies reviewed above strongly support the existence of a septal-hippocampal projection which may use Ach as a transmitter. The precise organization of septal terminals in the dentate gyrus is still controversial since some studies (Lynch et al, 1972; Storm-Mathisen, 1974) report a substantial input terminating in the supragranular zone as well as

in the hilus whereas others (Swanson and Cowan, 1975; Rose et al, 1976) only report substantial innervation of the hilus. These differences are relevant for the interpretation of data based on intensification of AchE staining in the re-innervated dentate (Lynch and Cotman, 1975) and to determine whether the septal input is directly onto granule cells and/or neurones in the hilus.

D. Monoamine Pathways

The development of the Falck-Hillarp histofluorescence method for visualizing monoamine containing cell bodies and fibres in the CNS (Falck, 1962; Carlson, Falck and Hillarp, 1962; Falck, Hillarp, Thieme and Thorp, 1962) combined with existing neuroanatomical and biochemical techniques have formed the basis for studying the monoamine projections to the hippocampus. As outlined below, the hippocampal formation contains substantial amounts of the monoamines noradrenaline (NA) and serotonin (5-HT) but only trace amounts of dopamine (for reviews see Ungerstedt, 1971; Moore, 1975). The low concentration of dopamine in the hippocampus suggests that it does not play an important role in synaptic transmission in this area aside from being a precursor for noradrenaline. The purpose of this section is to describe the source and postulated physiological role of NA and 5-HT containing terminals in the hippocampal formation.

Noradrenaline (NA):

Fuxe (1964, 1965) first described NA containing cell bodies located in various nuclei in the brainstem. Fluorescence histochemical mapping of their projections indicated that these nuclei give rise to fine axons which descend and ascend for long distances reaching widespread areas in the CNS (Anden, Dahlstrom, Fuxe, Larsson, Olson and Ungerstedt, 1966; Fuxe, Hokfelt and Ungerstedt, 1969; Ungerstedt, 1971). Most relevant to the present thesis is the identification of a nucleus in the dorsal pons, the locus coeruleus (LC), consisting of tightly packed cells most of which contain NA (Anden et al, 1966; Ungerstedt, 1971). Axons from the LC project caudally and rostrally. The most prominent rostral projection is through the dorsal NA bundle which projects into the medial forebrain bundle to terminate throughout the forebrain, including the septal region, hippocampus and the entire cortex (Fuxe, 1965; Anden et al, 1966; Fuxe, Hamberger and Hokfelt, 1968; Ungerstedt, 1971; Lindvall and Bjorklund, 1974, Jones and Moore, 1977; Moore, 1978). Electrolytic lesions in the LC or dorsal NA bundle result in a marked loss of NA fluorescence in the above areas (Anden et al, 1966; Ungerstedt, 1971). Biochemical experiments provide additional support for an NA containing projection to the hippocampal formation since destruction of the dorsal bundle severely reduces NA content of the hippocampus (Moore, 1973; Kobayashi,

Palkovits, Kopin and Jacobowitz, 1974) whereas electrical stimulation of this pathway causes a release of NA in the hippocampus (Arbuthnott, Crow, Fuxe, Olson and Ungerstedt, 1970).

The NA containing fibres reach the hippocampus via the fornix or by looping around the corpus callosum and entering through the cingulum (Ungerstedt, 1971; Lindvall and Bjorklund, 1974; Moore, 1975). However, using an immunofluorescence technique to visualize dopamine-beta-hydroxylase (DBH), Swanson and Hartman (1975) failed to find DBH-containing fibres in the fornix-fimbria suggesting that the caudal route may be more important.

The difficulties in discriminating between NA terminals and fibres based on NA or DBH fluorescence make a precise description of their distribution tentative. However, combined with autoradiographic techniques (Jones and Moore, 1977), a layered NA distribution in the hippocampus may be described. The densest terminal region is the dentate hilus and adjacent mossy fibre-CA3 zone followed by moderately dense projection to the stratum lacunosum of CA1 (Swanson and Hartman, 1975; Jones and Moore, 1977). The molecular layer of the dentate and the area of pyramidal cell bodies were only sparsely labelled. More recently, a histofluorescence technique for visualizing adrenergic receptors was used to study the distribution

of possible NA receptor sites (Melamed, Lahav and Atlas, 1977). In contrast to NA histofluorescence and autoradiographic studies described above, this study reports a high density of beta-adrenergic receptor sites (i.e. sites which bind a fluorescent analogue of propranolol) in the dentate granule cell layer.

The effects of micro-iontophoretically applied NA on neurones in the hippocampal formation indicate that it produces a long latency inhibition of pyramidal cells (Herz and Nacimiento, 1965; Salmoiraghi and Stefanis, 1965; Biscoe and Straughan, 1966; See Straughan, 1975 for review). Segal and Bloom (1974 a,b) attempted to relate the action of iontophoretically applied NA and the effects of electrical stimulation of LC on hippocampal pyramidal cells. These authors reported that LC stimulation or direct application of NA inhibit pyramidal cells. Moreover, these effects were blocked by beta-adrenergic antagonists and were absent in 6-hydroxydopamine-treated rats (Segal and Bloom, 1974b). Although these data are suggestive, there is still no firm physiological evidence implicating NA-containing terminals in synaptic function of the hippocampal formation.

Serotonin (5-HT):

The 5-HT innervation of the hippocampal formation originates from neurones within the midbrain raphe nuclei (Dahlstrom and Fuxe, 1965; Anden et al, 1966). Biochemical studies by Lorens and Guldberg (1974) indicate that the median raphe (nucleus centralis superior) and not the dorsal raphe is the source of hippocampal 5-HT. Histofluorescence (Fuxe et al, 1970; Bjorklund et al, 1973) and autoradiographic (Moore and Halaris, 1975) studies indicate that axons arising from the median raphe ascend in the medial forebrain bundle to reach the hippocampal formation via the fornix and the cingulum.

Within the hippocampal formation the distribution of 5-HT is almost impossible to study using histochemical methods due to the low fluorescence and small size of the terminals. Even the glyoxylic acid method (Lindvall and Bjorklund, 1974) which improves the localization of catecholamines does not aid in the detailed analysis of 5-HT systems. However, data based on autoradiographic procedures suggest that the distribution of axons arising from the median raphe nucleus essentially overlaps the NA innervation described in the previous section (Conrad, Leonard and Pfaff, 1974; Moore and Halaris, 1975). The densest termination in the hippocampal formation is restricted to a band approximately 65 um wide, along the ventral

border of dentate granule cells (Moore, 1975). It cannot be determined whether these terminals are on cell bodies of granule cells or subjacent hilar neurones. There is sparse to moderate innervation of the dentate molecular layer (See Moore, 1975 Fig. 4).

It must be kept in mind that the above terminal pattern does not necessarily reflect 5-HT innervation since some raphe neurones are not serotonergic. More recently Halaris et al (1976) have shown that injections of radiolabelled 5-HTP into the MR result in a similar pattern of termination to the amino acid. Furthermore, "selective" destruction of 5-HT terminals with 5,6-dihydroxytryptamine results in greatly reduced axonal transport from the median raphe to the hippocampal formation. Both of these observations support the conclusion that the pattern of innervation described above reflects the distribution of 5-HT containing terminals in the hippocampal formation.

Serotonin (5-HT) has been iontophoretically applied to pyramidal cells in anaesthetized cats (Stefanis, 1964; Biscoe and Straughan, 1965) and rats (Segal and Bloom, 1974a). In most cases 5-HT caused a short latency (2-15 sec) depression of neuronal discharge which, unlike the depressent action of NA, did not persist after the drug application had been stopped. In fact, NA was a weak depressant of neuronal firing relative to 5-HT when comparable ejection

currents were used (Biscoe and Straughan, 1966).

Neither the mechanism by which 5-HT depresses hippocampal neurones nor the physiological significance of this action are known. For these reasons any postulated role for 5-HT or NA in synaptic transmission in the hippocampus must be tentative; many cells may have receptors for substances not normally released onto them as transmitters.

Major Hippocampal Efferents

Although the efferent connections of the hippocampal formation have been studied for more than a century, our understanding of their organization is comparatively vague. Only recently have detailed descriptions of the hippocampal efferents other than the fornix been attempted. The new data have prompted a re-evaluation of the widely held view that the fornix is the major, if not the only, efferent from the hippocampal formation (Maynert, 1872; Von Gudden, 1881; Cajal, 1911; Nauta, 1956; Guillery, 1957) and have emphasized the importance of caudally-directed efferents to cortical and subcortical regions (Chronister and White, 1975; Hjorth-Simonsen, 1971, 1973; Swanson and Cowan, 1977).

Another new development has been in regard to what regions of the hippocampal formation give rise to extrahippocampal projections. At the simplest level,

the dentate gyrus does not have any extrahippocampal projections since its outflow consists almost entirely of the mossy fibre projection to CA3-CA4 pyramidal cells. Likewise the CA4 pyramidal cells have no extrahippocampal projection but do give rise to associational and commissural projections to the ipsilateral and contralateral dentate, respectively (Zimmer, 1970; Gottlieb and Cowan, 1973). Field CA3 gives rise to extensive intrahippocampal associational/commissural projections as well as an extrahippocampal projection to the septal area (Gottlieb and Cowan, 1973; Raisman et al, 1966). Perhaps the most substantial extrahippocampal projection arises from CA1 and projects through the precommissural fornix bilaterally to the lateral septal area (Swanson and Cowan, 1977) and through the alveus to the adjacent subiculum (Hjorth-Simonsen, 1973; Swanson and Cowan, 1977). The subiculum, in turn, gives rise to the massive post commissural fornix (Raisman et al, 1966; Swanson and Cowan, 1977).

On the basis of the above data, the hippocampus proper contributes only to the precommissural fornix whereas the subiculum is the source of the entire post-commissural projection to the anterior thalamic and mammillary nuclei. In addition to these direct extrahippocampal projections described in more detail below, indirect connections of the hippocampal formation through thalamic nuclei may influence the

frontal and parietal lobes thereby completing the so-called Papez (1937) circuit.

A. Fimbria-Fornix System

Although Elliot Smith (1897) concluded that the arrangement of the fornix conforms to the same fundamental plan in all vertebrates, many subsequent studies have shown significant differences between species and in different strains of the same species (Edinger and Wallenberg, 1902; Simpson, 1952; Cowan and Powell, 1955; Valenstein and Nauta, 1959). In the rat, the direct hippocampal projections contained in the precommissural fornix project to the septal nuclei and the preoptic region and through the columns of the fornix to the mammillary body and the medial hypothalmic region (Cajal, 1911; Nauta, 1956; Swanson and Cowan, 1971). Only a few fornical fibres reach the midbrain in the rat. In contrast, the cat fornix has a massive projection to the rostral mid-brain and few fibres to the medial hypothalamic region (Nauta, 1959). In primates the dorsal fornix projections are less pronounced than they are in rodents and do not appear to terminate caudal to the mammillary bodies (Paletti and Creswell, 1976).

Several proposals have been made regarding the topographical organization of the hippocampal efferents via the fimbria-fornix. Raisman et al (1966) suggested

that CA1 projects principally to the medial septal region while CA3 projects to the lateral septum. In contrast Siegel et al (1974) also using rats, suggest that the medial and lateral septal regions are innervated by the dorsal and ventral hippocampus, respectively. The postcommissural fornix originating in the subiculum may also be topographically organized in the dorsoventral axis. Swanson and Cowan (1977) suggest that the dorsal subiculum projects predominantly upon the mammillary complex and the ventral subiculum projects to the rostral hypothalamus and the basal forebrain including the nucleus accumbens and the bed nucleus of the stria terminalis.

Plasticity Of Synaptic Transmission In The Hippocampal Formation

Since the major afferents to the hippocampal formation occupy discrete dendritic layers which can be identified using both neuroanatomical and electrophysiological techniques, it is an ideal system for evaluating structural and functional modifications of synaptic transmission. Structural plasticity has been evaluated by determining the anatomical readjustments which occur following elimination of a major afferent to the same region. Functional plasticity has been studied primarily by determining the changes in efficacy of synaptic transmission resulting from previous activation of the same pathway.

Both of these approaches may contribute to an understanding of the mechanisms associated with processing and storage of information, however, only functional plasticity has direct relevance to the present work and will be reviewed below. Several reviews on terminal proliferation and synaptogenesis in the hippocampal formation following deafferentation are available (Lynch and Cotman, 1975; McWilliams and Lynch, 1978).

A feature of all excitatory afferents in the hippocampal formation studied so far is the increase in synaptic transmission during repetitive stimulation. The increased efficacy is seen as an enhancement in both the amplitude and rate of rise of extracellular field responses and an increase in the number of cells which are activated by the test pulse (Lomo, 1971b; Bliss and Lomo, 1973). The simplest example of this phenomenon was provided by Lomo (1971b) using double pulse stimulation of the perforant path-dentate projection in the rabbit. The monosynaptic test EPSP and population spike (the synchronous discharge of a number of cells) responses were both potentiated by a preceding (20-300 mesc) conditioning volley. The test EPSP was potentiated as much as 100% while the population spike could be potentiated up to 300%. These observations have been recently confirmed in the rat (Steward et al, 1976; Assaf and Miller, 1978) and in the <u>in vitro</u> hippocampal slice (Dudek et al, 1975).

If some excitatory synapses in the hippocampal formation are stimulated with particular frequencies (usually 4-15/sec) there is a gradual increase in the amplitude of evoked potentials until a maximum response is obtained after 10-30 stimuli (Bliss and Lomo, 1973; Andersen, 1975). With higher stimulation rates, the duration of the enhanced potentials is shorter, giving way to a period of depression usually lasting less than 5 minutes. This so-called "frequency facilitation" is more obvious in the mossy fibre-CA3 pathway than in the perforant path-dentate projection (Andersen, 1975).

Tetanic stimulation gives rise to an increased responsiveness of the stimulated pathway to single volleys delivered several hours later (Bliss and Lomo, 1973). Similar post-tetanic potentiation has recently been demonstrated in _in vitro_ hippocampal slice (Lynch et al, 1977; Tyler and Alger, 1975; Andersen et al, 1977; Dunwiddie and Lynch, 1978). The observed potentiation is not dependent on changes in the presynaptic fibre volley and is not observed in adjacent synaptic inputs which were not tetanized (Lynch et al, 1977; Andersen et al, 1977). In fact, Dunwiddie and Lynch (1978) recently reported a depression of the non-tetanized pathway which occurs simultaneously with potentiation of the stimulated pathway.

Mechanisms which mediate the above forms of functional plasticity are not known. Andersen (1975) suggests that they may reflect a changed ability of the presynaptic terminal to deliver increased amounts of transmitter. More recently, Dunwiddie, Madison and Lynch (1978) observed that synaptic transmission is necessary for producing long-term potentiation. These and other possibilities are discussed in more detail at the end of Chapter 8.

1.1.2 The Septal Area

The septal area comprises a group of nuclei located between the anterior horns of the lateral ventricles. Their extent is bounded ventrally by the decussations of the anterior commissure; dorsally by the corpus callosum; rostrally by the frontal cortex and caudally by the descending columns of the fornix (Hines, 1922; Young, 1936; Andy and Stephan, 1965). On the basis of its cytoarchitecture the septal area of the rat may be subdivided into medial, lateral, posterior and ventral divisions (Swanson and Cowan, 1974). The medial septal region consists of the medial septal nucleus dorsally and the nucleus of the diagonal band of Broca ventrally. The distinguishing feature of the medial septal region is the mass of large neurones which in Golgi preparations have long spine-free dendrites (Tombol and Petsche, 1969; Swanson and Cowan, 1974). These large bipolar neurones are interposed by

many small neurones. The lateral septal region consists primarily of medium sized cells which can be grouped into dorsal, intermediate and ventral subdivisions (Swanson and Cowan, 1974). The posterior septal region consists of the septo-fimbrial nucleus which is embedded within the precommissural fornix and the triangular septal nucleus which contains tightly packed cells within the ventral hippocampal commissure. The ventral septal region is poorly defined with the exception of the bed nucleus of the stria terminalis which receives afferents from the amygdala (Andy and Stephan, 1965, 1974; Tombol and Petsche, 1969; Swanson and Cowan, 1974).

Although the above description and terminology are based primarily on investigations in the rat, they may be applicable to other mammalian species since the septal region retains the above features in primate evolution. This uniformity is maintained despite the progressive enlargement and relative development of the primate septum (Andy and Stephan, 1965, 1974).

Major Afferents

The septal region receives its major afferents from the hippocampal formation via the fornix system, from the cortico-medial amygdala via the stria terminalis and from hypothalamic and midbrain regions by way of the medial forebrain bundle. More recently, ascending monoamine systems originating in the pons and midbrain have been shown to extensively innervate the septal region. On the basis of these connections, the septal region occupies a pivotal position between midbrain and forebrain areas.

A. Hippocampal Inputs

The major input from the hippocampal formation via the precommissural fornix terminates in the lateral septal region (Powell et al, 1957; Raisman et al, 1966; Swanson and Cowan, 1977). Raisman et al (1966) observed that lesions in fields CA1 and CA3 result in degeneration distributed to the dorsal and lateral parts of the lateral septal nucleus. More recently, Swanson and Cowan (1977) suggested that the above pattern of degeneration does not indicate that the sole source of the precommissural fornix is the hippocampus proper since fibres originating from the subiculum are also destroyed by these lesions. On the basis of autoradiographic studies, Swanson and Cowan (1977) concluded that the major source of these precommissural

fibres may be the ipsilateral subicular region. The afferents to the lateral septal region are topographically organized such that dorsal hippocampal regions project exclusively to the dorsal aspect of the lateral septal nucleus and ventral hippocampal fields project to the ventral portions of the lateral septal area (Raisman et al, 1966; Siegel and Tassoni, 1971; Swanson and Cowan, 1977). The Electrophysiological studies of McLennan and Miller (1974b,1976) support this topographical organization since short latency field responses were recorded in the lateral septal area following stimulation of the fimbria.

These data, taken together with the previously described septal-hippocamapal projection (section 1.1.1), establish a reciprocal link between the septal area and the hippocampal formation. The precommissural fornix terminates in the lateral septal nucleus and this, in turn, projects to the medial septal area which sends efferents to the hippocampal formation.

B. Hypothalamic Inputs

The medial forebrain bundle (MFB) is the major input from the hypothalamus and the midbrain (Milhouse, 1969). Since the MFB consists of fibres originating in both of these regions, it is difficult to assess the contribution of hypothalamic nuclei to this system, however, Guillery (1957) suggested that a hypothalamo-septal group of fine fibres originates in lateral hypothalamic areas and projects to the lateral septal region. On the other hand, large fibres appear to originate in the midbrain and project primarily to the medial septal region (Guillery, 1957; Nauta and Kuypers, 1958; Morest, 1961; Wolf and Sutin, 1966). This anatomical differentation is partly supported by the observations that electrical stimulation in the lateral hypothalamus results in desynchronization of hippocampal electrical activity and disruption of the bursting discharge of septal neurones; in contrast, stimulating more medially produces the opposite effects (Anchel and Lindsley, 1972; Wilson et al, 1975). However, any conclusions based on the above anatomical or functional data should be re-evaluated in light of the possibility that electrodes in these sites may stimulate ascending monoamine-containing fibres which pass through this area to terminate in the septal area (see below).

C. Amygdaloid Inputs

The major input from the amygdala is via the stria terminalis which originates in the corticomedial amygdaloid nucleus and innervates the bed nucleus of the stria terminalis in the ventral septal region (Fox, 1940; Gloor, 1955; Nauta, 1966; Cowan et al, 1965; Leonard and Scott, 1971). A second input originates in the basolateral amygdaloid nuclei and projects via the ventral amygdalofugal pathway to the diagonal band region (Fox, 1940; Nauta, 1956; Leonard and Scott, 1971). It is noteworthy that both the stria terminalis and the amygdalofugal pathways also project ventrally past the septal region to terminate in the preoptic area, hypothalamus and into the medial forebrain bundle. On the basis of electrophysiological evidence Renaud (personal communication) suggests that the same neurone in the amygdala can project to septal and hypothalamic sites.

D. Monoamine Pathways

Noradrenaline (NA):

On the basis of Falck-Hillarp and glyoxylic acid histochemical studies, the regional distribution of NA terminals within the septal area has been found to be dense in lateral septum and sparse in the medial septal area (Moore et al, 1971; Lindvall and Bjorklund, 1974).

Recently, Moore (1978) has noted that the bed nucleus of stria terminalis receives a very dense NA innervation. Moreover, the glyoxylic acid preparations reveal numerous fine terminals near cell bodies and dendrites which may make en passage synapses in the septal area and then continue to more rostral forebrain and cortical areas (Ungerstedt, 1971; Moore et al, 1971; Lindvall and Bjorklund, 1974; Moore, 1975)

As in the case of other forebrain regions, the septal area has been shown to receive NA-containing afferents from the locus coeruleus (LC) (Ungerstedt, 1971; Segal and Landis, 1974; Jones and Moore, 1971). However, Moore (1978) has recently shown that lesions of LC result in only a 48% decrease in septal NA content, suggesting that a substantial proportion of septal NA originates in other regions. Since transections caudal to LC also result in substantial (47%) depletions of of septal NA, the other sources of septal NA may be in more caudal brainstem sites.

Dopamine (DA):

At one time, the catecholaminergic innervation of the septal area was considered to consist almost exclusively of NA nerve terminals (Moore et al, 1971). More recently, histofluorescence studies have demonstrated a high density of DA terminals in the medial aspect of the lateral septal region (Lindvall,

1975; Moore, 1975, 1978). Furthermore, Lindvall (1975) has shown that transection of the medial forebrain bundle at the level of the rostral substantia nigra, or bilateral electrolytic lesions of the A10 mesencephalic cell group result in a complete loss of septal DA terminals.

Small injections of HRP into the lateral septal area result in extensive labelling of neurones in the ventrolateral extent of the A10 area at the level of the interpeduncular nucleus (Assaf and Miller, 1977; Moore, 1978). Taken together these anatomical and biochemical studies suggest a direct dopaminergic fibre system which originates in the A10 region of the ventromedial tegmentum and innervates the lateral septal area . In contrast, there are relatively few fibres to the medial septal region.

The functional role of DA in the septal region is not yet clear. Assaf and Miller (1977) have shown that electrical stimulation in the region of A10 results in a short latency excitation of septal neurones which is dependent on an intact dopamine system. The distribution of the activated neurones is similar to that of the dopamine innervation based on the histochemical experiments mentioned above.

Serotonin (5-HT):

On the basis of autoradiographic and biochemical data, serotonin-containing cell bodies localized to the midbrain raphe nuclei have been shown to extensively innervate the septal region (Moore and Heller, 1967, Moore, 1974; Conrad et al, 1974; Lorens and Guldberg, 1974). Lesions in the midbrain raphe or medial forebrain bundle significantly reduce 5-HT content of the septal area (Moore and Heller, 1967; Kuhar et al, 1972; Lorens and Guldberg, 1974; Jacobs et al, 1974).

The proximity of the dorsal and median raphe nuclei make it difficult to localize the exact origin of septal 5-HT using lesioning techniques. However, the results of autoradiographic (Conrad et al, 1974; Halaris et al, 1976) and HRP tracing techniques (J.J. Miller, unpublished observations) indicate that the median raphe contributes substantially more fibres to the septal area than does the dorsal raphe.

Unlike the catecholamines, 5-HT does not fluoresce well in Falck-Hillarp preparations and thus mapping its distribution within the septal area is difficult. To overcome this difficulty the distribution of raphe projections to the septum was studied using autoradiographic tracing of amino acids and the 5-HT precursor 5-HTP (Conrad et al, 1974; Halaris et al, 1976). These studies indicate that raphe axons run forward into the medial forebrain bundle and reach

the septal area via the diagonal band. There is dense innervation throughout the medial septal nucleus except for a dense band in its most lateral aspects (Moore, 1975). Halaris et al (1976) caution that some of the the fibres in the medial septal nucleus are fibres of passage which continue into the fornix. Some of these fibres probably project to the hippocampus since lesions in the medial septal nucleus significantly reduce HRP transport from the hippocampus to the median raphe (Assaf and Miller, 1978).

Previous studies have implicated 5-HT in the control of septal unit discharge. Stefanis (1964) reported that the iontophoretic application of 5-HT depresses the spontaneous discharge of septal neurones. More recently, Segal (1976) reported that raphe stimulation results in a long latency (>25 msec) inhibition of septal neurones.

1.1.3 Rhythmical Activity In The Septal-Hippocampal Axis

The slow electrical activity of the neocortex and hippocampus have been studied in relation to the level of 'arousal', the sleep-waking cycle, attention and movement (Moruzzi and Magoun, 1949; Green and Arduini, 1954; Lindsley, 1960; Vanderwolf, 1972). These studies indicate that low voltage fast activity in the neocortex and rhythmical slow activity recorded in the hippocampal formation may be considered as 'activation patterns'. The subcortical mechanisms underlying these patterns are not clear, although there is extensive evidence that ascending inputs from the brainstem determine the patterns of neocortical and hippocampal activity. Furthermore, it has been suggested that the rhythm for slow electrical activity of the hippocampus is not due to the direct action of brainstem afferents on the hippocampus but may be generated by the rhythmical bursting discharge of septal neurones which, in turn, relay it to the hippocampus (Petsche, Stumpf and Gogolak, 1962). The aim of the present section is to review the studies which have led to the above concepts.

Spontaneous Patterns Of Hippocampal Electrical Activity

In the terminology of clinical electrophysiology slow electrical waves are described on the basis of the frequency and pattern of their occurrence, amplitude, and shape. The electrical activity of the rat hippocampus consists of a mixture of large (up to 2mV) and small waves having frequencies ranging between 2-50 Hz. However, the occurrence of these different types of waves is not random and patterns which are dominated by certain frequencies and amplitudes have been recorded.

The most conspicuous is a rhythmical slow activity (RSA) consisting of a train of roughly sinusoidal waves with a frequency varying between 3-12 Hz. . The first observation of RSA was made by Jung and Kornmuller (1938) in the rabbit's hippocampus and in accordance with the terminology of clinical electrophysiology is designated a 'theta rhythm' since its frequency ranged between 4-7 hz. Although in some species the frequency of RSA is beyond this range, the term 'theta' is still used to denote the regular hippocampal activity independent of its frequency.

In contrast to RSA, a desynchronized activity of a mixture of large irregular waves and fast low voltage activity is also recorded from the hippocampus (Green and Arduini, 1954; Vanderwolf, 1971). Both types of activity may be recorded spontaneously, but RSA is more

prominently during states of arousal (Green and Arduini, 1954, See sections 1.3.3).

Sources Of RSA

Although patterns of electrical activity similar to those recorded in the hippocampus have been observed in other regions of the brain, notably the thalamus (Green and Arduini, 1954), the hippocampus has been established as a source of 'theta' since 1) a clear null zone and phase reversals have been demonstrated in the hippocampus (Green et al, 1960) and 2) hippocampal activity has been related to the discharge of local neurones which were observed to fire in bursts in phase with the regular slow waves (Kandel and Spencer, 1961).

Based on the above criteria which establish the source of the electrical activity, it was initially thought the pyramidal cell layer was the sole generator of RSA (Green, Maxwell,Schindler and Stumpf, 1960). More recently, two generators of RSA have been described (Winson, 1974;1975; Bland et al, 1976). One generator is located in stratum oriens of CA1 and the other more ventral in the molecular layer of dentate gyrus. If recordings are taken simulatneously from

these 'independent' generators, the RSA waves are 180 degrees out of phase. The physiological significance and the independence of the two different generators have not yet been established.

The events underlying RSA are not known. Von Euler and Green (1960) suggested that RSA is a reflection of a series of declining spikes generated within the same neurone and compared it to 'inactivation process' described by Granit and Phillips (1956). Furthermore, they suggested that phasic afferent inputs from the septal area cause synchronously occurring 'inactivation processes' in a population of neurones and thereby RSA. On the other hand Kandel and Spencer (1961) and Andersen et al (1964) have suggested that intrinsic mechanisms including delayed after-polarization of pyramidal neurones and recurrent basket inhibition (Gloor, 1963) favour the development of 'theta' in the presence of a steady and sustaining excitatory inputs. Although there is still controversy over whether RSA is an intrinsic property of the hippocampus or the result of rhythmic afferent impulses, there is general agreement that it is related to the discharge pattern of septal neurones.

Role Of Ascending Systems In The Generation Of RSA

The first suggestion that the production of RSA was related to ascending influences from the midbrain was made by Green and Arduini (1954) who observed that peripheral sensory stimulation elicited hippocampal RSA and neocortical desynchronization. In addition, electrical stimulation of the reticular formation produced RSA and neocortical desynchronization simultaneously. Since Moruzzi and Magoun (1949) had earlier used neocortical desynchronization as an indicator of 'arousal', Green and Arduini (1954) proposed that RSA is the 'hippocampal arousal pattern'.

The initial observation of Green and Arduini (1954) that electrical stimulation of the reticular formation elicits RSA has been confirmed by many studies (Torii, 1961; Yokata and Fujimori, 1964; Kawamura, Nakamura and Tokizane, 1961; Petsche et al, 1962; Anchel and Lindsley, 1972; Macadar et al, 1974). However, the term 'reticular formation' is a functional classification of collections of nuclei having a caudal-rostral extent from the medulla to the midbrain. These nuclei contain cells which have long descending and ascending axons projecting to many regions including the forebrain and cortex (Brodal and Rossi, 1955; Scheibel and Scheibel, 1958; Nauta and Kuypers, 1958; Magni and Willis, 1963). In an attempt to map more precisely the origins of these ascending systems

which elicit RSA Macadar et al (1974) demonstrated that electrical stimulation of anatomically distinct mesencephalic and pontine regions elicited characteristic hippocampal patterns. They reported that sites in reticularis pontis oralis, locus coeruleus, reticularis gigantocellularis and the midbrain tegmentum elicit RSA whereas stimulation of raphe nuclei and nucleus reticularis pontis caudalis produced hippocampal desynchronization. Torii (1961) had earlier proposed that some distinct ascending systems may produce hippocampal desynchronization whereas adjacent systems elicit RSA. This proposal was based on the observation that electrical stimulation in the medio-ventral midbrain tegmentum or lateral hypothalmus produces an increase in the fast (15-30 Hz) activity of the rabbit hippocampus whereas stimulation in the dorso-lateral tegmentum and medial hypothalamic region elicits RSA.

Although the above studies demonstrate that ascending inputs from the brainstem are capable of eliciting characteristic hippocampal patterns they do not prove that the brainstem is necessary for the production of RSA. Stimulation of sites such as the posterior hypothalamus are also effective in producing hippocampal RSA (Torii, 1961; Yakota and Fujimori, 1964; Bland and Vanderwolf, 1972). This may be due, in part, to stimulation of ascending fibres coursing through the hypothalamus. However, a rostral midbrain

transection at the meso-diencephalic junction (high cerveau isole) does not block RSA generated by stimulation of the posterior hypothalamus (Kawamura and Domino, 1968). Furthermore, RSA is recorded in cats which have a chronic high cerveau isole (Olmstead and Villablanca, 1977).

Although these studies suggest that sites other than the brainstem may play a role in the generation of RSA, these areas are not essential since massive hypothalamic lesions do not abolish RSA (Whishaw and Vanderwolf, 1973). Rather, these studies indicate that ascending systems which produce RSA are very diffuse and neither the brainstem nor the posterior hypothalamic regions are absolutely necessary for the production of RSA. Therefore small and large lesions of various regions from the medulla to the diencephalon do not eliminate RSA altogether. The only exceptions are lesions involving the medial septal-diagonal band area. In chronic and acute preparations electrolytic lesions of the medial septum abolish all RSA produced by sensory stimulation or electrical stimulation of the various sites discussed above (Green and Arduini, 1954; Brucke, Petsche, Pillat and Deisenhammer, 1959; Stumpf, 1965 for review). Furthermore, RSA does not recover following large septal lesions (Brugge, 1965) and even the RSA normally associated with walking or active sleep is abolished (Brugge, 1965; Kolb and Whishaw, 1977). These data suggest that the septal area plays a

critical role in the generation of RSA.

Role Of The Septal Area In The Generation Of RSA

Following the initial lesion experiments described above, a relationship between the discharge pattern of septal neurones and hippocampal electrical activity was described by Petsche et al (1962). Many cells in the medial septal-diagonal band region fire in rhythmic bursts which are synchronous with RSA. The same neurones fire in an irregular or random manner during hippocampal desynchronization (Petsche et al, 1962; Stumpf, Petsche and Gogolak, 1962; Petsche et al, 1965; Gogolak, Petsche, Sterc and Stumpf, 1967). Reticular or peripheral sensory stimulation initiates the bursting discharge pattern of medial septal neurones and RSA (Petsche et al, 1965; Wilson et al, 1976).

The mechanisms underlying the relationship between the bursting discharge pattern of septal neurones and hippocampal activity are still not clear. McLennan and Miller (1974,1976) demonstrated that septal unit activity is under hippocampal control. Electrical stimulation of the fimbria, the major efferent projection from the hippocampal formation to the septum, resulted in an activation-inhibition sequence of lateral septal neurones and a synchronization of the bursting discharge pattern displayed by medial septal neurones. On the basis of these data they suggested

that RSA is not simply the consequence of a septal 'pacemaker' but that the hippocampal output initiate the bursting discharge pattern of medial septal neurones via feedback loop within the septal region. These studies have prompted a re-evaluation of the role of the septal area in the generation of RSA. The only evidence that the septal area may in fact initiate RSA is based on the observations that 1) electrolytic lesions of the septal area block RSA (reviewed above) and 2) some septal cells continue to burst when RSA is not present in the vicinity of a recording electrode in the hippocampus (Petsche et al, 1962; Ranck, 1975). In the model proposed by McLennan and Miller (1976) septal lesions could be abolishing hippocampal RSA by either disrupting midbrain afferents which may pass through the septal area to reach the hippocampus or opening the feedback loop through the septal area. The second observation that some septal cells continue to burst when RSA is not present is harder to account for using McLennan and Miller's (1976) model, however, it must be remembered that in the experiments of Petsche et al (1962) hippocampal RSA was eliminated by inducing seizures in the hippocampus. Perhaps a steady input from hippocampus to the lateral septum is sufficient to cause the bursting discharge of medial septal neurones.

Regardless of the mechanisms involved, it is apparent that the septal area is of critical importance in the generation of RSA.

Role Of Pharmacologically Distinct Systems In The Control Of Septal-Hippocampal Activity

Many investigations have attempted to correlate effective brainstem sites which elicit hippocampal RSA with particular anatomical substrates (Torii, 1961, 1966; Anchel and Lindsley, 1972; Macadar et al, 1974). The results of these studies indicate rather diffuse systems originating in brainstem regions without implicating specific nuclei. Another approach has been to delineate these ascending systems on the basis of their neurotransmitters. By far the most studied putative transmitter is acetycholine (Ach), but the, more recent identification of ascending monoamine systems has prompted an investigation of their role in hippocampal and septal activity.

A. Acetycholine

Bremer and Chatonnet (1949) first observed that an intracorotid injection of Ach produces neocortical desynchronization in rabbits and cats. A similar effect was obtained by increasing the availability of Ach at synaptic sites by inactivation of acetycholinesterase (AchE) using physostigmine (eserine) or diisopropylflurophosphate (DFP) (Bremer and Chatonnet, 1949; Rinaldi and Himwich, 1955b; Monnier and Romanouski, 1962). A possible anatomical system mediating the effects of Ach on neocortical 'arousal'

was described using histochemical staining for AchE (Krnjevic and Silver, 1965; Lewis and Shute, 1967; Shute and Lewis, 1967). Further evidence that Ach may be involved in neocortical activity was provided by studies showing an increased cortical release of Ach simultaneous with neocortical desynchronization produced by electrical stimulation of the midbrain reticular formation (Celesia and Jasper,1966; Phillis, 1968).

A cholinergic system has also been implicated in the production of hippocampal RSA. Intracarotid injections of Ach or DFP produce long trains of RSA in rabbits (Rinaldi and Himwich, 1955a) and eserine when administered via a variety of routes elicits hippocampal RSA in every species tested (Brucke, Sailer and Stumpf, 1950; Bradley and Nicholson, 1962; Monnier and Romanouski, 1962; Stumpf et al, 1962; Vanderwolf, 1975). Eserine also initiates the bursting discharge pattern of septal neurones (Stumpf et al, 1962). On the other hand, cholinergic muscarinic antagonists (atropine and scopolamine block hippocampal RSA (Bradley and Nicholson, 1962) and the bursting pattern of medial septal units (Stumpf et al, 1962). However, Vanderwolf (1975) has recently reported that movement related RSA (3-12 Hz) is not blocked by atropine whereas immobility related (4-7 Hz) RSA is blocked. On the basis of these data Vanderwolf (1975) suggests that there are at least two pharmacologically distinct forms

of RSA which have different frequencies and relations to behaviour.

The effects of Ach and eserine on the generation of RSA are dependent on an intact septal area. Following septal lesions eserine is ineffective in producing RSA, however, it continues to enhance the fast activity of the hippocampus (Stumpf, 1965; Torii and Wikler, 1966). The evidence for a cholinergic septo-hippocampal projection (Reviewed in Section II) suggests that eserine may be enhancing the activity of this system.

Electrical stimulation of midbrain reticular formation or the medial septal region enhance Ach release in the dorsal hippocampus (Smith, 1972; Dudar, 1975,1977;. Furthermore, electrolytic lesions of the medial septal area block the release of Ach evoked by brainstem stimulation (Dudar, 1977). Although this suggests that the septo-hippocampal cholinergic system may mediate the effects of eserine on RSA they do not eliminate the strong possibilities that 1) eserine enhances the activity of an extrinsic cholinergic input to the septal area or 2) eserine enhances cholinergic transmission in synaptic mechanisms intrinsic to the septal area. The above studies taken together suggest that Ach is involved in the generation of RSA, but the anatomical substrates for these actions are not known.

B. Noradrenaline

The first suggestion that adrenergic systems may place a role in neocortical or hippocampal 'arousal' is based on the observations that intravenous adrenaline or NA produce neocortical desynchronization (Rothballer, 1956). However, injections of adrenaline or NA directly into the cerebral circulation via the carotid aretery rat produce a similar desynchronization (Mantegazzini, Paeck and Santibonez, 1959) suggesting that systemic effects may mediate the observed desynchronization. The additional observations that vasopressin (Capon, 1960) and compression of the descending thoracic aorta (Baust et al, 1963) increase blood pressure and produce neocortical desynchronization have strengthened the proposal that adrenaline and NA act indirectly to produce cortical 'arousal'.

More recently, a role for the dorsal NA bundle originating in the locus coeruleus (LC) in neocortical and hippocampal arousal has been postulated. Jones et al (1973) electrolytically lesioned LC, causing depletion of forebrain NA and observed a dramatic decrease in waking time and cortical activation. Likewise 6-hydroxydopamine injections into the LC and catecholamine depleting drugs decrease cortical desynchronization and waking time (Lidbrink, 1974; Jouvet, 1974).

It has also been suggested that NA containing neurones originating in the LC are involved in the production of RSA. This suggestion was based on the observations that electrical stimulation of LC (Macadar et al, 1974) and systemic injections of amphetamine produce RSA (Bradley and Nicholson, 1962; Longo, 1962; Vanderwolf, 1975; Assaf, Barbour and Miller, unpublished results). The effect of amphetamine may be related to its ability to increase release of NA and dopamine or its direct action on dopamine receptors (Moore, 1977).

C. Dopamine

Although, at this time, there is no direct evidence, other than the effects of amphetamine and apomorphine (Brucke et al, 1957) relating dopamine to RSA, it has been suggested to play a role in the production of cortical 'arousal' (Hornykiewicz, 1966). An increase in brain dopamine levels following systemic injections of L-Dopa results in neocortial desynchronization (Montegazzini and Glasser, 1960; Hawer and Domaw, 1970; Thut and Rech, 1972). This effect appears to be related to an increase in DA and not to NA since it is not blocked with disulfiram which prevents the conversion of DA to NA (Hawer and Domer, 1970). An indirect action mediated by the mesolimbic DA system projecting to the septal area (Lindvall, 1975;

Assaf and Miller, 1977) is more likely.

D. Serotonin

Serotonin (5-HT) has been implicated in cortical 'arousal' based on the initial observation that cerebral levels of 5-HT changes during the diurnal cycle (Jouvet, 1974). The additional observation that electrolytic destruction of the midbrain raphe nuclei reduces forebrain 5-HT and significantly decreases slow-wave sleep provided further support for the above relationship (Jouvet, 1974).

The role of 5-HT in the control of hippocampal electrical activity is also controversial. Domer and Longo (1962) first reported that the intravenous infusion of the 5-HT precursor 5-hydroxytryptophan resulted in hippocampal desynchronization. More recently, Macadar et al (1974) reported that electrical stimulation in the region of the midbrain raphe in cats results in desynchronization of hippocampal activity. In contrast, stimulation of the same site in the rabbit produces cortical activation and hippocampal RSA (Polc and Monnier, 1970). However, the latter study did not relate the effects of stimulation in the region of the raphe to 5-HT levels, in fact, large doses of methylsergide (10-20 mg/kg,i.p.) and p-CPA (500 mg/kg i.p.) do not block the ability of stimulation in the region of the raphe to elicit RSA (Robinson, 1978).

Therefore, some of these observed synchronizing effects may be due to non-serotonergic systems passing adjacent to the raphe nuclei.

E. Other Transmitters

Glutamic acid may be involved in cortical activation since the release of glutamic acid from the neocortex is reduced following lesions of the reticular formation (Jasper, Khan and Elliot, 1965) and increased following electrical stimulation of sites in the reticular formation (Jasper and Koyama, 1969). Although glutamic acid has been postulated as the transmitter of the perforant path input to the hippocampus (Nadler et al,1976), it has not been implicated in the generation of RSA. Electrical stimulation or transection of the perforant path did not disrupt the generation of RSA (Adey, Merilles and Sunderland,1956; Andersen, Bland, Myhrer and Schwartzkroin, 1977).

The inhibitory amino acid gamma-aminobutyric acid (GABA) may also be involved in the generation of RSA. McLennan and Miller (1974a) have shown that the bursting discharge pattern of medial septal neurones was disrupted by electrophoretic application of the GABA antagonist bicuculline. In addition, the systemic injection of bicuculline disrupts the bursting discharge of septal neurones (Segal, 1976) and hippocampal RSA (Assaf and Miller, unpublished observations). The

possibility that GABA mediates the recurrent inhibition of hippocampal neurones (Andersen et al, 1964b) suggests that it may be involved in the generation of RSA.

Relationship Of Septal And Hippocampal Activity To Behaviour

Hippocampal RSA has been related to changes in overt movement (Vanderwolf, 1969) and to inferred processes including information processing (Routtenberg and Kramis, 1968), attention (Bennet et al, 1973), reward and punishment (Gray, 1970), motivation (Konorski et al, 1968; Grastyan et al, 1966) and learning (Elazar and Adey, 1967; Winson, 1978). Vanderwolf (1969) first observed that certainovert motor behaviours of rats were consistently accompanied by RSA, while other behaviours were not (Vanderwolf et al, 1975 for review). In the freely moving rat voluntary behaviours such as walking, swimming and manipulation of objects are accompanied by 7-12 Hz RSA, while immobility or 'stereotyped' behaviours such as chewing, urination and pelvic thrusting are accompanied by irregular hippocampal activity (Wishaw and Vanderwolf, 1973) . One major exception to the relationship between overt motor behaviour and RSA is active (REM) sleep since the hippocampal electrical activity consists of RSA (Vanderwolf, 1971; Harper, 1971).

The above hypothesis relating hippocampal electrical activity to movement is still controversial. Bennet (1969) and others (Feder and Ranck, 1975) have observed that movements such as walking and lever pressing are not always accompanied by RSA. However, Whishaw and Vanderwolf (1973) have suggested that the failure to observe a close relationship between RSA and walking or lever pressing is due to an interaction between the electrode placement and type of response such that fast activity generated in neighbouring sites cancel out RSA in the immediate vicinity of the electrode. Another challenge to the movement hypothesis arises from Klemm's (1970,1971,1976) observations that RSA can be recorded during immobility. More recently, Vanderwolf et al (1975), primarily on the basis of pharmacological data (reviewed below), have proposed that there exists two different ascending systems to the hippocampus, one producing low frequency RSA during immobility (Type I) and the other higher frequency RSA which is correlated with movement (Type II).

Another approach that has been taken to relate the septal-hippocampal axis to behaviour is that of recording the activity of single hippocampal and septal neurones and attempting to relate discharge patterns to overt and inferred processes. Ranck (1973), recording single units in the dorsal hippocampal formation of unrestrained rats, divided the recorded neurones into two groups on the basis of their firing repertoire. One

group, which he called 'complex spike cells', fire a burst containing different size spikes and the other type was called 'theta cell' since it fired simple action potentials which were related to ongoing RSA. Ranck (1973,1975) observed that 'theta' cells increase their firing rate during hippocampal RSA and pointed out that Vanderwolf's (1971) description of 'voluntary movement' related RSA is the best description for the relationship of 'theta' cells to behaviour. In contrast 'complex spike' cells had no simple relation to RSA, but there was a correlation between the firing of these cells and the behaviour of the rat. The most common relationship between the firing of a 'complex spike' cell and behaviour was an increase in discharge rate during approach and successful appetitive behaviour.

Similar recording in the septal area also reveal a relationship between the discharge pattern of septal neurones and ongoing behaviour. In the medial septal nucleus two major types of cells were identified 'theta cells' which increase their firing rate only during hippocampal RSA and 'tight group cells' whose firing occurred during specific consumatory behaviours (Ranck (1974). The firing pattern of 'theta cells' recorded by Ranck (1974) suggests that they may be the same as B-neurones recorded by Petsche, Stumpf and Gogolak (1962) in the rabbit and by Assaf and Miller (1978) in the rat. Other studies relating the discharge of septal neurones to behavioural or physiological states suggest

an increase in discharge rate during dehydration and sensory stimulation(Hayat anf Feldman, 1974).

At this time, the relationships between the activity of the septal-hippocampal axis are not understood sufficiently to allow an appreciation of the role of the septum and hippocampus in behaviour. However, several theories which try to account for some of the above observations have been proposed. Vinogradova (1975) postulated that the hippocampus receives generalized signals through reticuloseptal input which do not contain qualitative information about stimuli reaching the hippocampus. The 'theta' bursts of septal neurones rhythmically modulate the dendritic activity of hippocampal neurones creating the conditions when comparison or matching of inputs will be possible only for signals which come in strictly determined time quanta. Nadel and O'Keefe (1974) suggested that the type of information which the hippocampus processes is spatial. According to this theory, the hippocampus functions as a cognitive map which is used by the animal to move to specific locations in its environment and to solve problems concerning spatial relations between objects in the environment. This theory accounts, in part, for deficits in spatial learning following hippocampal and septal lesions (Winson, 1978) and the relationship between the discharge pattern of hippocampal neurones

and unique places in the animal's spatial environment (Nadel and O'Keefe, 1974).

1.2 The Present Study

On the basis of the evidence reviewed above different patterns of hippocampal electrical activity are initiated by anatomically diffuse regions in the brainstem. The medial septal area is critically interposed between the brainstem and the hippocampal formation and may mediate RSA. Neuroanatomical and neurochemical evidence for monoamine projections to the septal area and the hippocampus suggest that the putative transmitters serotonin (5-HT) and Noradrenaline (NA) may be involved in the generation of distinct patterns of septal-hippocampal activity. The first part of this thesis examines 1) the role of the septal area in the generation of hippocampal electrical activity in the urethane anaesthetized rat (chapter 3) 2) the role of the 5-HT projection from the median raphe nucleus in the control of septal-hippocampal activity (chapter 4) and 3) whether the dorsal NA bundle from the locus coeruleus produces rhythmical hippocampal activity (chapter 5).

A second feature of the hippocampal formation that will be explored in this thesis is the potentiation of neuronal transmission between the entorhinal cortex and the dentate gyrus. Previous studies have indicated that conditioning stimulation of the perforant path (PP), the entorhinal projection to the dentate, results in a marked potentiation of the field responses evoked by a

subsequent test volley. However it is not known how the activity of other afferents to the dentate such as monoamine systems affect PP-evoked field responses. The second part of this thesis was therefore designed to: 1) characterize the field potentials recorded in the dentate gyrus following PP stimulation and to study the features of homosynaptic potentiation of this input (chapter 6) and 2) determine if extrinsic afferents, particularly the monoamine projections described above, alter the field responses evoked in the dentate by a test PP volley (chapters 7-9). In chapter 7 a laminar profile of the commissural input to the dentate will be presented in order to verify previous suggestions that this input terminates adjacent to PP synapses in the molecular layer of the dentate gyrus. The effects of conditioning stimulation of this input on field responses evoked by the PP will be compared to results obtained following conditioning stimulation of the monoamine systems which do not project to the area of the PP synapses. This approach may be useful in determining whether heterosynaptic potentiation of neuronal transmission is a feature of the hippocampal formation.

2.0 GENERAL METHODS

2.1 Surgical Preparation:

Male Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing between 250-400 g were used for acute recording experiments. The animals were anaesthetized with interperitoneal injections of ethyl carbamate (urethane 1.0-1.5g/kg) and supplemental doses to produce a state of light anaesthesia were given as necessary though a previously implanted jugular cannula. Body temperature was monitored by a rectal thermistor probe and maintained between 36.5-37 C using a heating pad regulated by a temperature control unit (EKEG Electronics).

The animals were placed in a Kopf stereotaxic frame (Model 1204) with the head rigidly fixed by the incisor bar at 4.5-5.0 mm below zero thereby positioning the skull in the horizontal plane. A midline incision approximately 15 mm in length was made through the scalp and skin flaps were retracted and held with mosquito forceps. The surface of the skull was then scraped and dried revealing bregma, lambda and the saggital suture. One or two pieces of bone (7x7 mm) were removed and the dura incised, so that the underlying cortical tissue was exposed over areas roughly corresponding to boundaries 2.0 mm anterior to bregma, 4.0 mm posterior to bregma and 4.0 mm on either

side of the saggital suture for forebrain placements and to boundaries 2.0 mm anterior to lambda, 3.0 mm posterior to lambda and 3.0 mm on either side of the saggital suture for placements in the brainstem. Extreme care was taken not to damage the superior saggital sinus when removing the bone or incising the dura. The exposed cortex was covered with warm saline to keep the area moist throughout the experiments. When pooling of saline was not possible warm mineral oil was used.

2.2 Stimulating And Recording Procedures

Stimulating electrodes were stereotaxically lowered into various regions, which are detailed in subsequent method sections, using a Kopf electrode holder. For microelectrode placements a Kopf hydraulic microdrive (Model 607W) was used to lower the electrode in 1 uM steps. The anterior-posterior (AP) stereotaxic zero for all forebrain electrode placements was measured from the junction of the saggital suture and bregma while brainstem placements were measured from stereotaxic zero at the tip of the ear bar. All lateral placements were taken from the midline position of the sagittal sinus and vertical coordinates were all measured from the surface of the cortex. The positions for all these placements were modified from the Koniq and Klippel (1963) atlas of the rat brain.

Concentric bipolar electrodes (SNE 100, Rhodes instruments) having a tip separation of 0.5 mm and DC resistance in normal saline of 75-100 K ohms were used for electrical stimulation. Single and repetitive monophasic rectangular pulses were delivered by an isolated stimulator (Digitimer type 2533) and unless specified otherwise pulse parameters were 0.05-0.1 msec in duration and 1-20 V intensity (20-400 uA). The stimulators were controlled by a four channel programmer (Digitimer Model D4030). The current spread from these electrodes was estimated to range between 0.15-0.5 mm from the tip when stimulating with intensities of 10-100uA (Bagshaw and Evans, 1976).

Single Unit Activity And Evoked Potentials

Extracellular unit activity and evoked field potentials were recorded using glass micropipettes containing 4M NaCl having tip diameters of 1-2 uM and DC resistance between 2-5 M ohms. These microelectrodes were prepared from Corning pyrex capillary tubing (outside diameter 1.5 mm) which were heated and pulled in a Canberra-type microelectrode puller and the resultant tips were broken back to the desired width under microscopic observation. The electrodes were filled under a dissecting microscope by injecting the electrolyte into the shaft of the electrode using a long 31 gauge hypodermic needle. The solution was pushed further down the shaft using a pyrex probe until

capillary action drew it to the tip of the electrode.

The microelectrode was connected to the positive pole of a custom made voltage follower using an insulated silver wire. The reference or negative pole was grounded to the stereotaxic frame. The recorded electrical activity was led through a Tektronix 5A22N amplifier and displayed on a Tektronix D44 oscilloscope and Tektronix D13 dual beam storage oscilloscope. Extracellular unit activity was filtered using a 100Hz-10kHz bandpass, while evoked potential recordings were filtered using a 10Hz-3KHz bandpass. In some cases both unit activity and evoked potentials were recorded simultaneously on the two beams of the oscilloscope. Amplifier outputs were connected to a custom made audiomonitor. The amplified signals were also led into a voltage discriminator (WPI Instruments, Model 120) which compared the recorded signals with a predetermined triggering potential so that action potentials having a minimum amplitude were represented by an output signal. The signals from the discriminator were fed into a Schmidt trigger and the resulting pulses were subsequently led into a PDP 11/10 (Digital Corp.) computer programmed to analyze the records as described in the next section. The analyzed data were plotted on a visual display terminal or an interactive digital plotter (Tektronix model 4662).

Electrical Activity Of The Hippocampal Formation

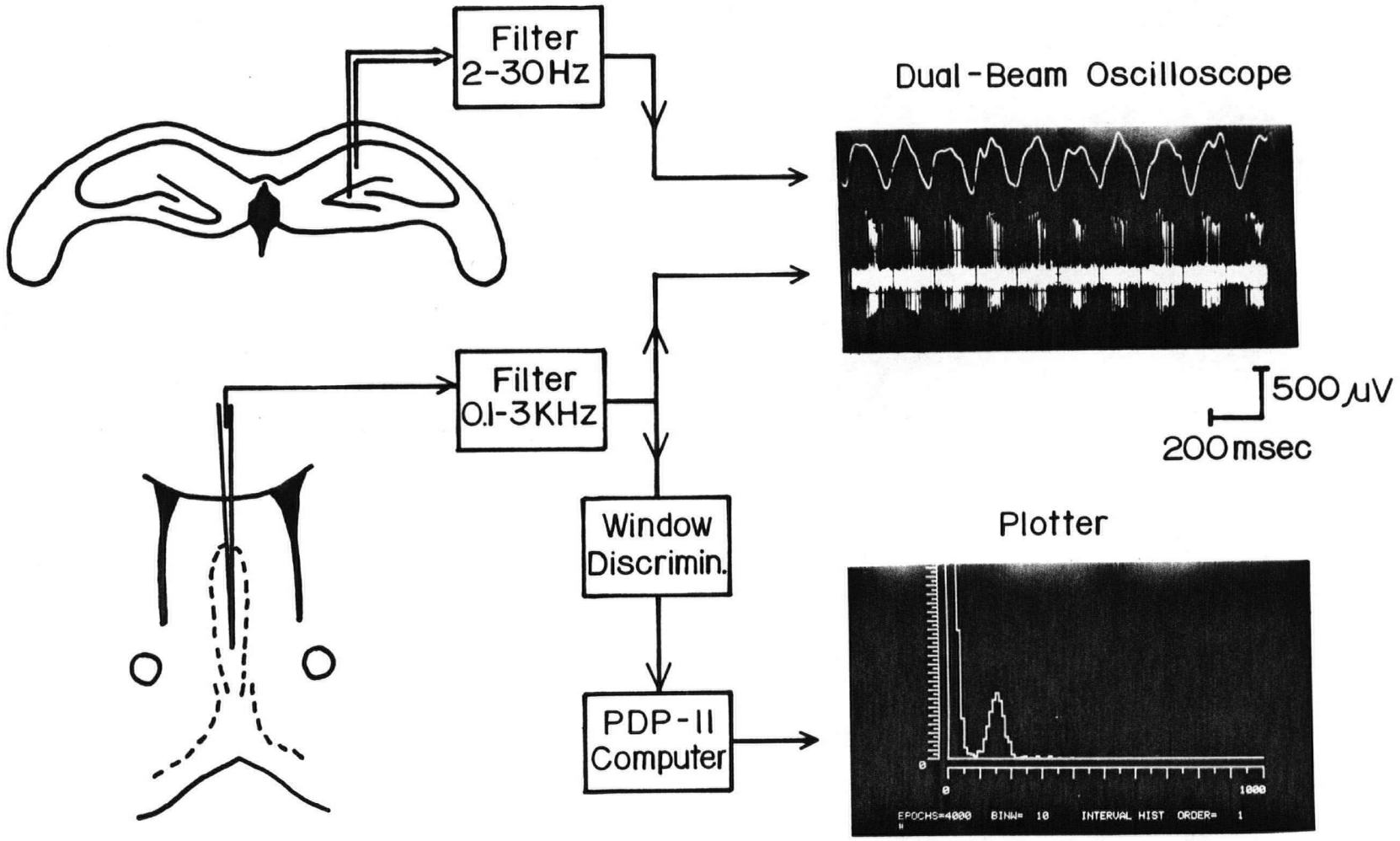
The electrical activity of the hippocampus and dentate gyrus was recorded using bipolar stainless steel electrodes (tip separation=0.5 mm). As shown in Fig. 2-1, the signals recorded from these electrodes were filtered (2-30 Hz), amplified and displayed on an oscilloscope and recorded on a polygraph (Gilson model M5P). One beam of a dual beam storage oscilloscope was used to display the recorded electrical activity and the second beam was used to display the simultaneous neuronal discharges in the septal area. A Tektronix Polaroid camera (model C-5) was used to photograph the resulting oscilloscope sweeps.

2.3 Data Analysis:

Single Unit Activity Several methods were used to analyze the spontaneous discharge pattern of a neurone and determine whether it was influenced by electrical stimulation of a particular region. The spontaneous discharge rate was analyzed using a first order interval histogram (IHT). A peak on an IHT shows a preferred interval between firings in a spike train. Similarily the nth order interval histogram is the distribution of the intervals between a given spike and the nth+1 spike. The sum of these IHT's of order 1 to n is known as the autocorrelation function whereby the peaks indicate the preferred periods of the spike

FIG. 2- 1: Schematic Illustration Of Extracellular Recording Technique.

Slow electrical activity is recorded from the dentate gyrus using bipolar stainless steel electrodes. Single unit activity is recorded simultaneously from the septal area using glass micropipettes filled with 4M NaCl. The signals are amplified and displayed on an oscilloscope for photography or plotted on an x-y graphics terminal.


Filter
2-30Hz
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Filter
0.1-3KHz
500 µV
200 msec
Window
Discrimin.
Plotter
PDP-11
Computer
EPOCHS=4000 BINW= 10 INTERVAL HIST ORDER= 1

train. Rhythmical neuronal discharge patterns result in an autocorrelogram displaying a damped oscillation whereas that corresponding to a randomly firing neurone is flat (Perkel, Gerstein and Moore, 1967). An additional statistical measure of the spontaneous discharge which was used is the joint interval density (Rodieck, Uring and Gerstein, 1962) in which the abscissa value of each point is the duration of a given interval and the corresponding ordinate value is the duration of the subsequent interval. This analysis gives a clear indication of the bursting discharge pattern of a neurone.

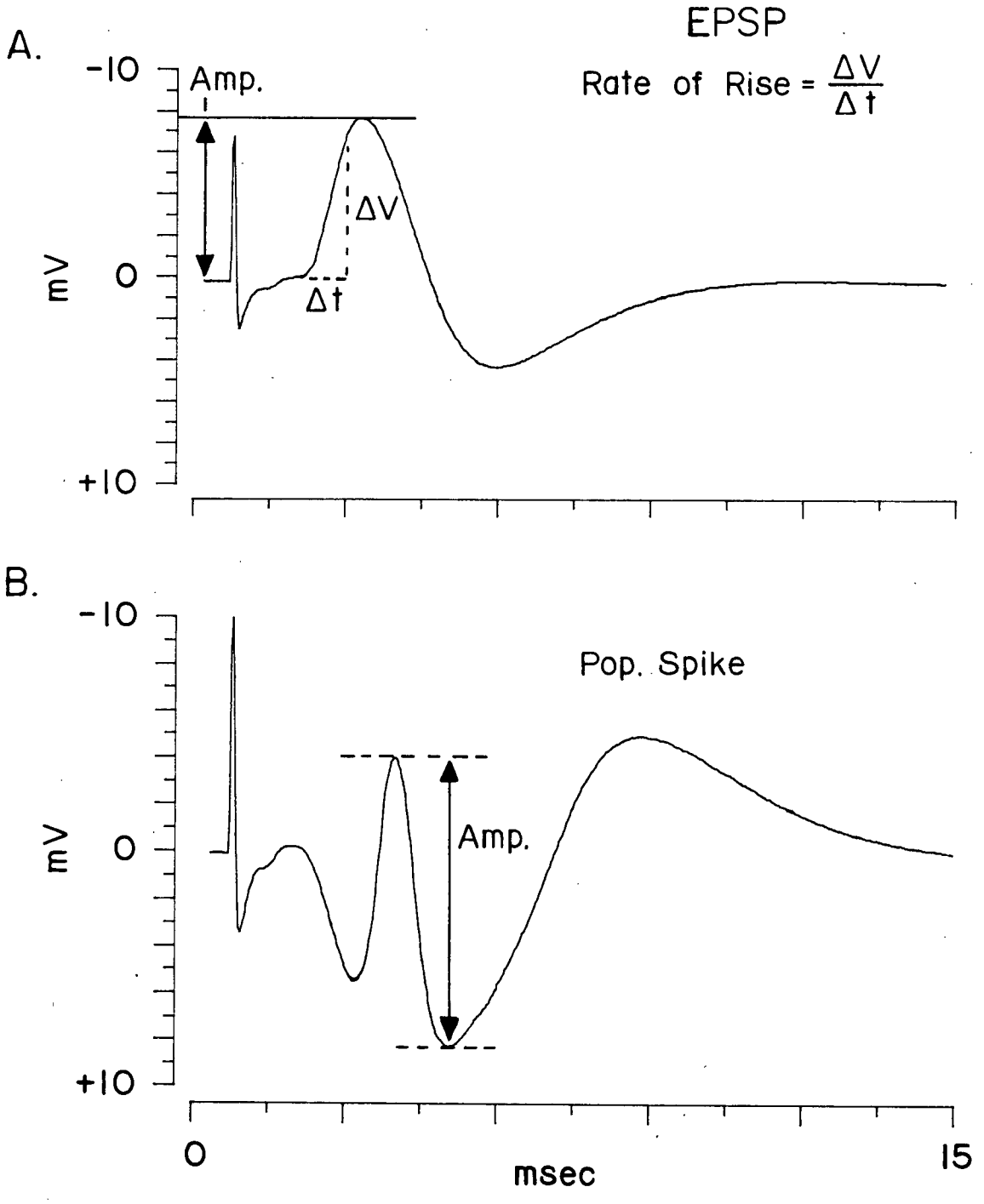
In stimulation experiments, a post stimulus time (PST) histogram was used to assess the changes in spike activity resulting from stimulation of a particular region against a background of spontaneous firing neurones. The distribution of spikes in time relative to the stimulus was summed over many repeated stimulus presentations or epochs. A peak on a PST histogram shows a preferred time of discharge relative to the stimulus (Gerstein and Kiang, 1960) whereas a flat PST indicates a lack of response to the stimulus. Post stimulus data were also displayed in a rastered form whereby each spike appears as a dot and the density of dots before and after the stimulus indicates the probability that the background discharge rate of the neurone was altered by presentation of the stimulus.

Evoked Fields Evoked field potentials were either photographed from the storage oscilloscope or plotted when computer averaging was used. The averaging was performed by converting the analog amplifier output to digital voltage values which were sampled in 35 microsecond bins by the PDP 11/10 computer. Twenty to thirty consecutive stimulus presentations (ISI= 1-5 sec) were used to obtain a typical average response. Latencies were measured from the beginning of the stimulus artifacts to the peak of the evoked wave (peak latency) and/or to the onset of the evoked wave (onset latency). The evoked responses were always displayed with negative polarity upwards as in Fig. 2-2. The amplitude of each field response was measured from baseline to peak. In addition the rate of rise of evoked fields was measured as the net voltage change from the onset of the field to a predetermined interval (usually 1.0-1.5 msec) after onset. The rate of rise was measured whenever amplitude measurements were likely to be contaminated by spike discharges occurring near the peak of the evoked field as in the case of EPSP's (Fig. 2-2). Special care was also taken to measure the amplitude of compound spikes, such as population spikes described in chapter 6 , which were sometimes superimposed on evoked waves. As shown in Fig. 2-2, the amplitude of the population spike was measured as the voltage change from maximum positive deflection to the peak negative deflection of the

FIG. 2-2: Measurements Of Amplitudes And Rate Of Rise Of Evoked Field Potentials.

A: field potentials such as the extracellular EPSP were analysed with respect to peak amplitude and rate of rise measured 1.0-1.5 msec after onset.

B: compound spikes which were superimposed on evoked waves were analysed with respect to their amplitude (maximum negativity to maximum positivity).


A.
EPSP
Rate of Rise = ΔV/Δt
-10
Amp.
ΔV
Δt
mV
0
+10
B.
-10
Pop. Spike
Amp.
mV
0
+10
0
msec
15

spike. This method of measuring population spike amplitudes was found to be as reliable as that previously used by Steward et al (1976) who expressed the spike amplitude as the average of the height of the ascending and descending limbs of the population spike. In addition, the present method is more suitable for automated data analysis. Alterations in evoked responses were expressed as a percentage of the control amplitude or rate of rise.

Rhythmical Electrical Activity

The slow wave activity of the hippocampus and dentate gyrus was analyzed with respect to its frequency, amplitude and shape. The electrical activity was classified as rhythmical slow activity (RSA) if it consisted of roughly sinusoidal waves with a frequency varying from 3-12 Hz and minimum amplitudes of 0.3 mV. Electrical activity that did not meet all three criteria was classified as irregular. No attempt was made to subclassify irregular activity, although its average amplitude was often noted (Fig. 3-1). When electrical stimulation or drug administration was used to alter the spontaneously occurring electrical activity an event marker on a separate channel of the polygraph was used to time the onset and termination of the stimulus train or injection period. The frequency of RSA was computed by manually counting the number of sinusoidal 'theta' waves occurring during 1 second

sampling periods. Typically, rates were calculated beginning one minute before the stimulus train until recovery to background activity.

Statistical Analysis: the statistical tests which were used for comparisons of data were all designed to disprove the null hypothesis. For comparisons between two independent populations such as the frequency of RSA before and during stimulation or potentiation of evoked responses a conventional Students t-test was used. When comparisons were made between two dependent measures , such as whether or not a population of cells is inhibited, the binomial distribution was used.

2.4 Lesioning Techniques

All lesions were performed when the animals were anaesthetized with sodium pentobarbital (50 mg/kg i.p.). The animals were positioned in the stereotaxic frame and the skull exposed as described in the previous sections. Small holes were drilled in the calvarium (1.0-1.5 mm in diameter) and a sharp needle was used to puncture the underlying dura and the lesioning electrode or cannula was then stereotaxically lowered to the desired depth. A lesion was then made using one of the methods described below. Following the lesions the incision was sewn up and the animals allowed to recover in a temperature controlled cage.

Electrolytic Lesions

The lesioning electrodes were made by scraping the insulation off the inner pole of an SNE-100 electrode under a dissecting microscope so that 0.5-0.75 mm of the tip was exposed. The positive pole of a Grass lesioning device (Model LM5A) was connected to the inner tip of the electrode and the negative pole was connected to the tail of the animal. A good connection between the negative pole and the animal was secured by wrapping a moist cotton swab around the animal's tail. The lesioning current (1.0-1.5 mA) was preadjusted before anodal DC current was passed for 10-15 seconds.

Acute And Chronic Transections

Acute transections were made using a modified surgical blade (1.0 mm X10.0 mm) which was mounted on a Kopf stereotaxic holder. The dura overlying the intended course of the transection was gently removed before the blade was stereotaxically lowered and moved to complete the transection. To transect the hippocampal commissure in the ventral psalterium the knife was lowered to a position 4.5 mm posterior to bregma; 0.5 mm from the midline and 4.0 mm below the surface of the cortex; the blade was then moved parallel to the saggital sinus to a position 2.0 mm anterior to bregma. To transect brainstem afferents to the forebrain the saggital sinus was tied off and

removed before the knife was lowered to a position 2.5-3.0 mm behind bregma; 4.5 mm from the midline and 2.5 mm below the surface of the cortex. As the knife was moved across the coronal plane it was gradually lowered further reaching a depth of 7.0-8.0 mm at the midline. At this point it was gradually retracted as it approached the contralateral side. The procedure was necessary to account for the shape of the base of the skull and avoid damaging the basilar arteries.

Chronic transection of the hippocampal commissure was performed by drilling two small holes in the skull, one positioned 1.0 mm from the midline and 2.0 mm anterior to bregma and the other 1.0 mm from the midline and 5.0 mm posterior to bregma. A sterile curved surgical needle (6-0) with attached thread was lowered through one hole and carefully brought up through the other. The two ends of the thread were then gently lifted thereby transecting the overlying fibres. The curve of the needle assured a minimum depth of 4.5 mm at the level of the ventral psalterium.

Neurochemical Lesions

Chemicals were injected intracerebrally using a Hamilton 30 gauge microsyringe attached to a Kopf stereotaxic holder. The microsyringe was filled taking special care to eliminate air bubbles. The microsyringe was stereotaxically lowered very slowly to minimize tissue damage. A predetermined volume was injected using a micrometer at the rate of 1.0 ul/10 minutes. At the end of the injection period the cannula was left in place for at least 10 minutes to allow undisturbed diffusion of the solution away from the tip before it was withdrawn.

The dorsal noradrenergic bundle (AP=+2.6; L=+1.1; V=-4.5 below cortex) was selectively destroyed using bilateral injections of 6-hydroxydopamine hydrobromide (6-OHDA, Regis Chemical; 6 ug dissolved 2.0 ul of 0.15M NaCl with 0.2mg/ml ascorbic acid). Previous studies have suggested that 6-OHDA is specifically taken up by catecholamine-containing axons resulting in their subsequent destruction. (Tranzer and Thoenen, 1967; Sachs and Jonsson, 1975) the degree of nonspecific tissue damage at the injection site resulting from the needle penetration and the injection did not exceed 0.25 mm in any penetration.

Central serotonin (5-HT) was selectively depleted using intragastric injections of p-chlorophenylalamine (p-CPA, 400 mg/kg; Pfizer Inc.) suspended in 2-3 ml of

saline. The suspension was prepared by adding a few drops of polysorbate (Tween 80) to wet the solid. The p-CPA was then completely dissolved at pH 11 by adding 5 M NaOH. The suspension was then diluted using 5M HCl and saline to final pH of 7.0. Given in this manner p-CPA has been found to block 5-HT biosynthesis possibly by inhibition of enzymatic tryptophan hydroxylation (Koe and Weissman, 1966). The resulting depletion of 5-HT is maximal 2-4 days following the injection.

Cell bodies in the septal area were destroyed using direct injections of kainic acid (1.0-3.0 ug in 1.0 ul of saline). Kainic acid is an analogue of the excitant amino acid glutamate which has been claimed to specifically destroy cell bodies in the area of the injection without destroying axons terminating in or passing through the injection site (Olney, Ho and Rhee, 1974; McGeer and McGeer, 1977).

2.5 Neurochemical Assays

The effectiveness of 6-OHDA and p-CPA in depleting central noradrenaline (NA) and serotonin (5-HT) ,respectively, was evaluated by assaying the concentrations of these substances in the caudate nucleus, the hippocampal formation and the septal area. At the termination of the acute electrophysiological experiments the animals were decapitated and the above brain areas promptly removed. The concentration of NA was measured using a radioenzymatic assay according to the methods of Coyle and Henry (1973). The concentration of NA in 6-OHDA treated rats was compared to levels in animals that that were also anaesthetized and used for electrophysiological experiments as well as to values obtained from unoperated controls.

Serotonin concentration was measured using a spectrofluorometric assay according to methods previously described by Richardson and Jacobowitz (1973). The animals were killed immediately after the electrophysiological experiments and the brain areas were dissected out and frozen at -40 degrees C until the following morning. They were them homogenized and assayed on a Turner model 430 spectroflourometer.

2.6 Histological Analysis

The location of stimulating sites was marked by passing low DC anodal current (10-15 uA for 10 seconds) through the stainless steel electrode resulting in small iron deposits from the electrode tip. The deposits were subsequently visualized as blue/green spots in histological sections when the preparation was perfused with a potassium ferrocyanide/formalin mixture (1g/100 ml). The cyanide reacts with Fe++ resulting in the Prussian blue reaction product which is easily observed under the light microscope. Microelectrode positions were determined by either ejecting pontamine sky blue dye (20% w/v in 4M NaCl) electrophoretically (Thomas and Wilson, 1965) or by breaking off the electrode tip so that the electrode track could be visualized after the fixation (for example See Fig. 3-2).

The animals were killed by an overdose of the anaesthetic agent and perfused intracardially with 100-200 ml of 0.9% NaCl followed by 100 ml of 10% buffered formalin. Histological verification of the electrode sites was made by cutting 50 uM frozen sections and staining them with cresyl violet or saffranin.

CHAPTER 3: ROLE OF THE SEPTAL AREA IN THE GENERATION OF HIPPOCAMPAL ELECTRICAL ACTIVITY

3.1 Introduction

As reviewed in chapter 1, the electrical activity of the hippocampal formation consists of a mixture of slow and fast waves having varying amplitudes which are related to ongoing physiological states. The most conspicuous activity is a synchronous or regular slow activity (RSA) which is recorded during sensory stimulation or direct activation of the ascending midbrain reticular formation. In rabbits, rhythmical hippocampal activity has been related to the discharge pattern of neurones in the medial septal (MS) region. Petsche et al (1962) observed that during RSA a population of MS neurones fire in bursts which occur during a certain phase of RSA waves, whereas, during desynchronization the same neurones fire in an irregular or random manner. The additional observation that electrolytic lesions of the septal area abolish RSA (Brucke et al, 1959) has led to the proposal that the septum 'paces' electrical activity of the hippocampal formation (Petsche et al, 1962; Stumpf, 1965).

A true pacemaker in the septal area would support the hypothesis that RSA is generated by phasic afferent inputs which produce periodic depolarization of

hippocampal neurones (Von Euler and Green, 1960). Recently, McLennan and Miller (1976) have provided electrophysiological evidence that, in the rat, the bursting discharge pattern of MS neurones may be a consequence rather than the cause of hippocampal RSA. This prompted a re-evaluation of the evidence that the MS initiates RSA.

The purpose of the present chapter is to 1) characterize the discharge patterns of septal neurones in the urethane anaesthetized rat and relate these patterns to ongoing electrical activity of the hippocampus and 2) describe mechanisms which could underlie the observed relationships.

3.2 Experimental Procedures

Slow electrical activity was recorded from the CA1 region of the dorsal hippocampus or the upper blade of the dentate gyrus in 71 rats using techniques detailed in chapter 2. The simultaneous discharge of single neurones in the septal area (0.2 mm anterior to 0.3 mm posterior to bregma; 0.1-0.5 mm from the midline; 3.5-6.0 mm below the surface of the cortex) was recorded extracellularly. Several recording electrode penetrations were made into the septal region and individual neurones were classified on the basis of the stereotaxic position of the recording electrode, the response to stimulation of the fimbria (McLennan and

Miller, 1974a) and the discharge pattern of neurones analyzed as outlined in chapter 2.

Hippocampal electrical activity was also recorded in animals which previously (3-20 days) had either an electrolytic lesion (1.5 mA anodal current for 10-15 seconds) or an intraseptal injection (1-3 ug in 1 ul of saline) of the neurotoxin kainic acid which, unlike electrolytic MS lesions, destroys neuronal perikarya without interfering with axons of passage (See appendix a). In 4 additional rats an acute transection was made at the level of the hypothalamus to evaluate the contribution of brainstem afferents to the discharge pattern of septal neurones.

3.3 Results

Spontaneous Electrical Activity Of The Hippocampus And The Dentate Gyrus

Electrical activity was recorded from the CA1, pyramidal cell layer and the upper blade of the dentate granule cell region. The records obtained from both of these sites consisted of either 1) an irregular or desynchronized activity consisting of slow and fast waves having varying amplitudes (Fig 3-1B or 2) a rhythmic pattern of slow waves having constant amplitudes and frequencies which ranged from 3-7 Hz (Fig 3-1a). The latter pattern, referred to as RSA or

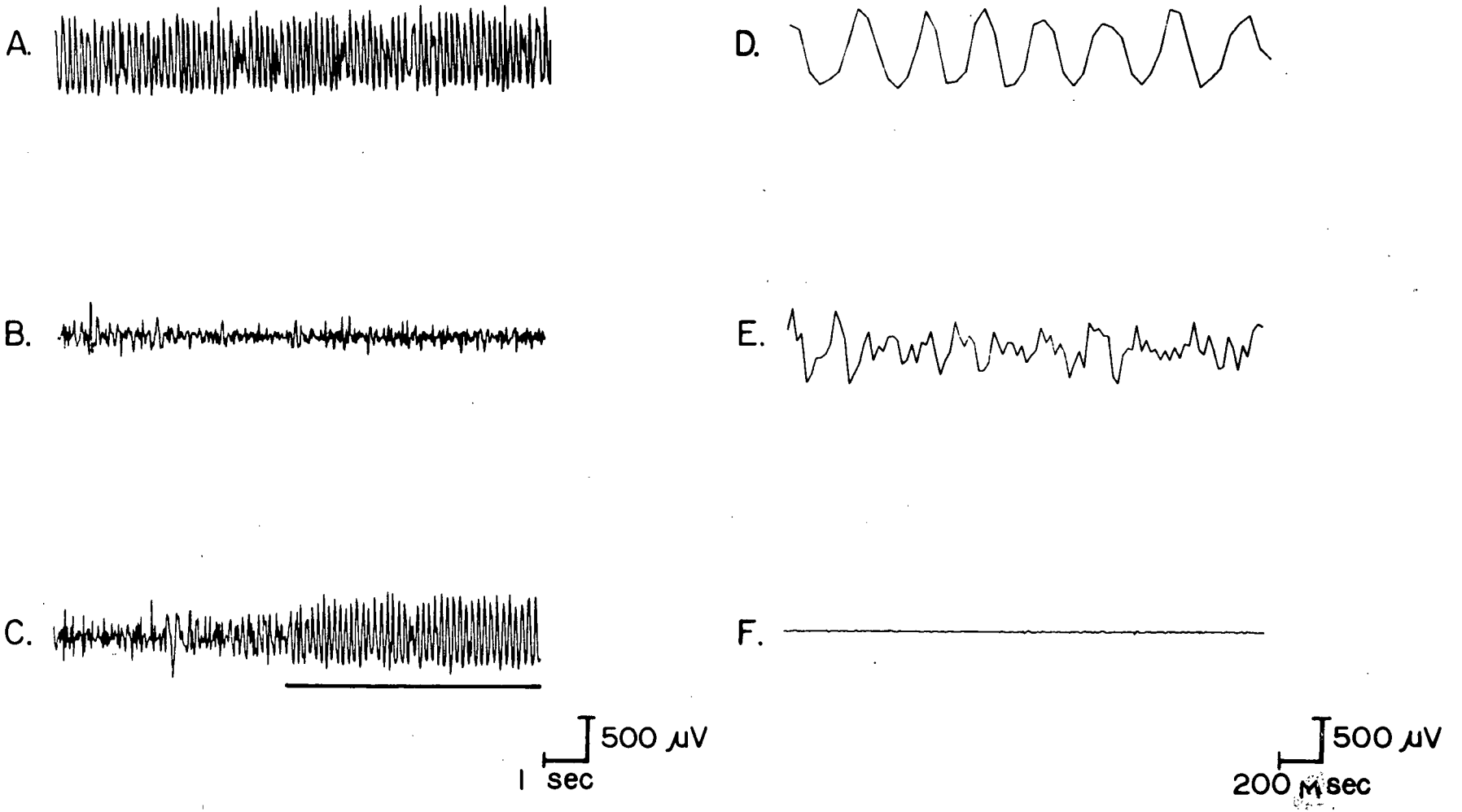
FIG. 3- 1: Patterns Of Electrical Activity Recorded From The Dentate Gyrus Of A Urethane Anaesthetized Rat.

A,D: spontaneously occurring RSA shown at fast and slow sweeps.

B,E: desynchronized electrical activity recorded from the same position 3 minutes after the previous records.

C: somatosensory stimulation (indicated by the solid line) shifts the electrical activity from desynchronization to RSA.

F: records obtained following the death of the animal indicating minimal contribution of electrical noise.


A.
B.
C.
D.
E.
F.
500 µV
1 sec
500 µV
200 msec

'theta', could also be elicited by somatosensory stimulation such as pinching the animal's tail (Fig 3-1C). The pattern of electrical activity recorded from CA1 was always similar to that recorded from the dentate with respect to its synchrony and frequency. Moreover, RSA was never recorded in CA1, in the absence of RSA from the dentate. For this reason only records obtained from histologically verified sites in the dentate (Fig. 3-2) will be presented in the present thesis.

The frequency of RSA observed in urethane anaesthetized rats (3-7 Hz) is significantly lower than the frequency of RSA in freely moving rats (3-12 Hz) previously reported by Vanderwolf (1975). Similar effects of urethane on RSA frequency have been observed in rabbits (Stumpf, 1965). The spontaneous occurrence of RSA was related to the depth of anaesthesia, but sensory-evoked RSA could be demonstrated at a wide range of anaesthetic doses. In order to eliminate the possibility of any extraneous movement artifacts which might be associated with the patterns of electrical activity, 5 animals previously anaesthetized with urethane were then immobilized with flaxedil (20 mg/kg, i.v.) and artificially ventilated. The spontaneous and sensory-evoked patterns of electrical activity recorded under these conditions did not differ from those obtained in non-immobilized rats. Furthermore, the contribution of electrical noise generated by the

FIG. 3- 2: Location Of Recording Electrode In The Dentate Gyrus.

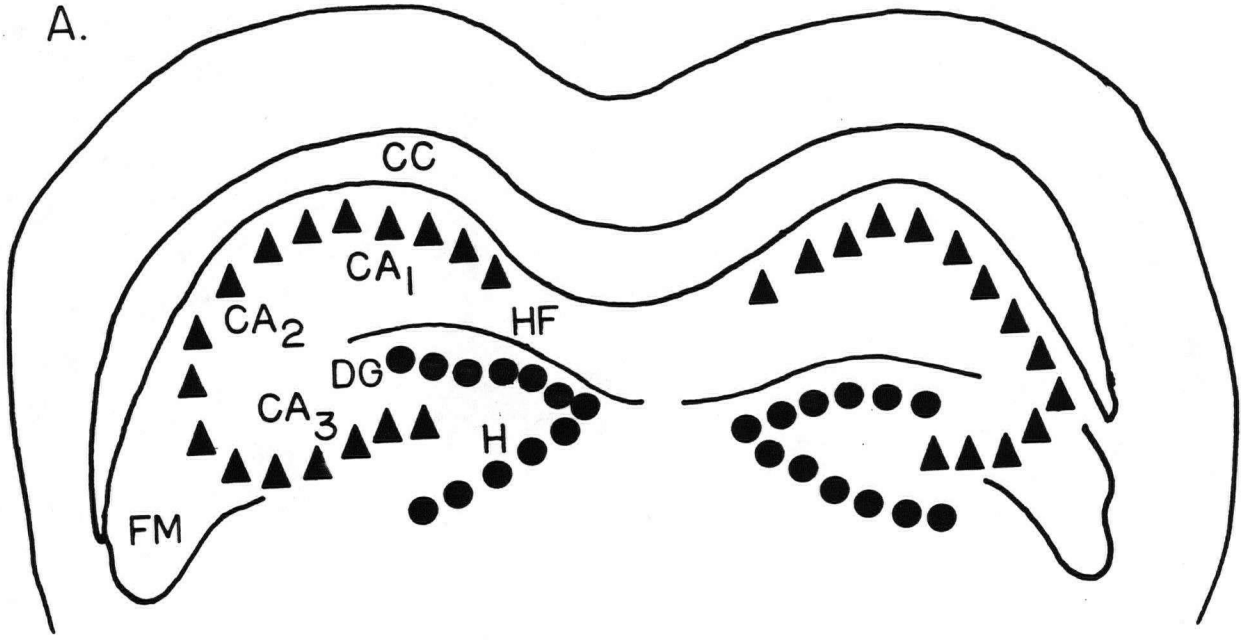
A: A schematic drawing of the hippocampal formation showing pyramidal cell layer of the hippocampus proper (triangles) and the granule cells of the dentate gyrus (circles).

B: Coronal section of the rat brain showing electrode positions (arrow) from which RSA was recorded in the upper blade of the dentate gyrus.

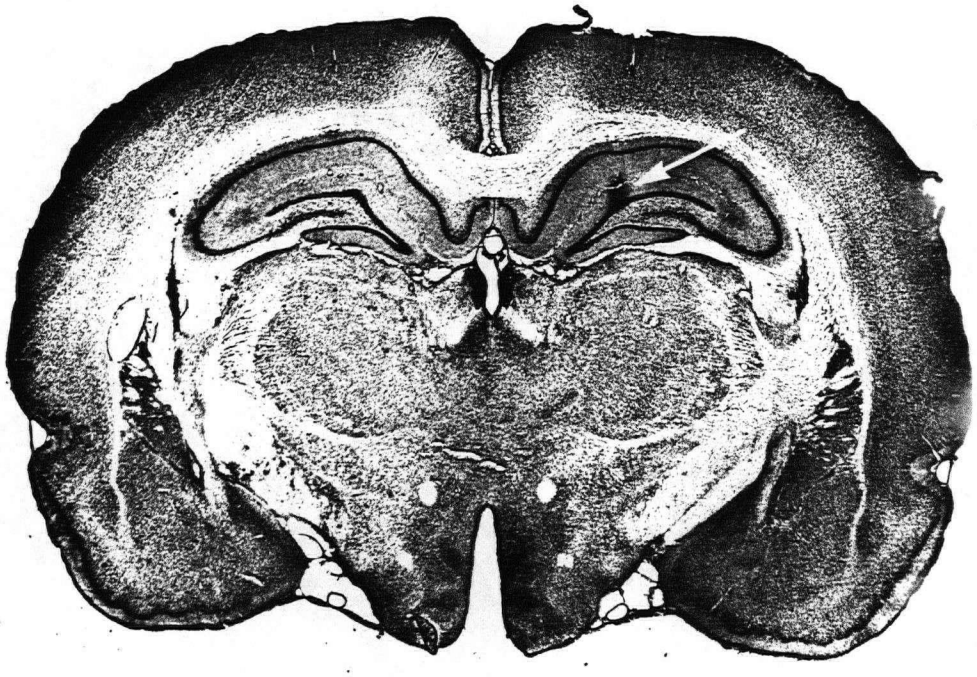
Abbreviations:

CA1-3	Pyramidal cell layer
CC	Corpus Callosum
DG	Dentate Gyrus
FM	Fimbria
HF	Hippocampal Fissure
H	Dentate Hilus

A.



B.



equipment and room fixtures was checked routinely by recording from the animal immediately after death. As shown in Fig 3-1f, such artifacts did not contribute substantially to the recorded activity.

Septal Unit Activity

Action potentials with signal to noise ratios greater than 4:1 (160:40 uV) were recorded from 431 neurones in the medial septal-diagonal band region. All neurones were spontaneously active and exhibited discharge frequencies ranging between 2 and 50 Hz. On the basis of their discharge patterns septal neurones were classified as either irregular (I -neurones) or bursting (B-neurones). I-neurones always discharged in an irregular or random pattern which did not exhibit any constant phase relationship to the patterns of hippocampal electrical activity (Fig. 3-3 A-C). B-neurones, in addition to firing in an irregular manner which was always associated with the desynchronized pattern of hippocampal activity (Fig. 3-3 E) were also observed to discharge in rhythmic bursts of 2-10 spikes (Fig. 3-3 D). This bursting pattern, which occurred either spontaneously or as a result of sensory stimulation, exhibited a constant frequency and phase relationship to hippocampal 'theta' waves.

A given B-neurone may discharge during any phase of the 'theta' wave and for each cell the discharge

FIG. 3- 3: Differences In The Discharge Patterns Of Septal Neurones Recorded During Rhythmical Or Desynchronized Hippocampal Activity

A,B,D,E: Single oscilloscope sweeps in which the top trace is hippocampal electrical activity and the lower trace the simultaneously recorded septal unit discharges. A and D were recorded during RSA whereas C and E were recorded during irregular activity.

C,F: Inter-spike interval histograms (corresponding to A and D) indicating irregular discharge of the I-neurone (C) and the bursting bimodal discharge pattern of the B-neurone (F). Number of spikes in each histogram is 2000 and the bin width is 3 msec.

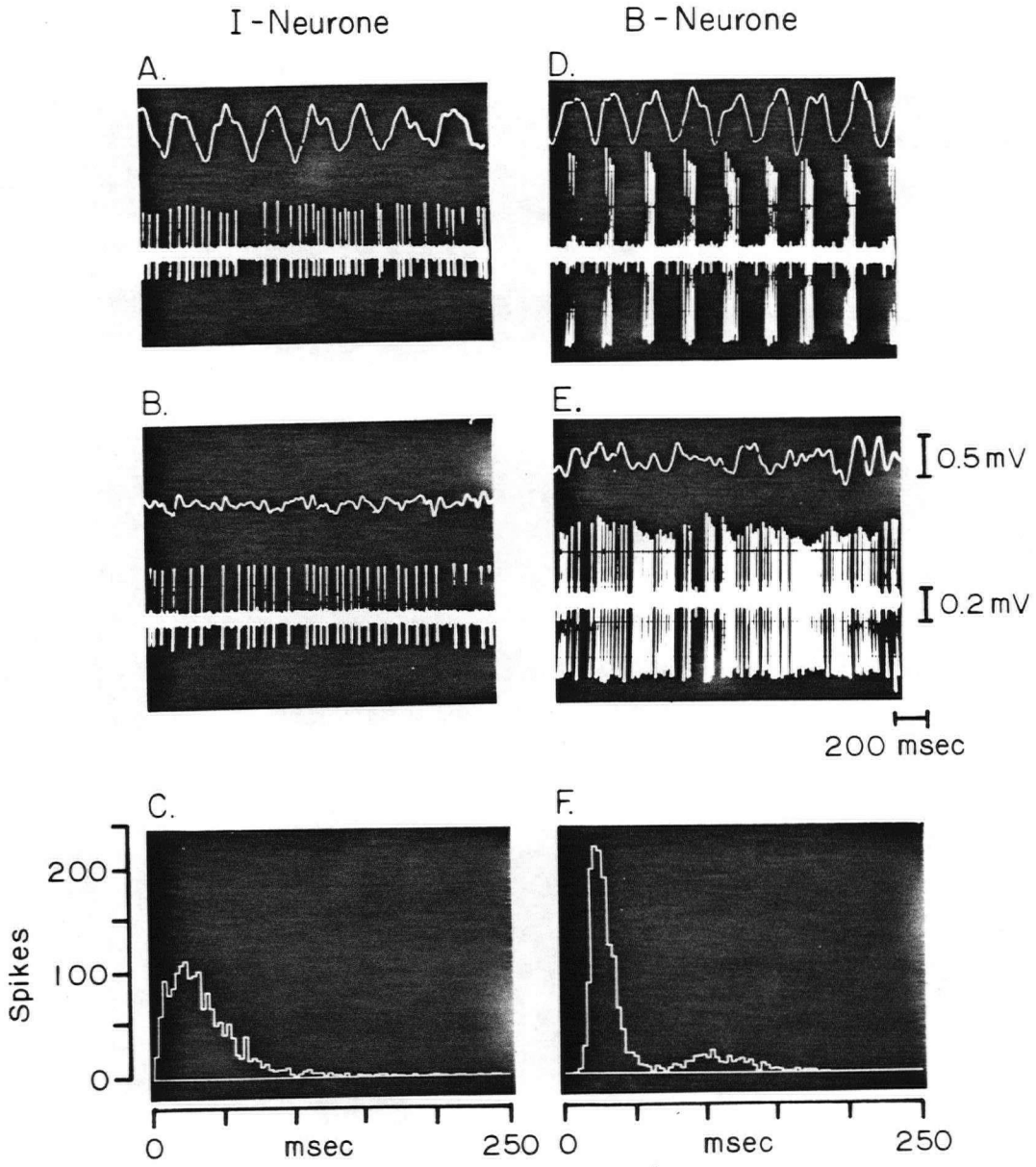
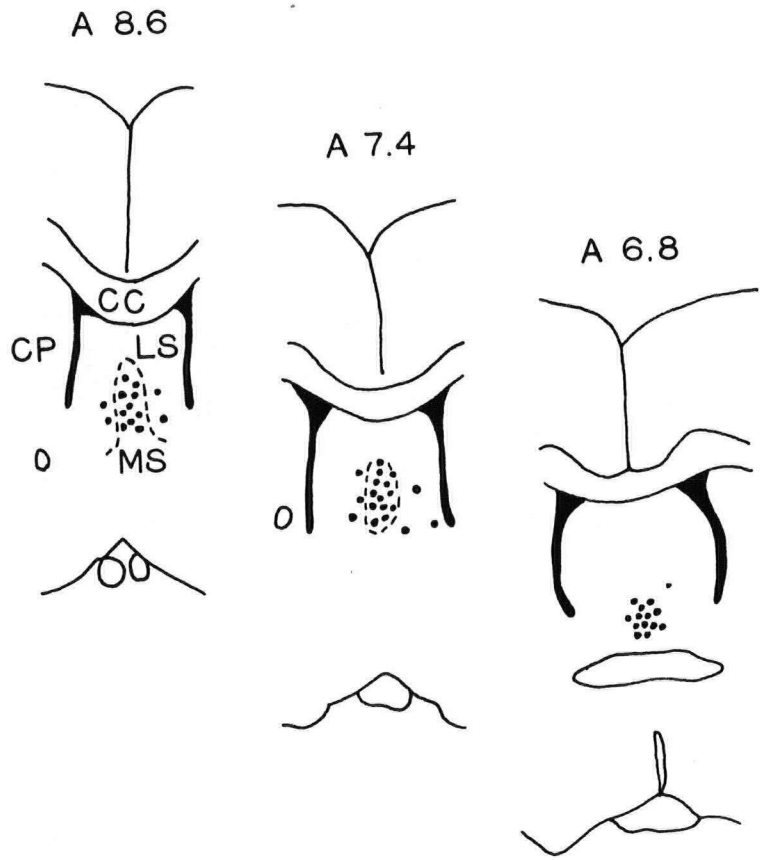

I - Neurone
B - Neurone
A.
D.
B.
E.
0.5 mV
0.2 mV
200 msec
C.
F.
Spikes
200
100
0
0
msec
250
0
msec
250

FIG. 3- 4: Localization Of B-neurones In The Septal Area.

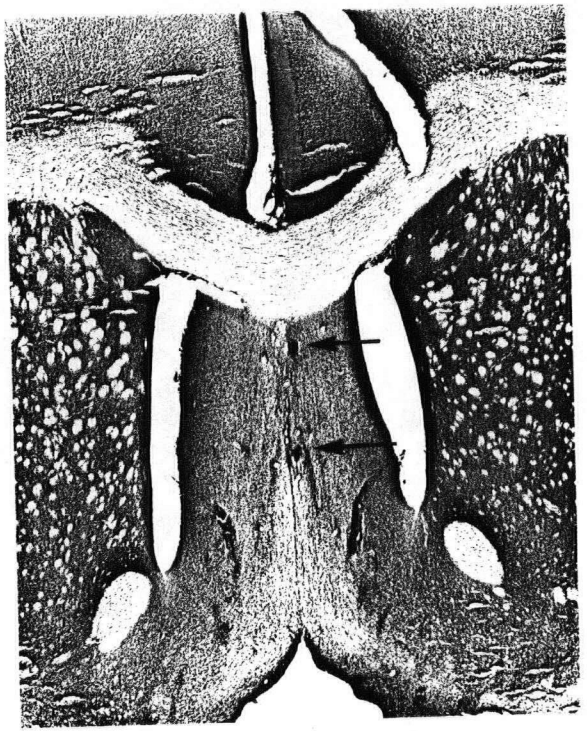
A: Coronal sections from stereotaxic atlas showing the distribution of neurones which were identified as B-neurones.

B: Histological verification of the position of the recording electrode by ejection of pontamine sky blue dye (indicated by arrows). Note that the top deposit was left at the site of non-bursting neurones in the dosal septum and the lower deposit was left where bursting neurones were first recorded in a track through the medial septal region.

A.



B.



distribution as revealed by serial correllograms remained fairly constant (see also Petsche et al, 1962 Fig. 2). In contrast to the conclusions drawn by Petsche et al (1962) for septal bursts in the rabbit, a small proportion (20-30%) of B-neurones in the rat display an amplitude decrement within a burst. However, intracellular recordings are necessary for a quantitative analysis and comparison of this decrement to the 'inactivation response' of hippocampal pyramidal cells described by von Euler and Green (1960). Similar patterns of neuronal discharges have been described in the rabbit. (Petsche et al, 1962; Stumpf et al, 1962; Apostol and Creutzfeldt, 1974).

Of the 431 neurones recorded throughout the septal region in this series of experiments 202 neurones (47%) were classified as I-neurones and 229 (53%) as B-neurones. However, there was a regional distribution of B-neurones whereby a high proportion was recorded in penetrations that were in the ventro-medial aspects of the septum (Fig. 3-4).

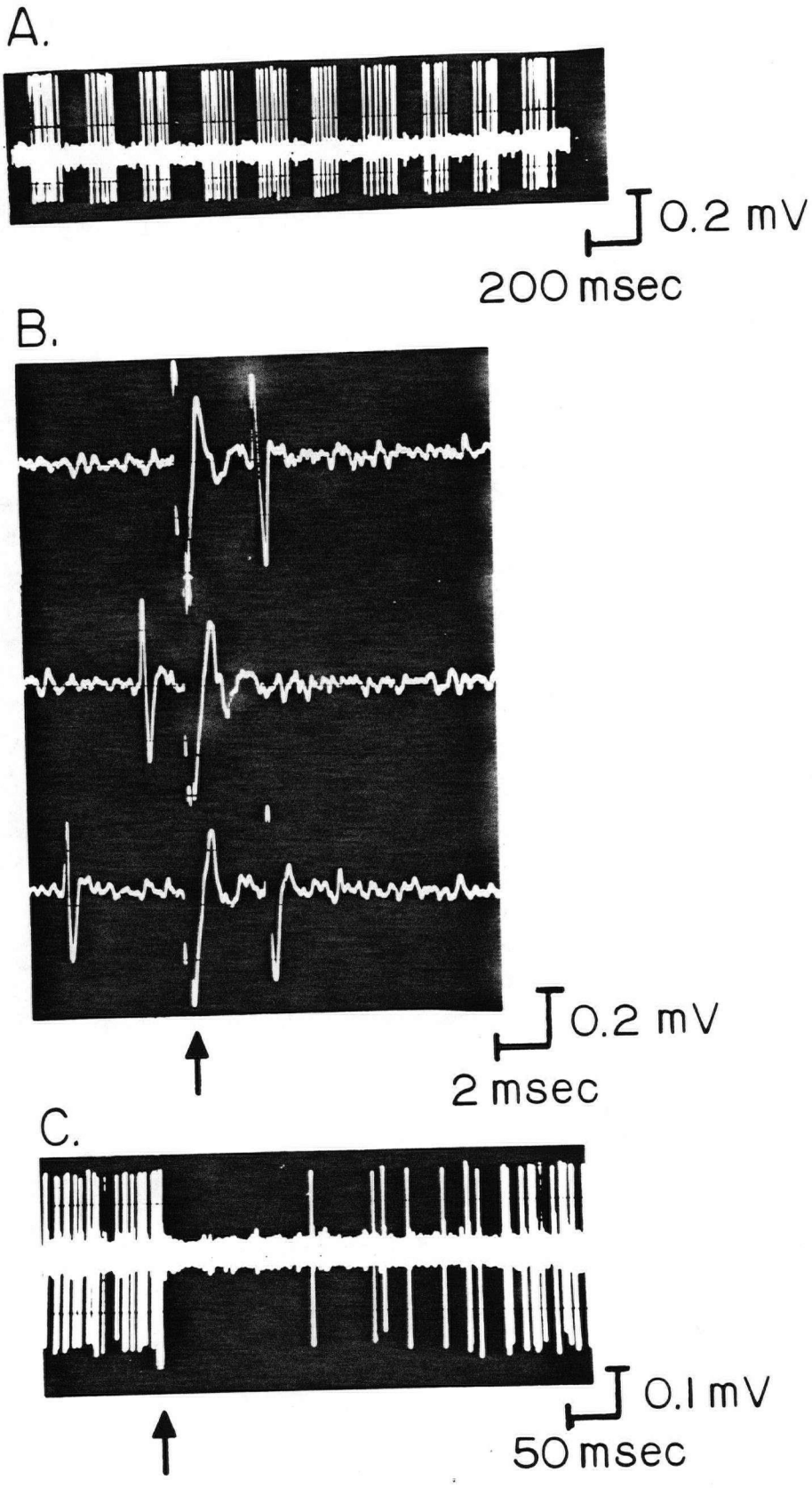
In addition to their characteristic discharge patterns, septal neurones were classified on the basis of their response to stimulation of the dentate gyrus. Of 51 B-neurones tested, 24 were antidromically activated following single pulse stimulation of the dentate at the same electrode position from which RSA activity was recorded (Fig. 3-5). In contrast, only one

FIG. 3- 5: Characteristics Of A Septal B-neurone.

A: Single oscilloscope sweep displaying the spontaneous bursting pattern.

B: Antidromic activation of the same neurone in response to stimulation of the dentate gyrus (DG). Collision-extinction with spontaneously occurring spike is illustrated in middle trace.

C: Inhibitory response of the B-neurone following antidromic activation of DG (25 superimposed sweeps). Arrows indicate stimulus artifacts.


A.
0.2 mV
200 msec
B.
0.2 mV
2 msec
C.
0.1 mV
50 msec

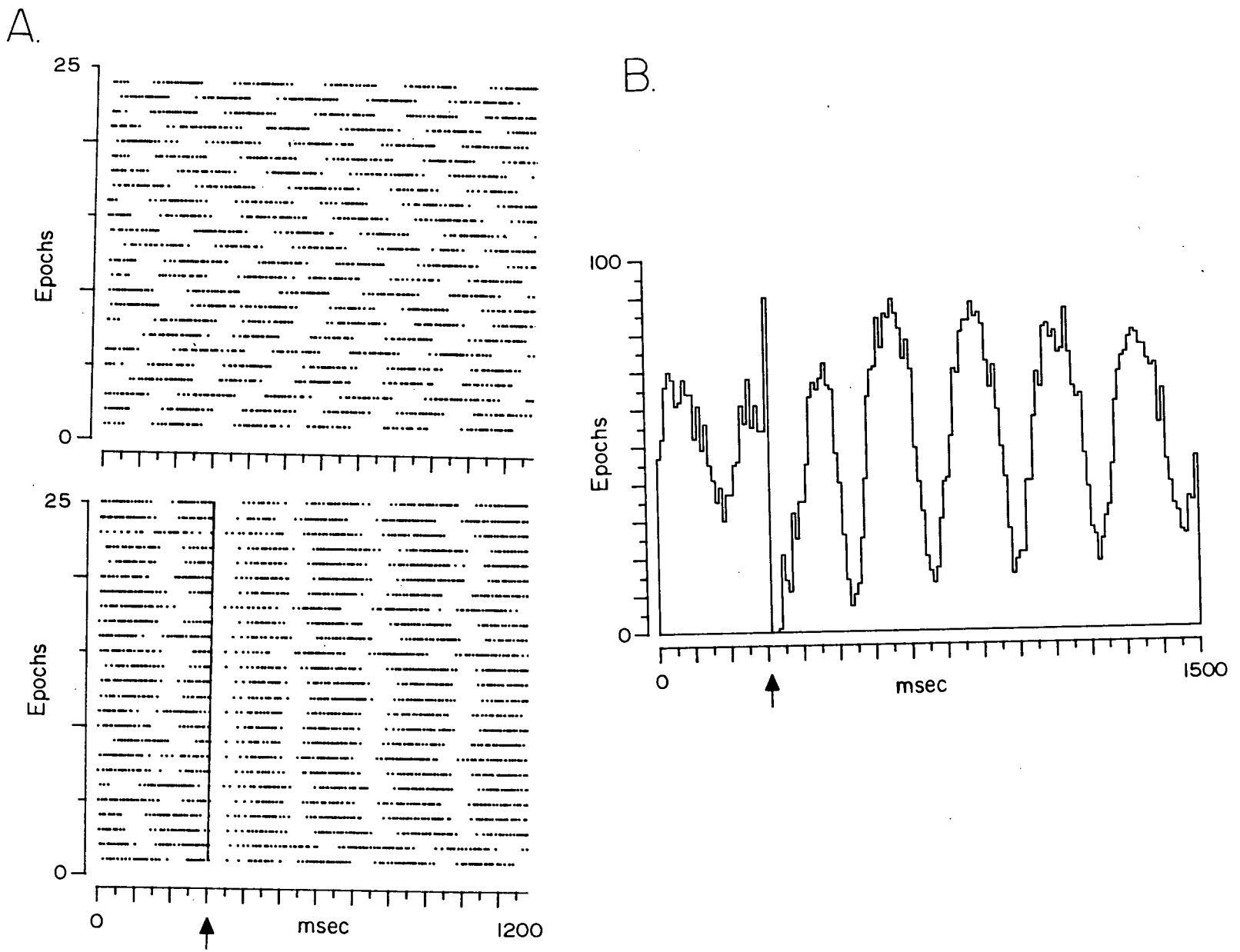
of 96 I-neurones examined could be antidromically activated. Several criteria were used to verify that spikes were antidromically evoked: (a) short and constant latency (1.0-3.0 msec) at threshold stimulation (3-5 volts), (b) collision-extinction between a spontaneous and the evoked spike at critical intervals up to 4.0 msec, (c) the presence of IS-SD break in the configuration of the evoked spike and (d) ability to follow high frequency stimulation (100-150 Hz). Single pulse stimulation of the fimbria also resulted in antidromic activation of the same septal neurones with a shorter latency (0.5-1.5 msec) than that observed during stimulation of the dentate gyrus. The spontaneous discharge of B-neurones was invariably inhibited for periods of 30-100 msec following dentate or fimbrial stimulation even when they were not antidromically evoked. (Fig. 3-5). This inhibition is similar to that reported by McLennan and Miller (1974a) and used for the identification of medial septal neurones. Two additional characteristics of MS response patterns following stimulation of the fimbria or the dentate gyrus were brief low amplitude burst discharges having low amplitudes preceding the period of inhibition and a high probability that MS neurones would begin to burst at the end of the inhibitory period (Fig. 3-6).

Although I-neurones were not antidromically activated following stimulation of the fimbria, they

FIG. 3- 6: Bursting Discharge Pattern Of Septal Neurones Following Hippocampal Stimulation.

A: rastered display of spontaneously occurring bursts (upper raster) which are synchronized by single pulse stimulation (arrow) in the dentate (lower raster).

B: post stimulus time histogram of the cell shown in A indicating a rhythmical discharge pattern.


A.
25
Epochs
0
25
Epochs
0
0
msec
1200
B.
100
Epochs
0
0
msec
1500

could be synaptically activated (37%) or inhibited (42%) by stimulation of sites that antidromically activated B-neurones.

Hippocampal Response Patterns After Electrolytic And Kainic Acid Lesions Of The Septal Area

Fig. 3-7 compares the electrical activity recorded from DG of unoperated control rats and rats having electrolytic or kainate lesions of the septal area. In control rats, the two distinct spontaneously occurring patterns of electrical activity, desynchronized and RSA, were recorded. RSA was also initiated by somatosensory stimulation such as pinching the animal's tail (T.P. in Fig. 3-7B) or electrical stimulation (1-40,100 Hz) in the region of the locus coeruleus. In contrast, spontaneous or evoked RSA was not recorded in electrolytic or kainate lesioned rats. However, irregular hippocampal activity was not significantly altered by kainate and electrolytic lesions of the medial septal region (Fig. 3-7D,G). In fact, there were no easily detectable differences between the electrical activity recorded from the dentate of rats having kainic or electrolytic lesions of the MS.

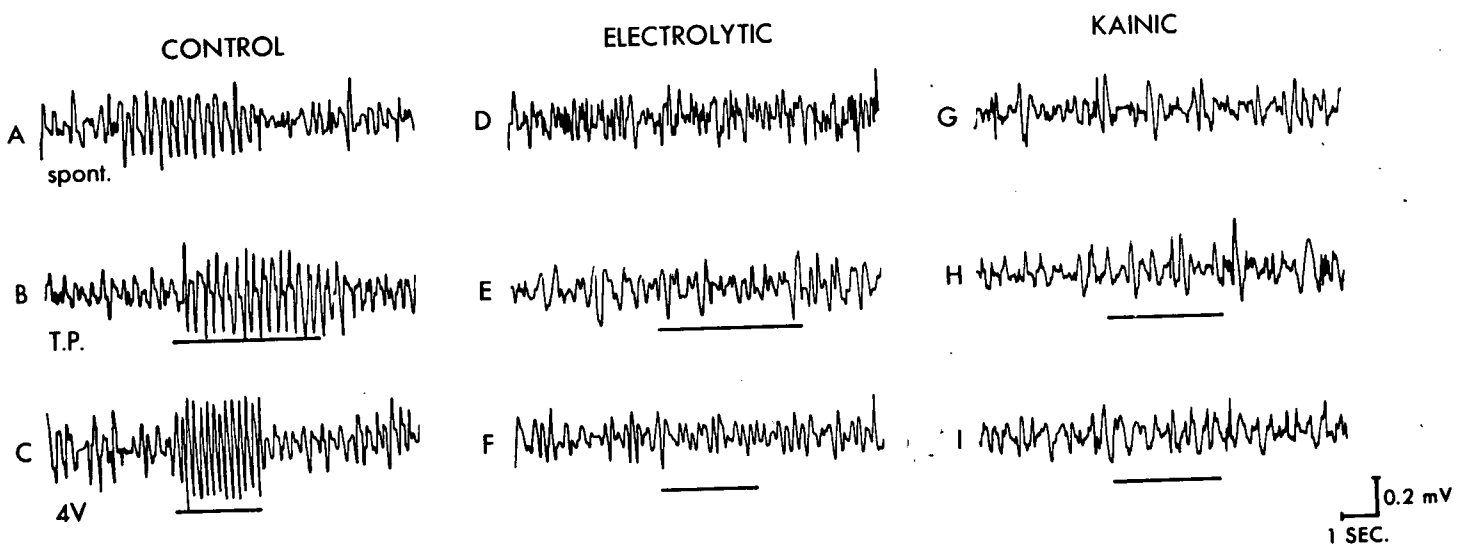
The extent of kainate and electrolytic lesions that resulted in the disruption of RSA are shown in Fig. 3-8A,B . In both cases, the damage was restricted to the medial septal-diagonal band region. Localization

FIG. 3- 7: Effects Of Electrolytic And Kainate Lesions Of The Septal Area On Hippocampal Electrical Activity.

A,D,G: spontaneously occurring activity showing the periodic appearance of RSA only in controls.

D,E,H: effect of pinching the animal's tail (T.P.) during the period indicated by solid lines.

C,F,I: electrical stimulation of the brainstem evokes RSA in control rats only.


CONTROL
ELECTROLYTIC
KAINIC
A
spont.
B
T.P.
C
4V
D
E
F
G
H
I
0.2 mV
1 SEC.

of the kainic acid lesion, as indicated by degeneration of neuronal perikarya and the marked proliferation of glia (Fig. 3-9), revealed that it extended from 0.5-0.7 mm anterior to bregma to 0.7-1.0 mm posterior to bregma. Fig. 3-9 also shows that the number of normal perikarya in the MS of a kainate lesioned rat is significantly less than in a control section taken at the same level.

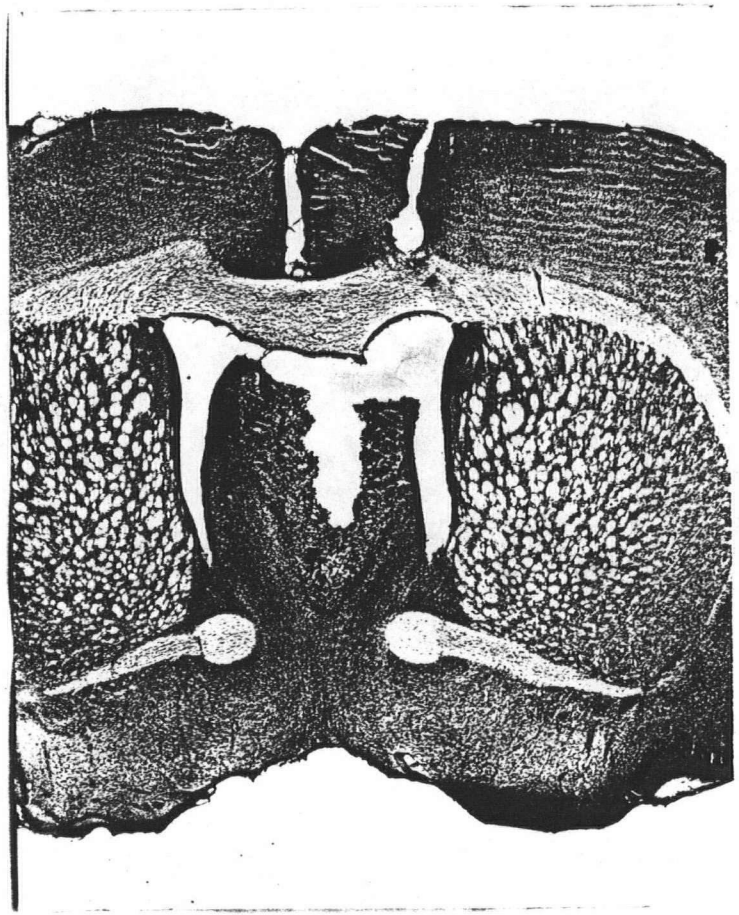
Inasmuch as microscopic investigation reveals loss of perikarya, the possibility that presynaptic terminals or axons of passage were also damaged by kainic injections can not be ruled out. In order to eliminate this possibility, the efficacy of the projection from the median raphe nucleus to the dentate gyrus was examined. This projection was selected since previous anatomical studies have shown that a proportion of its axons project through the septal area to terminate in the dentate (Halaris et al, 1976). As previously reported (Assaf and Miller, 1978c), efficacy of transmission was the same in control and kainate lesioned rats but was markedly attenuated in rats with electrolytic destruction of the septal area.

RSA was only blocked by histologically verified injections of kainic acid into the septal area. Control injections into the lateral ventricles, which caused neuronal degeneration in a restricted region of CA3, did not abolish RSA recorded in the dentate gyrus.

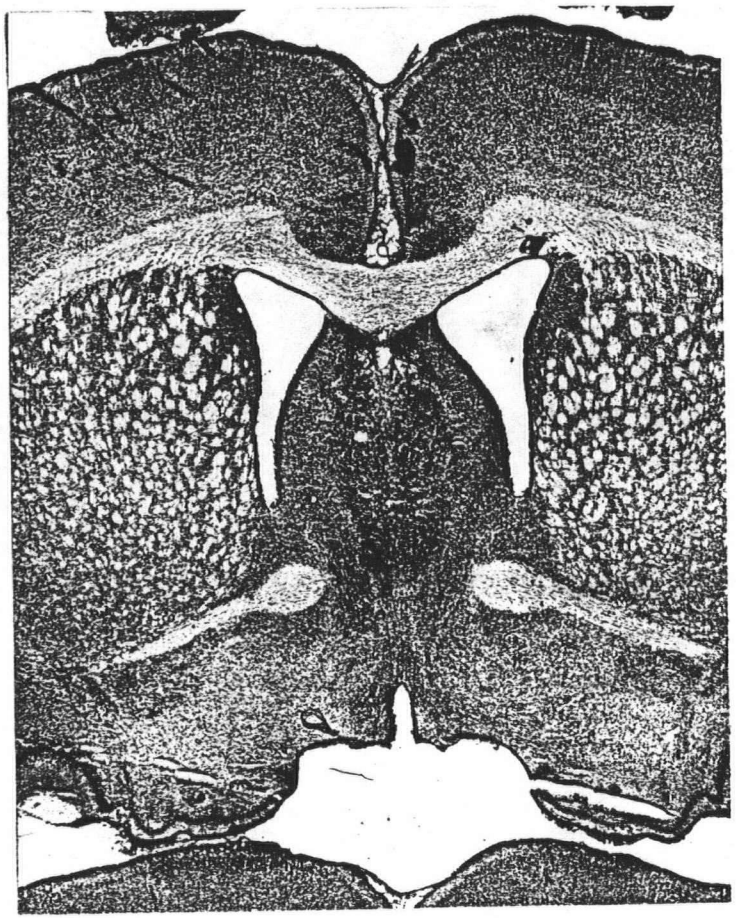
FIG. 3- 8: Extent Of Electrolytic And Kainic-induced Lesions In The Septal Area.

A: coronal section of the forebrain at the widest extent of the electrolytic lesion showing loss of tissue restricted to the septal area.

B: section taken at the same level following the intraseptal injection of kainic acid (2.0 ug).



Electrolytic

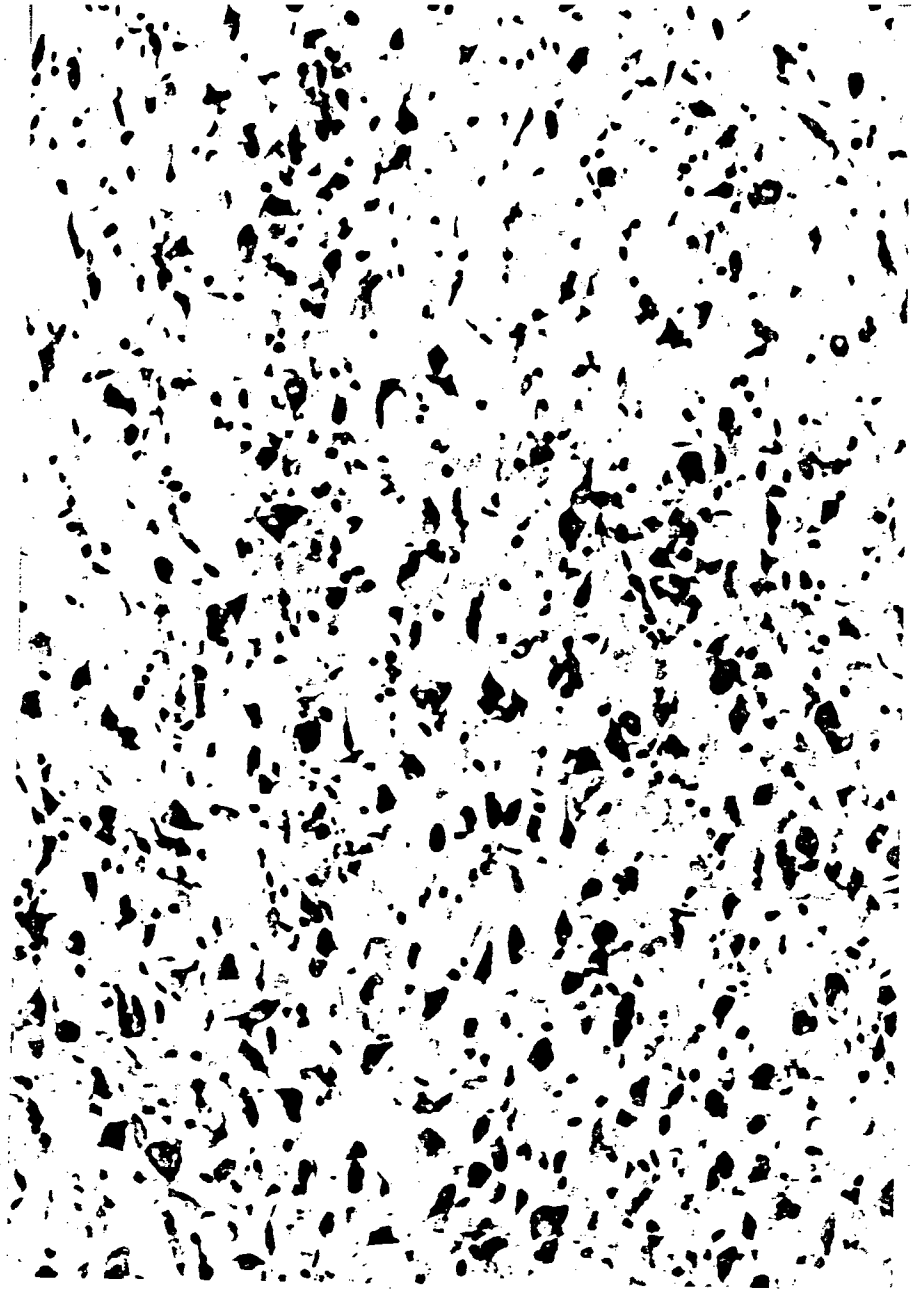


Kainic

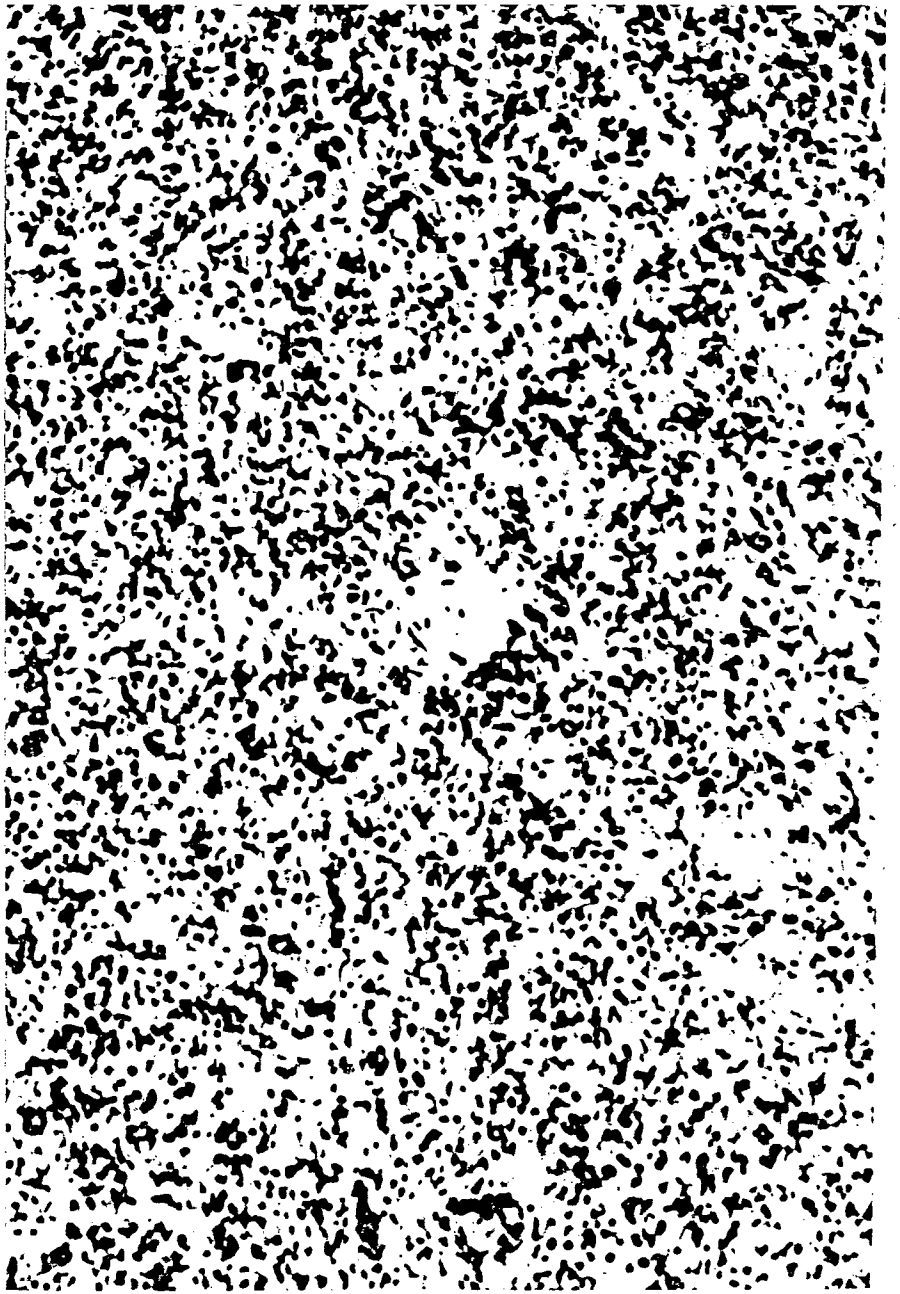
FIG. 3- 9: Neuronal Degeneration Following Kainic Injections Into The Septal Area.

A: a photomicrograph of the medial septal nucleus taken from a control rat showing abundance of neurones.

B: medial septal nucleus following Kainic injection indicating loss of normal perikarya and proliferation of glia. Sections were stained with cresyl violet and magnified 100X.



Control



Kainic

Therefore the effects of intraseptal injections were not due to diffusion of kainic acid into the ventricles.

Discharge Pattern Of Septal Neurones In The Isolated Forebrain

In the previous section it was shown that an intact septo-hippocampal axis is necessary for the generation of RSA. In order to investigate whether the bursting pattern of septal neurones is synaptically relayed by phasic, brainstem influences, the discharge pattern of B-neurones was recorded before (n=43) and after (n=22) a complete transection of the diencephalon at the level of the hypothalamus (Fig. 3-10). Stable recordings were obtained up to 4 hrs after the transection.

In 4 of 6 animals the integrity of the sagittal sinus and the basilar artery was preserved and only data from these animals were included in the analysis.

Before the knife cut 31 of 43 (72%) spontaneously active neurones recorded in the medial aspects of nucleus medialis septi were observed to discharge in rhythmical bursts at an average frequency of 5.3±0.5 Hz. After the knife cut only 5 of 22 neurones (23%) were recorded in the bursting mode of discharge at a frequency (4.3±0.4 Hz) which is significantly ($p < 0.01$) lower than that observed before the knife cut.

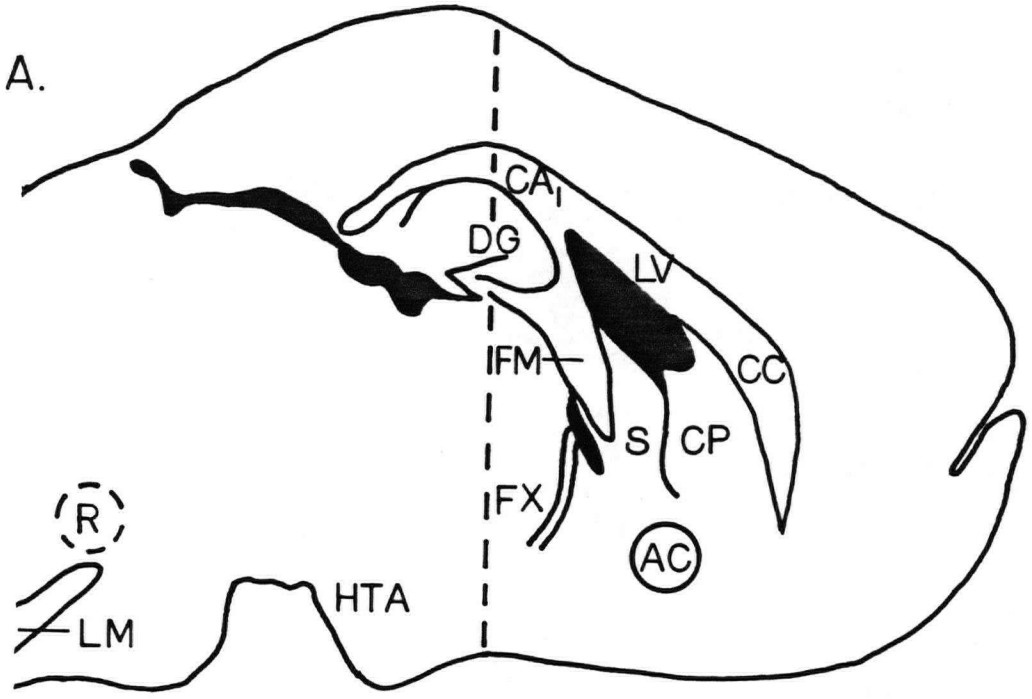
FIG. 3-10: Acute Transection Of Forebrain.

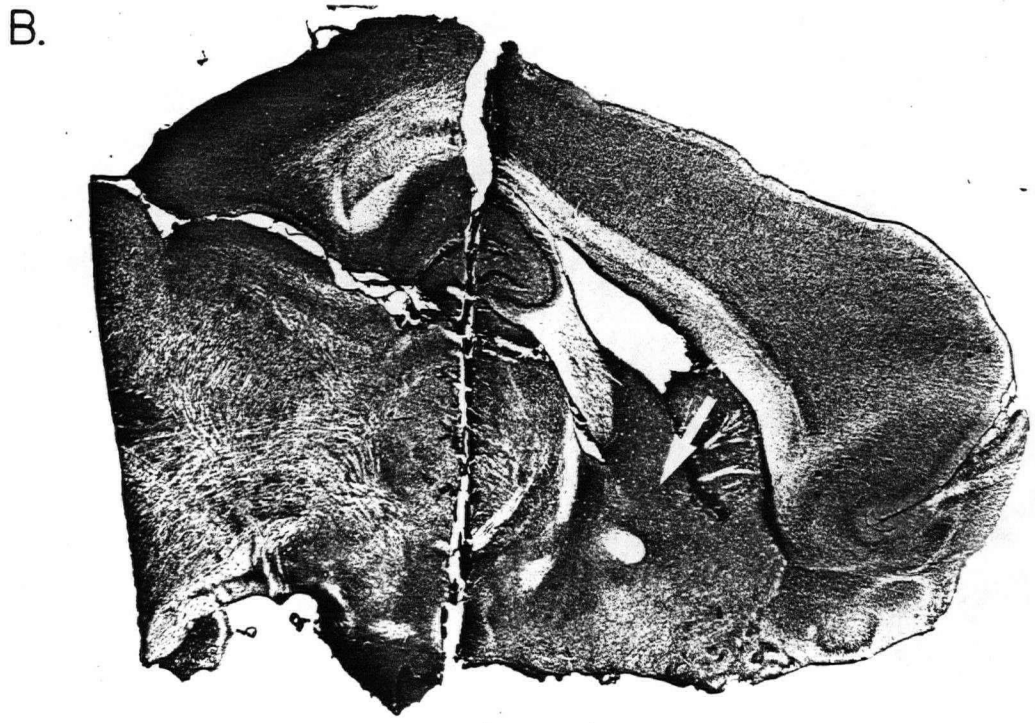
A: A schematic showing the location of the knife cut (dotted line) in relation to indicated anatomical landmarks.

B: A saggital section of brain at about 200 um from the midline. The arrow indicates the level of the recording electrode in the septal region.

Abbreviations

CA1	Pyramidal cell region of hippocampus.
CC	Corpus Callosum
AC	Anterior Commissure
CP	Caudate Nucleus
DG	Dentate Gyrus (Fascia Dentata)
FM	Fimbria
FX	Fornix
HTA	Hypothalamus
LM	Medial Lemniscus
LV	Lateral Ventricle
R	Red Nucleus
S	Septal Area


A.
CA1
DG
LV
IFM
CC
S
CP
FX
R
AC
HTA
LM


B.

Direct activation of the brainstem or sensory stimulation did not alter the discharge pattern of septal neurones recorded in the isolated forebrain preparation (Fig. 3-11). On two occasions recordings were obtained from the same cell before and after the knife cut and as shown in Fig. 3-11C, one of those cells continued to burst after the knife cut. In cases where bursting was observed in the isolated forebrain, the spikes within a burst were not regular as shown by the wide interval histogram shown in Fig. 3-11B.

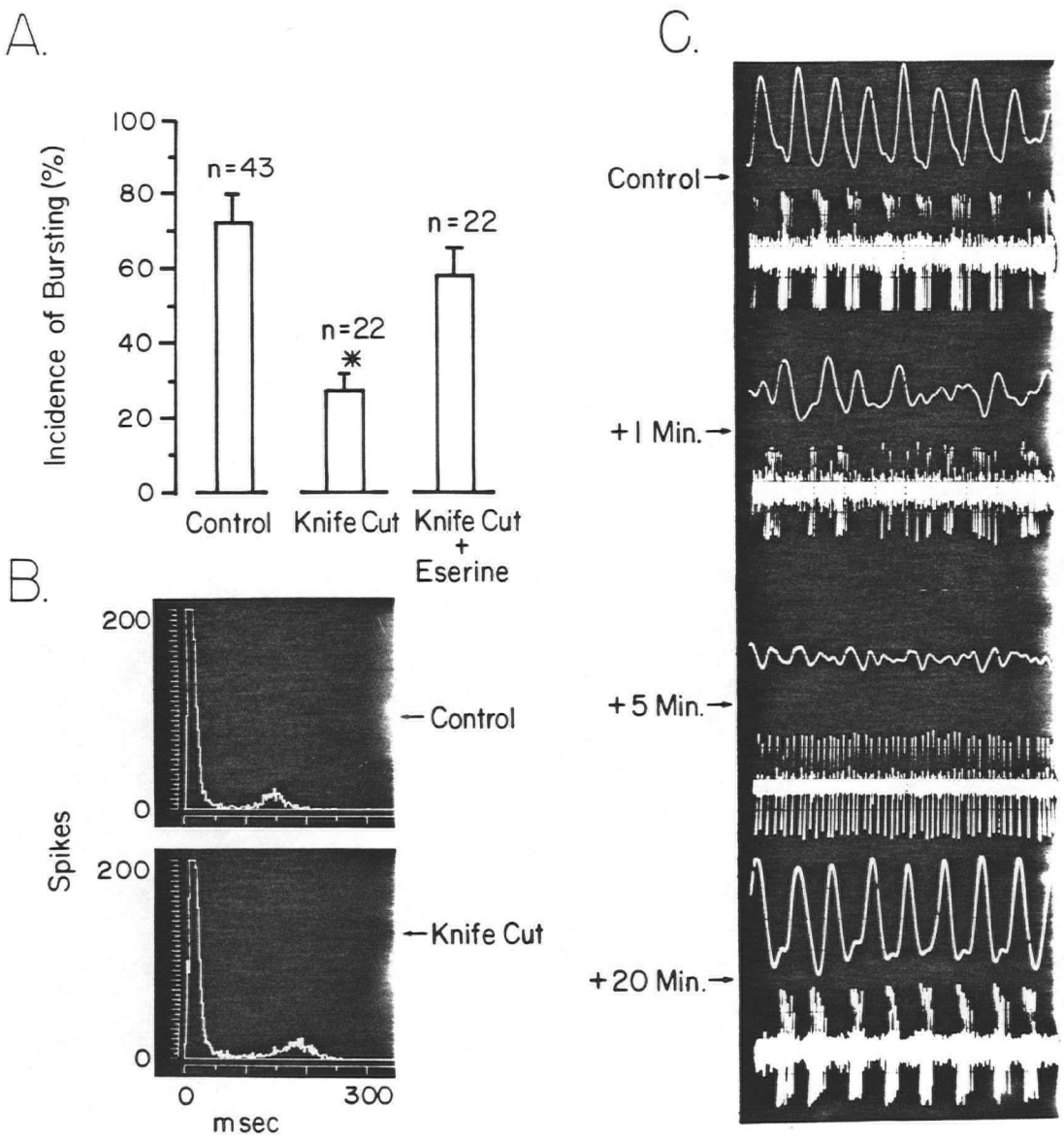
Since it has been shown that physostigmine (eserine) enhances the burst activity of septal neurones (Petsche et al, 1962; Assaf and Miller, unpublished results), the response of the 22 neurones in the isolated forebrain was recorded following an intravenous injection of physotygmine (0.1 mg/kg). As shown in Fig. 3-11A, 13 of the 22 neurones (59%) exihibited the bursting mode of discharge following the injection. The response to eserine had a latency of 90-100 seconds and was maximal 15-20 minutes post injection.

FIG. 3-11: Discharge Patterns Of Septal Neurones Before And After Transection Of The Forebrain

A: The number of medial septal neurones (expressed as a percentage of the total number of cells recorded) displaying the bursting mode of discharge.

B: Interval histogram of a neurone recorded before and after the knife cut. Bursting discharge as indicated by the bimodal distribution continued after the cut.

C: discharge pattern of a neurone (bottom sweeps) and a corresponding slow potentials recorded from the same electrode (top sweeps) before and at the indicated times after a transection of the forebrain. Eserine was injected 15 minutes after the transection to re-instate bursting discharge.


A.
Incidence of Bursting (%)
100
80
60
40
20
0
n=43
n=22
n=22
Control
Knife Cut
Knife Cut + Eserine
B.
Spikes
200
0
Control
200
0
Knife Cut
0
300
msec
C.
Control
+1 Min.
+5 Min.
+20 Min.

3.4 Discussion

These data indicate a relationship between the discharge pattern of septal neurones and electrical activity of the hippocampus in the urethane anaesthetized rat similar to that previously shown in the rabbit (Petsche et al, 1962; Apostol and Creutzfeldt, 1974). In addition, at least two cell populations in the medial septal region may be differentiated on the basis of their discharge patterns and whether or not they project to the hippocampus.

Discharge Pattern Of Septal Neurones

I-neurones, which are not antidromically activated by hippocampal stimulation, discharge in a random or irregular manner that is unrelated to hippocampal activity. In contrast B-neurones discharge in bursts which are phase related to electrical activity of the hippocampus and are antidromically activated by stimulation of sites shown to generate RSA. Since antidromic activation of B-neurones is followed by inhibition an axon collateral may be postulated to activate inhibitory interneurones or perhaps those cells presently classified as I-neurones. The observation that evoked discharges precede the inhibition of B-neurones and that a proportion of I-neurones are synaptically activated by hippocampal stimulation supports the latter suggestion.

Correlations With Morphological Studies

Studies of the histological organization of the medial septal region (Tombol and Petsche, 1969; Andy and Stephan, 1964) also indicate a heterogenous cell population consisting of two fundamentally different cell types (1) small ovoid neurones with profusely branching axon collaterals and (2) medium to large neurones whose axons leave the medial septum in a dorsal direction. Since the terminals of the small ovoid cells could be traced to the dendrites of the larger cell type, Tombol and Petsche (1969) suggest these neurones are intrinsic to the septal area. On the other hand the large neurones are presumably output cells projecting to hippocampal regions. The close relationship between the discharge of B-neurones and hippocampal RSA observed in this and other studies (Petsche, et al, 1962; Gogalak et al, 1962; Apostol and Creutzfeldt, 1974) together with the fact that only these cells could be antidromically activated by stimulation of RSA generating zones in the dentate gyrus suggests that these neurones correspond to the output cells described by Tombol and Petsche (1969). The irregular firing I-neurones may reflect activity in the intrinsic cell population.

Regulation Of Hippocampal Activity

Petsche et al (1962) emphasized the importance of the medial septal region as a relay station transforming influences of the brainstem into a discontinuous pulse train which initiates RSA in the hippocampus. The present data tends to support this suggestion since destruction of the septal area abolished rhythmical electrical activity in the hippocampus. The additional observation that kainate injections also block RSA suggests that intact septal cell bodies and not direct brainstem afferents to the hippocampus are absolutely necessary for the generation of rhythmical hippocampal activity.

Neurones in the midbrain and pons may synaptically relay bursting patterns to septal neurones. However, the observation that in the isolated forebrain preparation septal neurones continued to fire in rhythmical bursts suggests that mechanisms within the forebrain, possibly the septal area itself, are capable of generating 'theta' rhythm. Removal of tonic brainstem influences would explain the lower incidence of bursting discharge in the isolated forebrain and the lack of responses to peripheral stimulation. Recent observations that hippocampal RSA persists in cats with chronically isolated forebrains (Olmstead and Villablanca, 1977) provides further support for the proposal that generation of rhythmical activity is

dependent on forebrain mechanisms.

Role Of Cholinergic System.

The intravenous injection of the anticholine-esterase, physostigmine, elicited the bursting discharge pattern of B-neurones in intact (See also Petsche et al, 1962) and isolated forebrain preparations. Likewise, anticholinesterases have potent actions on the production of hippocampal RSA in intact, cerveau isole and isolated forebrain preparations (Bradley and Nicholson, 1962; Monnier and Romanowski, 1962; Olsmstead and Villablanca, 1977; Vanderwolf, 1975). Eserine could be potentiating the action of cholinergic afferents to the septal area or the activity of septal cholinergic neurones. The observation that following septal lesions eserine is ineffective in producing RSA (Torii,1966 Stumpf, 1965) suggests that the action of eserine is dependent on septal mechanisms. Since eserine potentiates the bursting of septal neurones in the isolated forebrain, it may be enhancing the activity of the collaterals of B-neurones onto neighbouring septal neurones. The additional observation that atropine blocks hippocampal RSA in urethane anaesthetized rats (Vanderwolf,1975; Assaf and Miller unpublished results; chapter 5) emphasizes the role of cholinergic systems in the production of rhythmical activity.

3.5 Summary

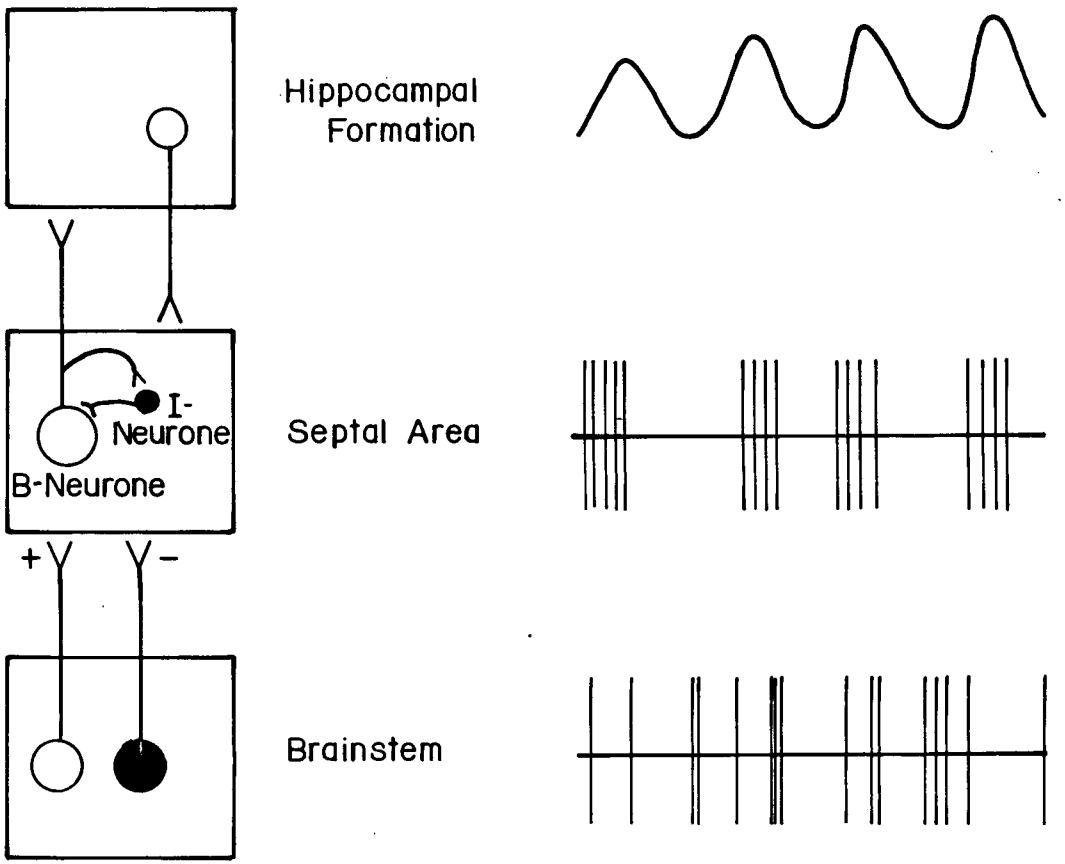
A summary of the proposed relationships between the septal area and the hippocampus based on the results of the present experiments is shown in Fig. 3-12. Stimulation of a RSA generating zone in the dentate gyrus antidromically activates only B-neurones indicating that these cells project to the hippocampus. Furthermore, it is the discharge of B-neurones but not I-neurones which shows a phase and frequency relationship to RSA waves. Since the antidromic activation of B-neurones is followed by inhibition, an axon collateral system may be postulated to activate inhibitory interneurones or perhaps those cells presently classified as I-neurones. Recent evidence that gamma-aminobutyric acid antagonists block the bursting of septal neurones (McLennan and Miller, 1974; Segal, 1976) and the inhibition of septal neurones elicited by hippocampal stimulation (McLennan and Miller, 1974a) suggests that this compound may be the inhibitory transmitter of the interneurones.

The observations that electrolytic or kainate induced lesions of the medial septal diagonal band area abolish hippocampal RSA suggests that the septal area is critical for the generation of rhythmical electrical activity in the hippocampus. Furthermore the bursting discharge pattern of septal neurones may be mediated by local forebrain mechanisms and is not synaptically

relayed by midbrain afferents.

FIG. 3-12: Schematic Illustration Of The Proposed Relationship Between The Brainstem, Septal Area And The Hippocampal Formation

The septal area transforms randomly arriving signals from the brainstem into rhythmical bursts which are relayed to the hippocampal formation by B-neurones and in turn generate RSA. Stimulation of the dentate antidromically activates B-neurones and subsequently inhibits their spontaneous discharge via recurrent collaterals onto interneurones in the septal area.


Hippocampal
Formation
I-
Neurone
B-Neurone
Septal Area
+
−
Brainstem

CHAPTER 4: ROLE OF A RAPHE-SEROTONIN SYSTEM IN THE CONTROL OF SEPTAL HIPPOCAMPAL ACTIVITY

4.1 Introduction

In the previous chapter it was shown that the septal area, particularly the medial region, plays a significant role in the control of hippocampal electrical activity. Earlier experiments have suggested that electrical stimulation of ascending systems from the hypothalamus and brainstem elicit contrasting patterns of hippocampal activity (Torii, 1961; Yokoto and Fujimori, 1964; Anchel and Lindsley, 1972; Macadar et al, 1974). In an attempt to localize the brainstem origins of these systems, Macadar et al (1974) demonstrated that electrical stimulation of anatomically distinct mesencephalic and pontine regions elicits the characteristic hippocampal patterns. Some of these regions correspond to histochemically identified monoamine-containing nuclei. Since recent neuroanatomical data have shown that these cell groups have extensive terminal projections upon the septum and hippocampus (see Chapter 1 for review), the possibility arises that these pharmacologically distinct systems may form the neural substates through which patterns of activity in the septum and hippocampus are controlled.

Of particular interest in this chapter is the serotonin fibre system whose cell bodies are located in

the mesencephalic raphe nuclei. The presence of a substantial projection originating in the median raphe nucleus (MR) and innervating the septum and hippocampus (Conrad,Leonard and Pfaff, 1974; Halaris et al, 1976) together with the observations that lesions of the MR result in a significant depletion of forebrain serotonin (Lorens and Guldberg, 1974) suggest that a serotonergic innervation of the septum may be involved in the control of septal-hippocampal function.

The aim of the present chapter is to 1) examine the effects of electrical stimulation of the MR on neuronal discharges in the septal area and related hippocampal activity and 2) determine whether the observed effects are dependent on serotonin containing systems originating in the MR.

4.2 Experimental Procedures

Experiments were performed on 54 rats anaesthetized with urethane (1.0g/kg i.p.). Surgical procedures and recording electrode placements in the hippocampus and the septal area were identical to those described in the previous chapter. In addition, a concentric bipolar electrode was stereotaxically lowered into the region of the median raphe nucleus (0.8 mm anterior to stereotaxic zero; 0.0-0.5 mm lateral to the midline; 6.0-6.7 mm below the surface of the cortex). Electrical stimulation of the MR consisted

of either single pulse of 0.05-0.1 msec duration and 10-20 msec duration and 10-20 V intensity or repetitive volleys of 40-100 Hz for 1-3 sec at intensities of 1-9V.

The signals recorded were filtered (0.1-3 KHz for septal unit activity and 2-50 Hz for hippocampal activity) and displayed on a dual beam oscilloscope and analysed using the method outlined in Chapter 2. Special care had to be taken to ensure that the discriminator window eliminated the contribution of stimulus artifacts in the analysis of septal unit discharge during repetitive pulse stimulation. In order to deplete 5-HT, a total of 6 rats were pretreated by para-chlorophenylalanine (p-CPA). An additional evaluation of the role of serotonergic mechanisms was made following the intravenous injection of the 5-HT precursor 5-hydroxytryptophan (5-HTP) 5-60 mg/kg and the presumed agonist quipazine (0.5-2.0 mg/kg; 2-(1-piperazinyl)-quinoline supplied by Pfizer Inc.). This compound was previously shown to activate central serotonin-containing systems (Rodrigues, Rojas-Ramirez and Drucker-Colin, 1973).

4.3 Results

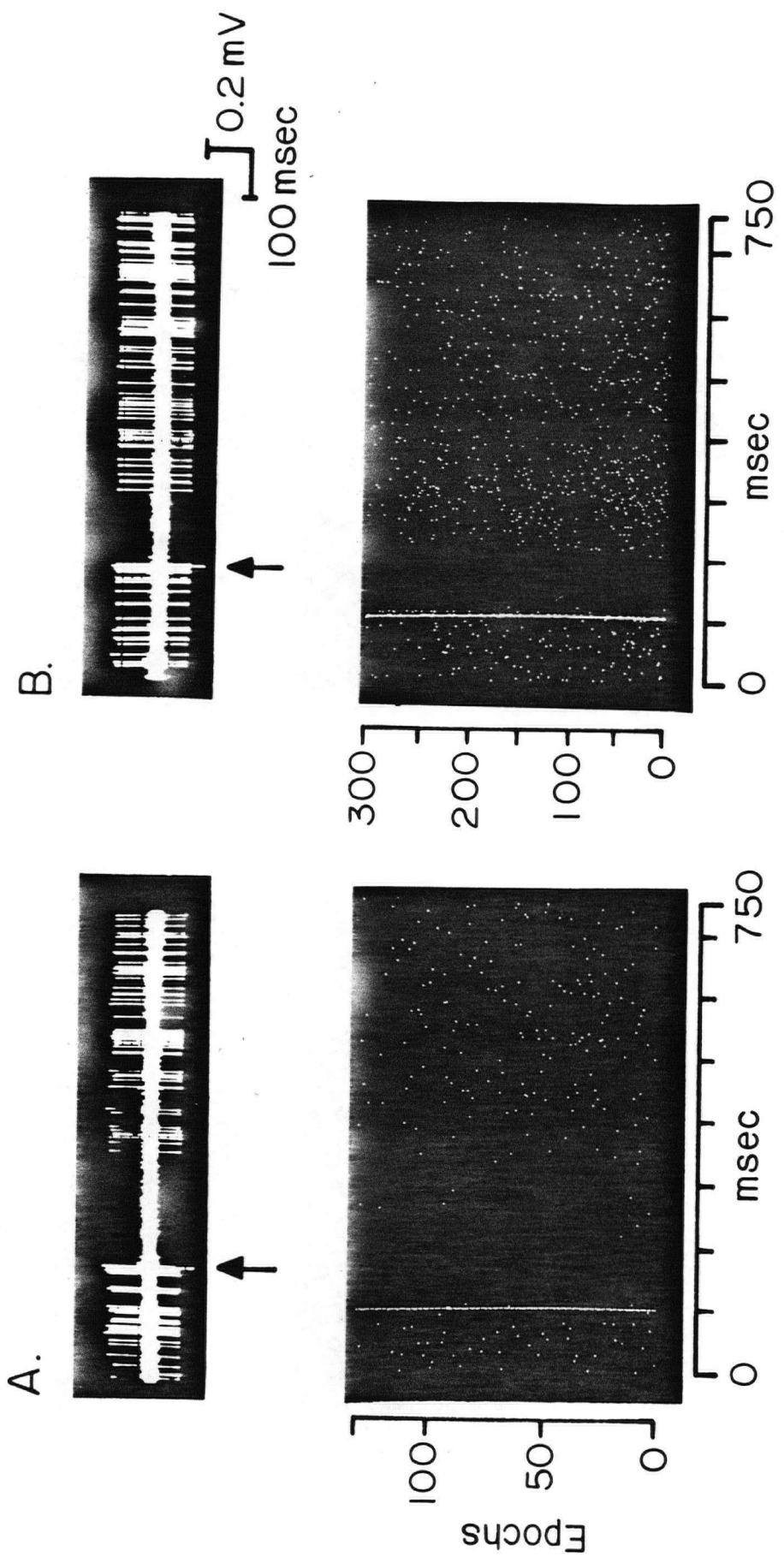
Since in the previous chapter it was shown that at least two populations of septal neurones can be classified on the basis of their discharge pattern and its relationship to hippocampal RSA, the effects of MR stimulation were analyzed separately for I-neurones and B-neurones.

Response Of I-Neurones To Raphe Stimulation

The discharge pattern of a total of 202 I-neurones were examined following single pulse stimulation of the MR at stimulus intensities ranging between 10-15 volts. One hundred and fifty-nine cells (78.7%) were inhibited for periods of 30-200 msec(mean=75.2±40.5 S.D.). The duration of the inhibition could be increased considerably if the MR was stimulated with two or more successive pulses (100 Hz) at threshold intensity or single pulses at suprathreshold (1.5 x T) intensity. The latency to the onset of the observed inhibition was always less than 25 msec following the stimulus (mean=12.5 msec) and in some cases the inhibition began immediately after the stimulus artifact (e.g. cell on the right in Fig. 4-1). Histological verification of stimulation sites which elicited the inhibitory response indicated that they were localized to the MR (Fig. 4-2B). Electrode sites in the region of the medial longitudinal fasciculus and the dorsal raphe

FIG. 4- 1: Response Of Septal I-neurones To Raphe Stimulation.

A,B: response of two neurones recorded in the medial septal nucleus of the same animal. The top records show 25 superimposed oscilloscope sweeps and bottom records are rastered PST responses of the corresponding cells. Note that in A the onset of inhibition is immediately after the stimulus artifact (vertical lines in the rasters) while in B the onset latency is longer.


A.
B.
0.2 mV
100 msec
300
200
100
0
0
750
msec
100
50
0
Epochs
0
750
msec

nucleus were either ineffective or sometimes elicited short latency (3-8 msec) spike activation with a small amplitude field response. When the same stimulating electrode was lowered into the MR proper, septal neurones previously unaffected were then inhibited (Fig. 4-2 A). When the stimulating electrode was located on the perimeter of the MR, a mixture of both activation and inhibition sequences was observed. The threshold for the inhibitory component of these responses was lower than that for activation and was related to the distance between the stimulating electrode and the centre of the MR .

The effects of stimulating the region of the dentate gyrus and CA3 area of the hippocampus were examined on a total of 96 I-neurones that were inhibited by MR stimulation. Some neurones (41.7%) were inhibited by both MR and hippocampal stimulation, an approximately equal proportion (35.5%) were inhibited by the MR and activated by stimulation of the hippocampus, and the remainder (20.8%) were not influenced by hippocampal stimulation.

FIG. 4- 2: Localization Of Stimulating Electrodes In The Region Of The Raphe And The Corresponding Response Of I-neurones.

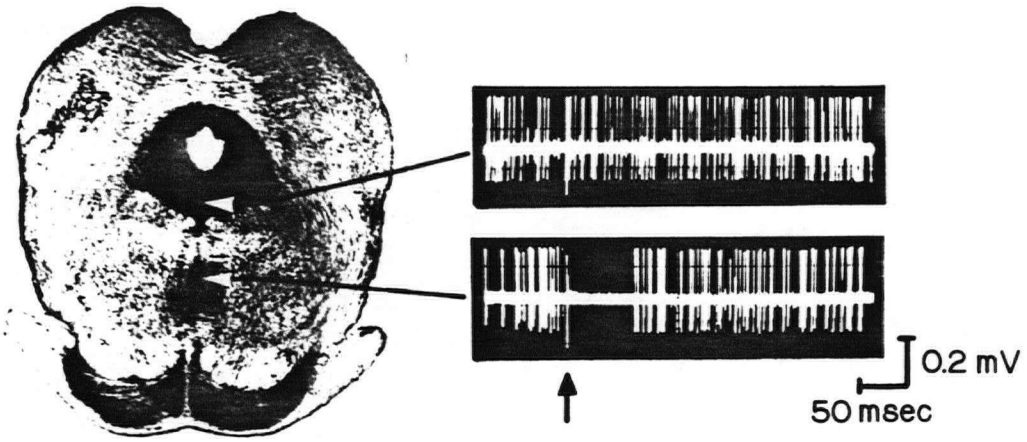
A: photomicrograph showing small iron deposits made at the site of an ineffective placement in the dorsal raphe (top arrow) and a placement in the median raphe that produced inhibition (lower arrow).

B: frontal sections through the raphe nucleus indicating the distribution of stimulating sites and their effects on septal unit activity: (0) ineffective, (+) activation, (-) inhibition.

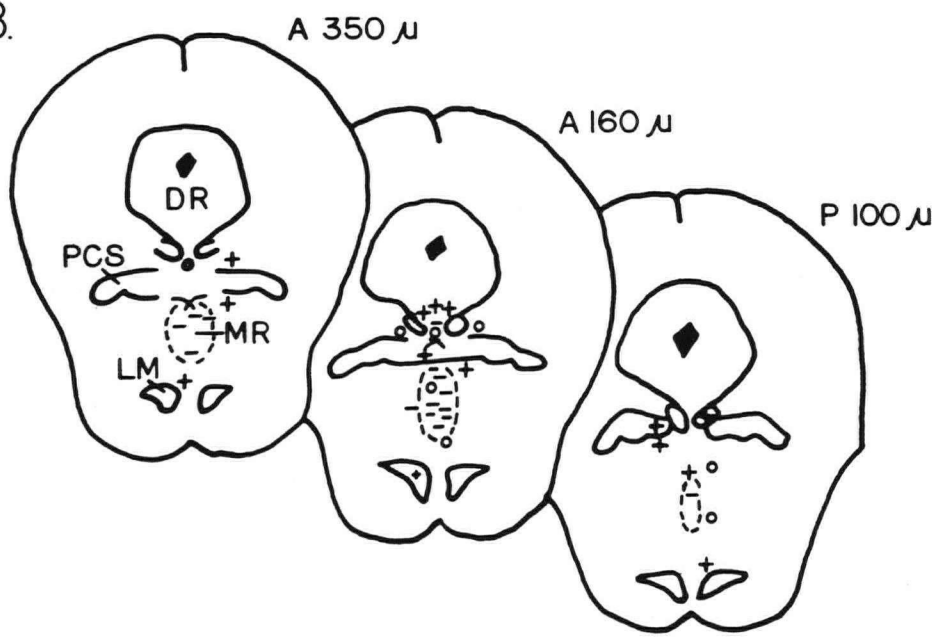
ABBREVIATIONS:

DR	Dorsal Raphe Nucleus
LM	Medial Lemniscus
MR	Median Raphe Nucleus
PCS	Superior Cerebellar Peduncle

A.



B.



Response Of B-neurones To Raphe Stimulation

In contrast to the inhibitory responses described above, single pulse stimulation of MR rarely influenced the discharge of identified B-neurones (Fig. 4-3). Inhibition was observed in only 5 of 229 B-neurones recorded during the irregular or bursting mode of discharge. Increasing the stimulus intensity (20-35 V) did not alter the proportion of B-neurones influenced. Likewise, double pulse stimulation of the MR did not alter the proportion of B-neurones that were inhibited. However, repetetive stimulation of the MR at low intensity (2-9 V) and frequencies of 40-100 Hz resulted in the disruption of bursting which was replaced by an irregular firing pattern (Fig. 4-4B). Of 220 B-neurones recorded during the bursting mode, 185 (84.1%) began to fire in an irregular pattern. In the majority of cases this response did not outlast the period of stimulation, however, some neurones (4 of 220) continued to fire in an irregular manner after the termination of MR stimulation (e.g. cell in Fig. 4-4 C). The average discharge rate during the repetitive train was not consistently altered in that a given neurone may fire with an increased or a decreased frequency during subsequent stimulus trains. The response of B-neurones described above was unique to stimulation of MR nucleus since stimulation of adjacent areas did not result in disruption of burst activity (Fig. 4-5) and sometimes changed the irregular

FIG. 4- 3: Characteristics Of A B-neurone During Single Pulse Stimulation Of MR.

A: rastered display of 60 successive stimulus presentations during both non-bursting (epochs 1-25) and bursting (epochs 26-60) modes of discharge.

B,C: single oscilloscope sweeps showing the relationship of the same unit (lower traces) to hippocampal electrical activity (top traces).

Epochs
60
0
A.
0
msec
1000
B.
C.
0.5 mV
0.2 mV

discharge of B-neurones into rhythmical bursts. The rhythmical bursts often outlasted the period of stimulation, persisting until the start of the next trial. Stimulation of sites such as the medial longitudinal fasciculus or the superior cerebral peduncle also activated I-neurones which were recorded in the medial septal area (Fig. 4-2 and 4-5).

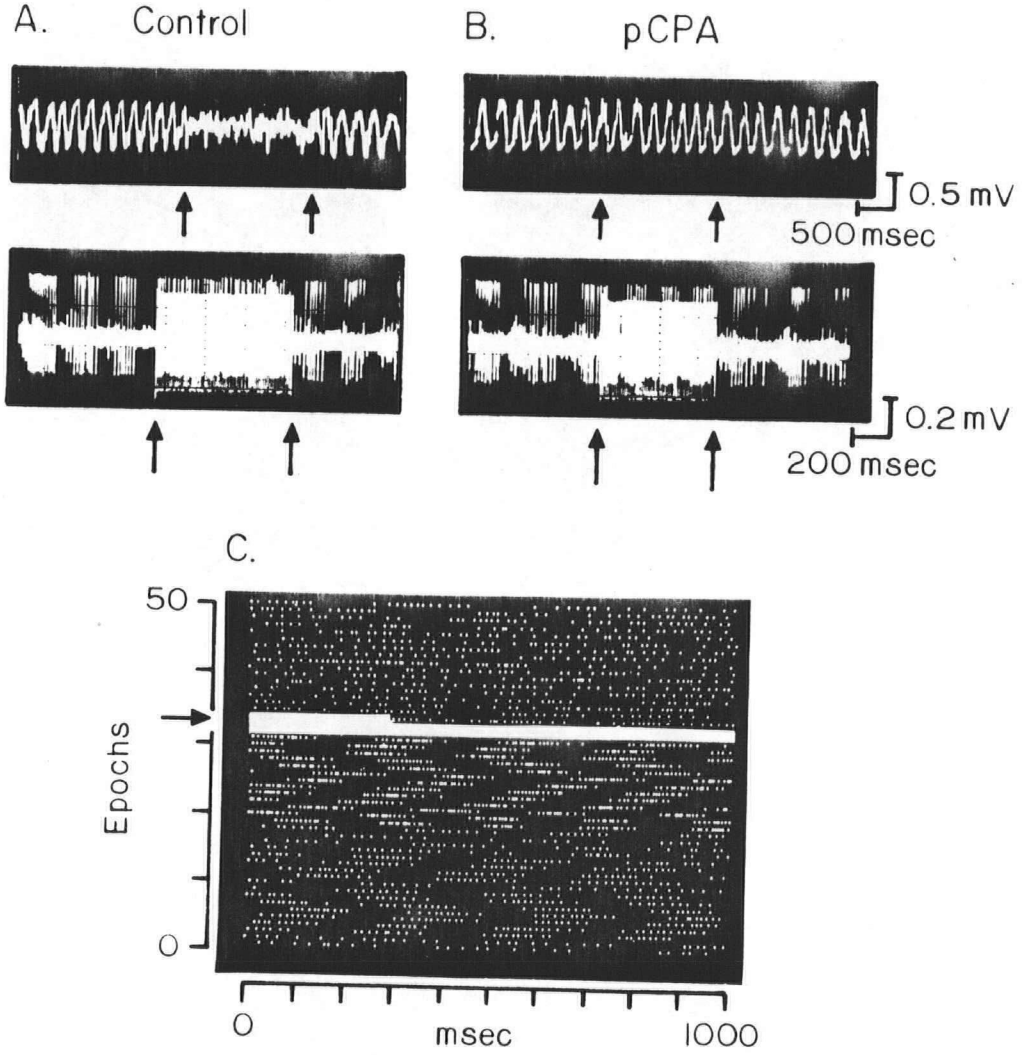
Effect Of Raphe Stimulation On Hippocampal Electrical Activity

The spontaneous electrical activity of the hippocampus was recorded simultaneously with the discharge of septal neurones. As shown in chapter 3, the bursting pattern of B-neurones was accompanied by RSA. Single pulse stimulation of MR which did not influence B-neurones also did not elicit any detectable changes in ongoing electrical activity recorded from CA1, or the dentate gyrus. However, repetitive stimulation of MR caused a shift from ongoing RSA to a fast low voltage desynchronized hippocampal activity (Fig. 4-4A, 4-6C,D). The disruption of B-neurone bursting and desynchronization of electrical activity were observed within 200 msec (average burst interval) of the stimulus presentation. However, it was not possible to determine which occurred first. It is important to note that low voltage (less than 5.0 V) stimulation of MR results in hippocampal desynchronization independent of the mode of the

FIG. 4- 4: Effects Of Repetitive MR Stimulation On The Bursting Discharge Pattern Of B-neuronesin Control And p-CPAtreated Rats.

A,B: top traces are hippocampal electrical activity and lower traces are septal unit discharges. MR stimulation occurs between the two arrows and the stimulus artifacts obscure a portion of the spikes in the lower traces. In (A) repetitive stimulation (3 V, 100 Hz, 600 msec) results in a desynchronization of the hippocampal activity and blockade of the cellular bursting pattern. In (B) the MR stimulation is ineffective.

C: a rastered display of the discharge pattern of a B-neurone recorded in a normal rat before and after a train of stimuli (3 V, 100 Hz, 3.2 sec) indicated by the solid white bar. Note that, in contrast to response in (A), the irregular discharge pattern elicited by MR outlasts the stimulus train.


A.
Control
B.
pCPA
0.5 mV
500 msec
0.2 mV
200 msec
C.
50
Epochs
0
0
msec
1000

preceding spontaneously occurring electrical activity. Stimulation of adjacent areas and sometimes the perimeter of MR resulted in RSA at low stimulus intensity, but at higher intensities (5-7 V) a desynchronization synchronization sequence occurred.

Effects Of P-chlorophenylanine On Septal-Hippocampal Activity

Pretreatment with p-chlorophenylanine (p-CPA) 3 days prior to electrophysiological experiments resulted in 60-80% depletion of forebrain 5-HT. In the treated rats (n=4) only 7 of 24 (29.2%) I-neurones examined were inhibited by MR stimulation, compared to 159 of 202 (78.7%) in control animals (Table 1). The p-CPA pretreatment did not abolish the short latency excitatory responses elicited from stimulation points in the vicinity of MR. Of 46 B-neurones recorded in treated rats only 9 (19.6%) were influenced by repetitive pulse stimulation of the MR at the same intensities (2-5v) which resulted in disruption of bursting discharge in the majority (84.1%) of B-neurones recorded in controls (Table 1). As indicated by the binomial distribution, both the occurrence of inhibition of I-neurones ($p<0.001$) and disruption of the bursting of B-neurones ($p<0.05$) were significantly lower than those observed in control rats.

As shown in Fig. 4-5, p-CPA pretreatment did not

TABLE 1

COMPARISON OF SEPTAL NEURONES INFLUENCED BY MR STIMULATION IN CONTROL AND p-CPA TREATED RATS

CELL TYPE	CONTROL (n=29)	p-CPA (n=4)
I-NEURONES	159/202 (78.7%)	7/24 (29.2%)
B-NEURONES	185/229 (84.1%)	9/46 (19.6%)

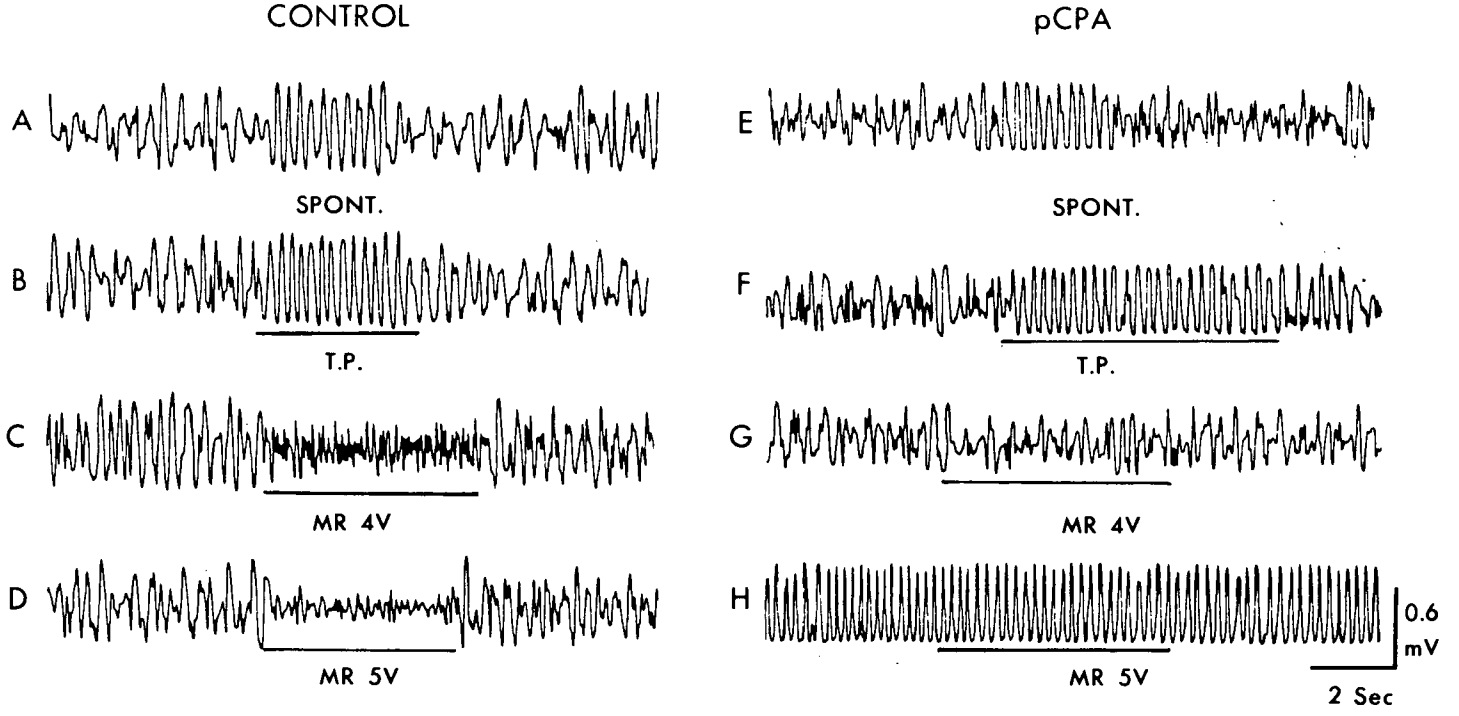
p-CPA (400 mg/kg) was administered intragastrically 3 days before recording experiments. Fluoremetric assays for forebrain serotonin levels were performed immediately following the recording sessions. Entries indicate number of cells influenced/number of cells tested (and percentages of total).

FIG. 4- 5: Effects Of Repetitive MR Stimulation On Hippocampal Electrical Activity In Control And pCPA Treated Rats.

A,E: spontaneously occurring electrical activity showing that RSA is recorded in both control and pCPA treated rats.

B,F: RSA recorded following a tail pinch (T.P.).

C,G,D,H: effects of 4 and 5 volt stimulation (100 Hz) of the MR on hippocampal electrical activity indicating that pCPA eliminates the MR evoked desynchronization.


CONTROL
pCPA
A
SPONT.
E
SPONT.
B
T.P.
F
T.P.
C
MR 4V
G
MR 4V
D
MR 5V
H
MR 5V
0.6
mV
2 Sec

significantly alter the spontaneous electrical activity of urethane anaesthetized rats. As in control rats RSA occurred spontaneously or as a result of somatosensory stimulation such as tail pinch (t.p. in Fig. 4-5). However, MR stimulation did not result in the low voltage desynchronized activity which was observed in control rats. In addition MR stimulation in p-CPA treated rats did not abolish ongoing RSA (Fig.4-5). In a separate group of 9 rats which received a central injection of the catecholamine-specific neurotoxin, 6-hydroxydopamine, MR stimulation resulted in desynchronization of RSA suggesting that the effects produced by p-CPA were due to the loss of 5-HT and not noradrenaline or dopamine.

Effects Of Quipazine On Septal-Hippocampal Activity.

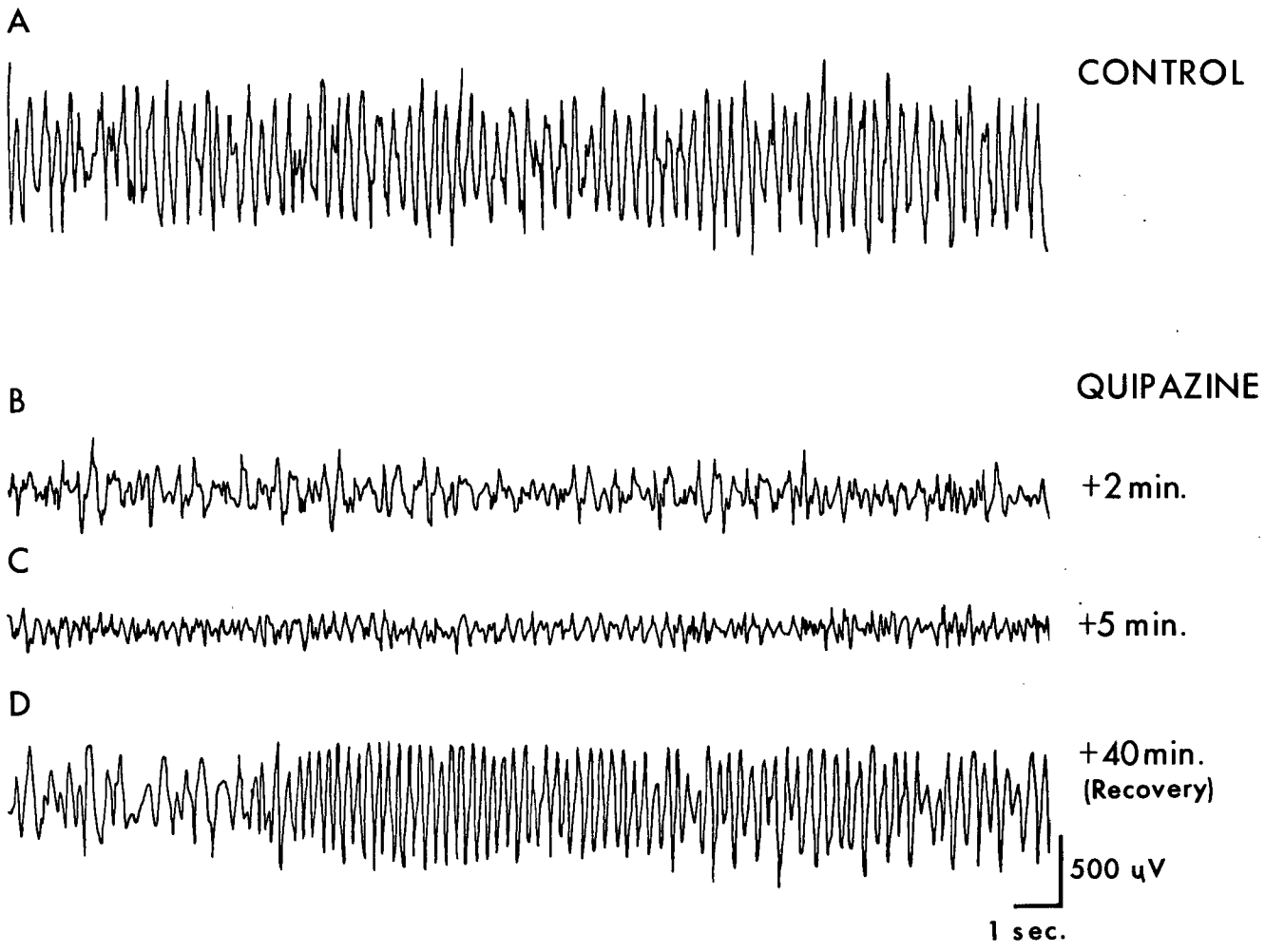
Six rats received an intravenous injection of quipazine (0.5-2.0 mg/kg) in saline solution. In 4 of them 0.6-1.0 mg/kg reliably resulted in disruption of spontaneously occurring RSA and a shift of hippocampal activity to low voltage desynchronization (Fig. 4-6). In the remaining 2 rats a dose of 2.0 mg/kg was necessary to produce any effects. The onset of desynchronization occurred 2-4 minutes following the injection and persisted for 1-3 hours. Full recovery of RSA was demonstrated in 3 rats which were studied for up 6 hours following the injection. In all animals sensory-evoked RSA recovered before the appearance of

FIG. 4- 6: Effects Of Quipazine On Hippocampal Electrical Activity

A: spontaneous occurring activity prior to quipazine injection.

B,C: activity recorded following the intravenous injection of quipazine (1.0 mg/Kg).

D: recovery of RSA recorded 40 minutes following the injecticn.


A
CONTROL
QUIPAZINE
B
+2 min.
C
+5 min.
D
+40 min.
(Recovery)
500 µV
1 sec.

spontaneously occurring RSA. An injection of the saline vehicle did not procduce any effects similar to those of quipazine and a subsequent injection of eserine through the same cannula resulted in the appearance of RSA. The latter injection verifies that quipazine does not irreversibly damage mechanisms which mediate RSA.

The effects of systemic injections of quipazine on the discharge pattern of 4 MS neurones (2 I-neurones and 2 B-neurones) were examined. Quipazine significantly lowered the spontaneous discharge of the I-neurones and attenuated the burst activity of B-neurones. The effect of quipazine on all septal units was overcome by somatosensory stimulation.

Effects Of 5-hydroxytryptophan On Hippocampal Electrical Activity

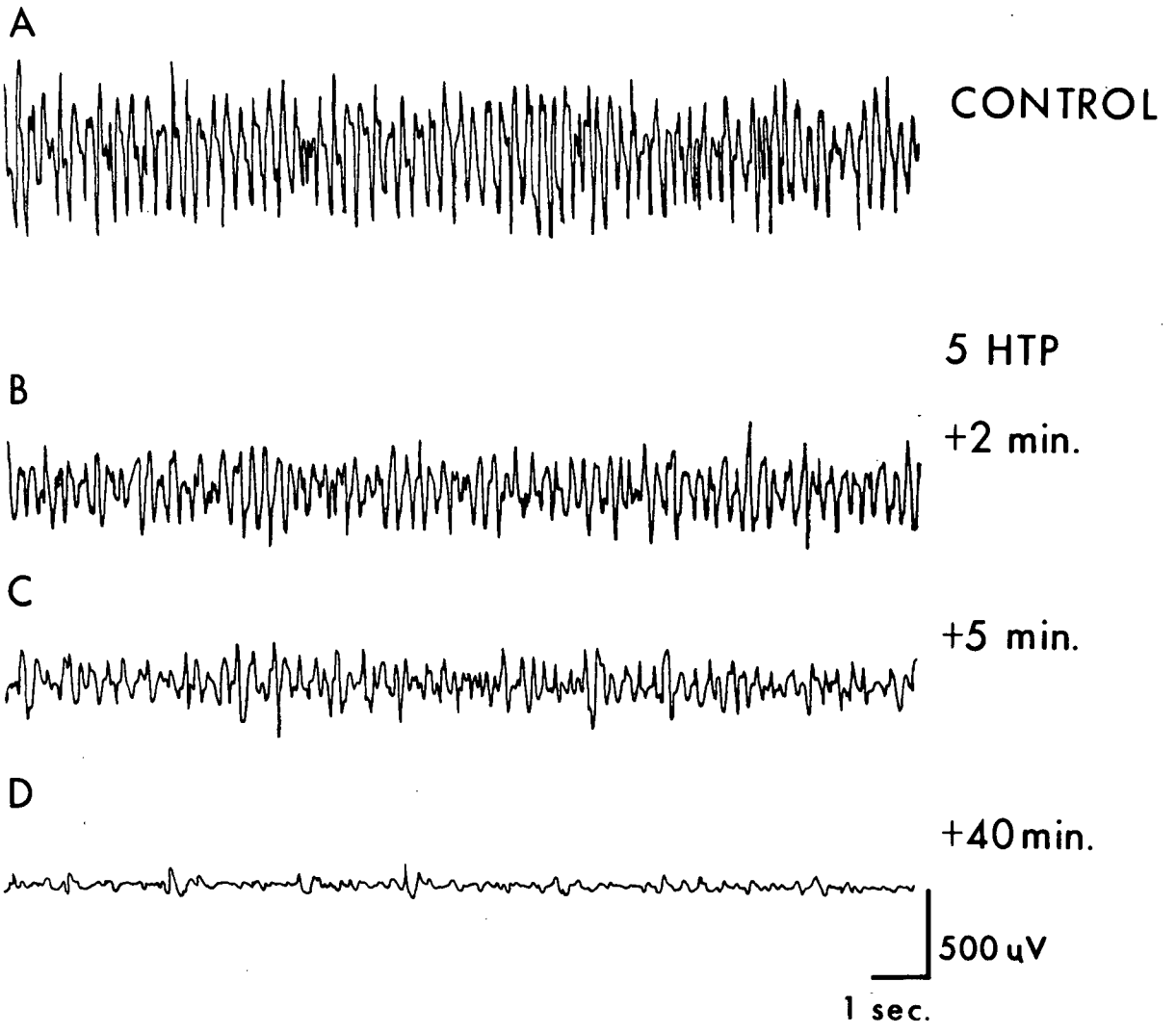
Some rats (n=5) received an intravenous injection of the serotonin precursor 5-HTP (5-80 mg/kg) which, unlike 5-HT, passes the blood-brain barrier (Domer and Longo, 1962). Doses lower than 20 mg/kg did not result in any detectable change in the spontaneously occurring or evoked patterns of hippocampal electrical electrical activity. Higher doses (40-80 mg/kg) produced a gradual desynchronization which occurred 5-7 min following the start of the injection. At 15-20 minutes post injection the amplitude of the electrical activity was greatly reduced (Fig. 5-8E). Recovery to the normal amplitude

FIG. 4-7: Effects Of 5-HT On Hippocampal Electrical Activity.

A: spontaneously occurring activity recorded from the dentate gyrus before injection.

B,C: records obtained 2-5 minutes after the injection of 5-HTP (60 mg/Kg).

D: low voltage activity recorded 40 minutes following the injection and persisting until the animal's death at 55 minutes post-injection.


A
CONTROL
5 HTP
B
+2 min.
C
+5 min.
D
+40 min.
500 uV
1 sec.

during the remainder of the recording session was not observed. Three of the four animals that received the higher doses (i.e. >40 mg/kg) of 5-HTP died within 30-180 minutes post injection.

4.4 Discussion

These data suggest that electrical stimulation of the MR results in inhibition of irregularly firing septal neurones (I-neurones), disruption of rhythmical activity of bursting septal neurones (B-neurones) and desynchronization of electrical activity of the hippocampus.

Effect Of MR Stimulation On I-neurones.

Single pulse stimulation resulted in short latency (mean=12.5 msec) inhibition of I-neurones and considering the distance between stimulating and recording sites (8-10 mm) allows for a conduction velocity of between 0.5 and 1.5 m/s. These velocities are compatible with previously reported conduction velocities of unmyelinated 5-HT fibres (Wang and Aghajanian,1977). The relative constancy of the onset of inhibition together with anatomical evidence for a direct projection from the MR to the medial septum (Conrad, Leonard and Pfaff, 1974; Halaris et al., 1976) suggests that this response is mediated by a monosynaptic input directly onto I-neurones. The

absence of clear inhibitory responses following stimulation of loci outside the MR indicates that the observed inhibition is not mediated by an extra-raphe system.

Effect Of MR Stimulation On B-neurones

In contrast to the response of I-neurones, B-neurones were unaffected by single pulse stimulation of the MR regardless of whether they were in bursting or non-bursting mode of discharge (See Fig. 4-3). However, the observation that repetitive stimulation disrupts the bursting of B-neurones without significantly altering their firing rate indicates a more complex relationship between the MR and those neurones than that suggested for I-neurones. Repetitive stimuli may temporally summate to inhibit local circuits, involving septal interneurones (Tombol and Petsche, 1968; McLennan and Miller, 1974a) or neurones presently classified as I-neurones. It is interesting that some septal neurones that are monosynaptically activated by stimulation of RSA generating zones in the hippocampus are I-neurones which are inhibited by MR stimulation. It is conceivable that a proportion of I-neurones are septal interneurones that are excited by axon collaterals of B-neurones which project to the hippocampus. An alternate explanation for the effectiveness of repetitve stimulation is that mechanisms within the MR itself may be initiated only

by repetitive stimulation. However, this is unlikely since single pulse stimulation of the MR results in inhibition of I-neurones and alters neuronal transmission in the dentate gyrus (See Chapter 8).

Regulation Of Hippocampal Activity

Chapter 3 has emphasized the importance of the medial septal region as a relay station transforming influences of the brainstem into a discontinuous pulse train which initiates hippocampal RSA. The results of the present chapter show that a system originating in the MR is capable of disrupting the bursting response of septal neurones which in turn results in a desynchronized hippocampal activity. Wilson et al (1975) demonstrated a similar effect on septal neurones and hippocampal activity following stimulation of the lateral hypothalmus. Since projections from the MR to the medial septum are known to course through the medial forebrain bundle, it is possible that desynchronization observed by these authors was due to the activation of fibres of passage of neurones originating in the MR .

Alternate mechanisms mediating a desynchronization of hippocampal activity have been proposed. Anchel and Lindsley (1972) observed that lesions of the fornix blocked synchronization but not the desynchronization elicited from hypothalamic sites thereby concluding

that the latter effect was mediated by an extra-septal system. McLennan and Miller (1976) proposed a frequency gating mechanism in the lateral septum which controls the discharge pattern of medial septal neurones such that either bursting or irregular firing will result depending on the level of hippocampal output mediated by the fimbrial pathway. At the present time the mechanisms regulating the activity of septal neurones and patterns of hippocampal activity have not been sufficiently elucidated to eliminate any of the above proposals.

Possible Role Of Serotonin

The present results suggest that a serotonergic system mediates the responses elicited by MR stimulation. This suggestion is based on the observation that p-CPA pretreatment which lowers forebrain 5-HT blocks the inhibition of I-neurones elicited by MR stimulation. In addition, repetitive stimulation of MR in p-CPA treated rats does not disrupt the bursting of B-neurones or desynchronize hippocampal activity. The additional observations that quipazine, a presumed agonist of serotonin(Rodriques et al, 1973) desynchronizes hippocampal activity strengthens the proposal that a serotonergic system mediates the observed desynchronization.

The observation that the effects of quipazine are

reversible inasmuch as RSA can be subsequently produced by eserine injection or somatosensory stimulation suggests that desynchronization is not due to nonspecific and permanent destruction of RSA generators. Likewise the observation that p-CPA does not abolish RSA indicates an intact septal-hippocampal axis. The proposal that serotonin is not essential for the generation of RSA is also supported by the failure to block RSA in freely moving animals following the systemic injection of the 5-HT antagonist methysergide (Robinson, 1978).

The results following the intravenous injection of 5-HTP were not as clear cut as the desynchronization produced by quipazine injection. Although desynchronization of hippocampal electrical activity was observed following the injection of 5-HTP, very large doses were required to produce reliable effects. Similar observations were previously made by Domer and Longo (1962) in the rabbit. Thus, it is possible that these doses required to elicit desynchronization produced systemic effects which ultimately kill the animals.

Previous studies have also implicated a serotonergic system in the control of septal unit activity. Stefanis (1964) reported that iontophoretic application of 5-HT inhibits the discharge of septal neurones. More recently, Segal(1976) observed that

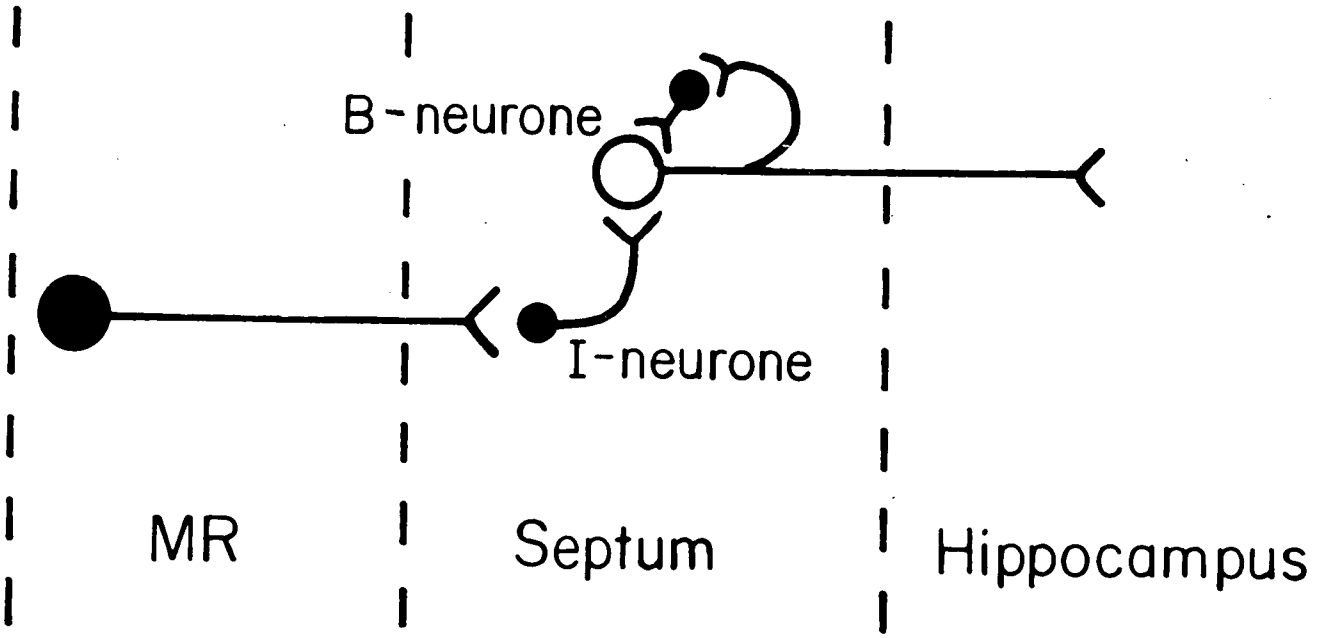
raphe stimulation results in a long latency (20-50 msec) inhibition of septal neurones. In the present experiments no inhibition with latencies longer than 25 msec was observed. Although it is difficult to account for this discrepancy between the latencies to inhibition, the fact that in both instances p-CPA pretreatment blocked the inhibition suggests that these effects were mediated by serotonin. The observation that stimuli of low intensity inhibited I-neurones only if the stimulating electrode was in the centre of the MR provides further support for the suggestion that the observed inhibition is mediated by a serotonergic system originating in the MR . Furthermore, the preliminary observation that systemic injection of quipazine decreases the firing rate of I-neurones and attenuates the bursting of B-neurones also implicate 5-HT in the control of septal unit activity. However, these effects can be more directly evaluated by the iontophoretic application of quipazine and 5-HT itself on identified septal neurones.

4.5 Summary

A model of the proposed relationship between MR, septal area and the hippocampus based on the results of this and the previous chapter is shown in Fig. 4-8. Single pulse stimulation of MR causes direct inhibition of non bursting I-neurones without inhibiting B-neurones suggesting that only I-neurones receive a monosynaptic input from MR. The desynchronization of the bursting of B-neurones and RSA may be mediated by temporal summation of inhibited I-neurones. Since B-neurones are antidromically activated by stimulation of RSA generating zones they project to the hippocampal formation. The subsequent inhibition of B-neurones suggests that they give off axon collaterals to neighbouring inhibitory interneurones. The observation that some I-neurones which are inhibited by MR are synaptically activated by hippocampal stimulation suggests that a population of I-neurones may in fact be the inhibitory interneurones. Recent evidence that antagonism of the inhibitory transmitter gamma-aminobutyric acid dissrupts the bursting of MS neurones (McLennan and Miller, 1974a) emphasizes the importance of inhibitory mechanisms in the generation of rhythmicity. Manipulations that initiate RSA including somatosensory or fimbrial stimulation (McLennan and Miller, 1974a) may provide a net excitatory drive onto I-neurones.

FIG. 4- 8: A Schematic Illustration Of The Proposed Synaptic Arrangements Between The MR, Septum, And Hippocampal Formation

MR neurones directly inhibit septal I-neurones which in turn may inhibit B-neurones. Stimulation of the dentate gyrus antidromically activates B-neurones and inhibits their spontaneous discharge via recurrent collaterals innervating interneurones in the septum. Black circles represent inhibition, white circles excitation.


B-neurone
I-neurone
MR
Septum
Hippocampus

CHAPTER 5: THE DORSAL NORADRENERGIC SYSTEM AND HIPPOCAMPAL ELECTRICAL ACTIVITY

5.1 Introduction

Previous studies (reviewed in chapter 1) have shown that stimulation of diffuse brainstem regions elicits rhythmical electrical activity in the hippocampus. Further characterization of these ascending pathways and the localization of these cells of origin is essential for understanding the mechanisms which underlie hippocampal and cortical activation.

The observation that electrical stimulation in the vicinity of the locus coeruleus, the site of origin of noradrenaline containing neurones which innervate the forebrain , elicits RSA (Macadar et al, 1974) suggests that this pharmacologically distinct system may initiate the generation of rhythmical activity in the septum and hippocampus. This suggestion is supported, in part, by the observations that amphetamine injections reliably elicit RSA (Vanderwolf, 1975) and that electrolytic lesions of the locus coeruleus result in a decrease in electrocortical arousal (Jones et al, 1973). However, a detailed analysis of the role of this pathway in the control of rhythmical hippocampal activity has not yet been carried out.

The aim of the present chapter is to 1) test the effects of electrical stimulation of LC on hippocampal

electrical activity, 2) determine whether these effects are dependent on the dorsal noradrenergic pathway and 3) determine whether this pathway mediates the well known effects of amphetamine on hippocampal electrical activity.

5.2 Experimental Procedures

Electrical stimulation: Bipolar stimulating electrodes were stereotaxically positioned in the vicinity of the locus coeruleus (1.5 mm posterior to stereotaxic zero; 0.9-1.3 mm lateral; 5.5-7.0 mm below cortical surface) in urethane anaesthetized rats. Stainless steel recording electrodes were lowered into the CA1 pyramidal cell layer or the upper blade of the dentate gyrus to a position which reliably produced spontaneous or sensory evoked RSA. The LC was then stimulated with a 3-10 second train of pulses (0.1 msec duration, 1-5 V) at a frequency of 10-300 Hz and the effects on hippocampal electrical activity were determined. The positions of the stimulating and recording electrodes were marked by producing a small lesion for subsequent histological verification.

Lesion experiments: In order to examine if the observed responses were mediated by the ascending NA projection, 9 rats received a bilateral injection of 6-hydroxydopamine(6-OHDA, 6 ug) directly into the dorsal NA bundle (AP=+2.6; L=+1.1; V=-4.5). In addition the LC

of 5 rats was electrolytically lesioned bilaterally using 1.0-1.5mA anodal current for 10-20 seconds. At least 5 days were allowed for recovery after which the rats were anaesthetized with urethane for recording experiments. The occurrence of RSA in control and lesioned rats was noted during spontaneous conditions and following peripheral sensory stimulation, intravenous injection of amphetamine (0.5-2.0 mg/kg), eserine (0.08-0.1 mg/kg) and atropine sulphate (1-5 mg/kg).

5.3 Results

Spontaneous And Sensory Evoked Patterns Of Electrical Activity.

The spontaneous electrical activity of the hippocampus in rats with electrolytic lesion of the LC or 6-OHDA lesion of the dorsal NA bundlewas not different from that recorded in control rats. A mixture of fast and slow waves with the periodic appearance of RSA was recorded in all three groups (Fig. 5-1). The frequency of RSA following sensory stimulation in control (mean=5.6±0.2) rats did not differ from that recorded in electrolytic (mean=5.3±0.4) or 6-OHDA treated (mean=5.3±0.2,) rats (Fig. 5-2). In addition there were no detectable differences in the amplitude or shape of spontaneously occurring and sensory-evoked RSA.

FIG. 5- 1: Rhythmical Electrical Activity Recorded In The Dentate Gyrus Of Control And Treated Rats.

RSA was elicited in each animal by a tail pinch during the period indicated by the solid line.

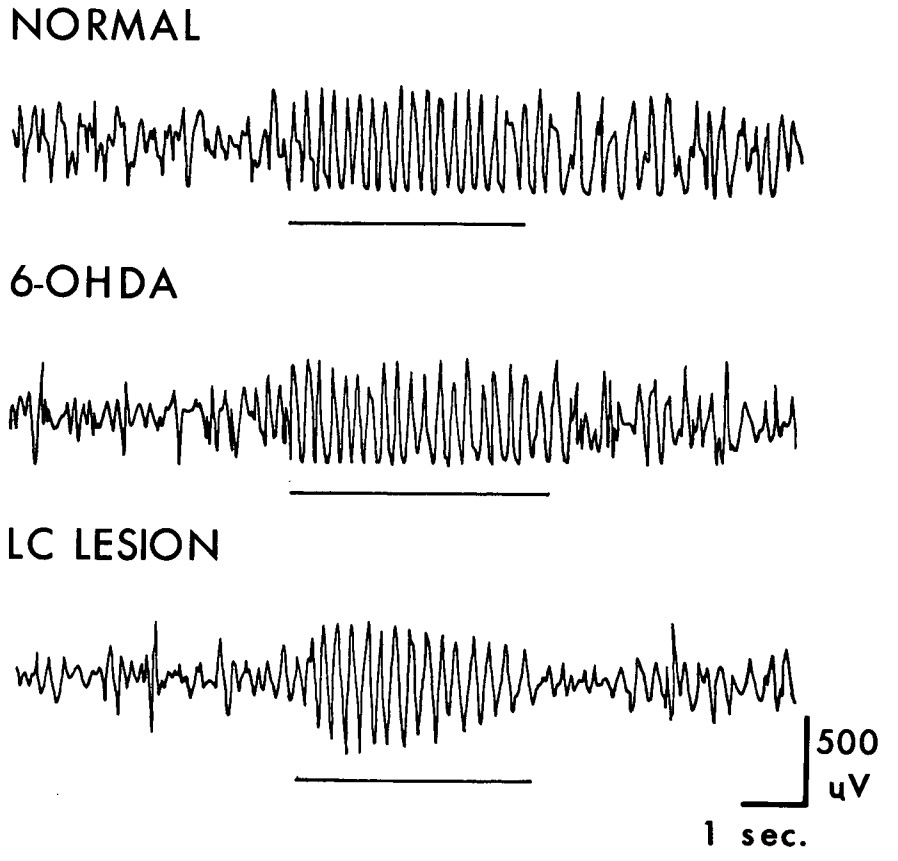
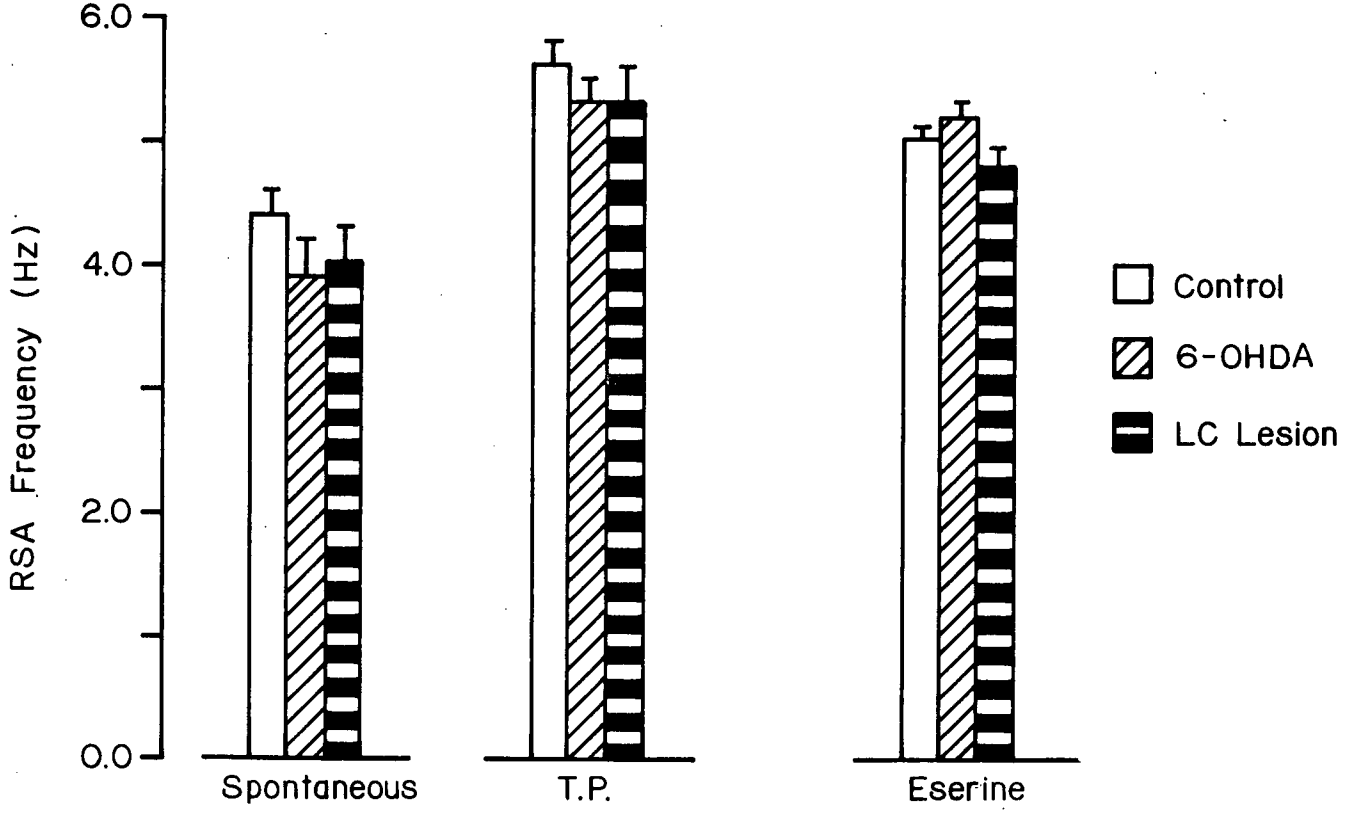

NORMAL
6-OHDA
LC LESION
500
uV
1 sec.

FIG. 5- 2: Freguency Of Spontaneously Occurring And Elicited RSA.

Each histogram represents the mean frequency of RSA recorded in normal (n=7), 6-OHDA-treated (n=9) and LC-lesioned (n=5) groups. The vertical bars are standard errors of the mean. There are no statistically significant differences between experimental groups. However, RSA recorded during tail pinch (T.P.) or following eserine was faster than spontaneously occurring RSA.


6.0
4.0
2.0
0.0
RSA Frequency (Hz)
Spontaneous
T.P.
Eserine
Control
6-OHDA
LC Lesion

In order to verify that rhythmical electrical activity is generated in each group of rats the frequency of RSA was examined (2-60 min.) following the intravenous injection of eserine (0.08-0.1 mg/kg). This treatment resulted in the continuous appearance of RSA in all three groups of rats for periods ranging between 20 min and 2 hrs. As in the case of spontaneous and sensory-evoked RSA, there were no significant differences in the frequency of RSA between control and lesioned rats (Fig. 5-2).

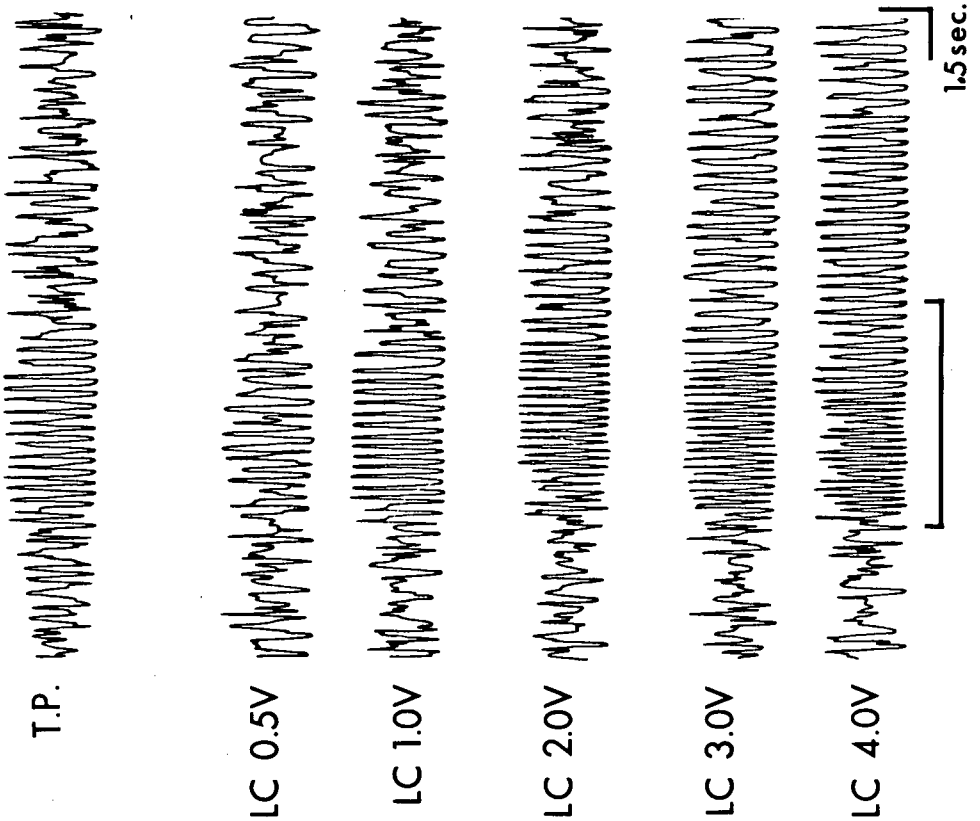
Effect Of LC Stimulation On Hippocampal Activity.

In control rats electrical stimulation (1-6 V) in the region of the LC reliably elicited RSA having a frequency of 4-7 Hz. As shown in Fig. 5-3, the frequency of elicited RSA was related to the intensity of stimulation. At lower stimulation intensities (1-2 V) the evoked RSA was stimulus bound , but at higher intensities RSA persisted for up to 30 seconds following termination of the train.

The stimulus frequency was selected by keeping the intensity and duration of the train constant and analyzing RSA at a range of stimulus frequencies (10-300 Hz). A stimulus frequency of 100 Hz was found to be optimal at 3-4 V intensity and this frequency was used in the remainder of the analysis.

FIG. 5- 3: Effects Of Stimulation In The Region Of The Locus Coeruleus On Electrical Activity Of The Dentate Gyrus

The top record shows RSA elicited by a tail pinch (T.P) and the remaining records show the effects of stimulating LC with the voltages indicated on the left during the period shown by the solid bar.


T.P.
LC 0.5V
LC 1.0V
LC 2.0V
LC 3.0V
LC 4.0V
500 μV
1.5sec.

As shown in Fig. 5-4, electrical stimulation of diffuse regions in the vicinity of LC also initiated RSA. An analysis of the relationship between the histologically verified site of the stimulating electrode and the current required to initiate RSA revealed several regions which were at least as effective as LC proper. These regions include the mesencephalic nucleus of the fifth nerve (MES V) and more ventral placements in nucleus pontis caudalis. Placements in the area of MES V also elicited facial movements which could indirectly initiate RSA through sensory-motor pathways (Vanderwolf, 1972).

Effects Of 6-OHDA On RSA Initiated By LC Stimulation

In control rats the concentration of NA in the hippocampal formationwas 0.41±0.05 ug/kg. Following a bilateral injection of 6-OHDA into the dorsal bundle the concentration was reduced to 0.02±0.3ug/kg (94% depletion) which is significantly lower ($p<0.01$) than normal.

Electrical stimulation of LC in 6-OHDA treated rats (Fig. 5-5) elicited RSA of comparable frequency and amplitudes to that recorded in control rats. As shown in Fig. 5-6, the stimulus-response curve of 6-OHDA rats is not significantly different from the normal curve, despite less than 10% NA remaining in the hippocampal formation.

FIG. 5- 4: Location Of Stimulating Electrodes In The Region Of LC And Intensity Threshold For The Generation Of RSA

A: Drawings of coronal sections showing the effectiveness of stimulating at each site as indicated by the key on the lower left.

B: Coronal section of the pons at about P 2.0. The arrow indicates the tip of the stimulating electrode on the perimeter of LC.

Abbreviations

Cer	Cerebellum
DTN	Dorsal Teqmental Nucleus
NV	mesencephalic nucleus of the fifth nerve
LC	Locus Coeruleus
RPOC	Reticularis Pontis Oralis Caudalis
IV	Fourth Cerebral Ventricle

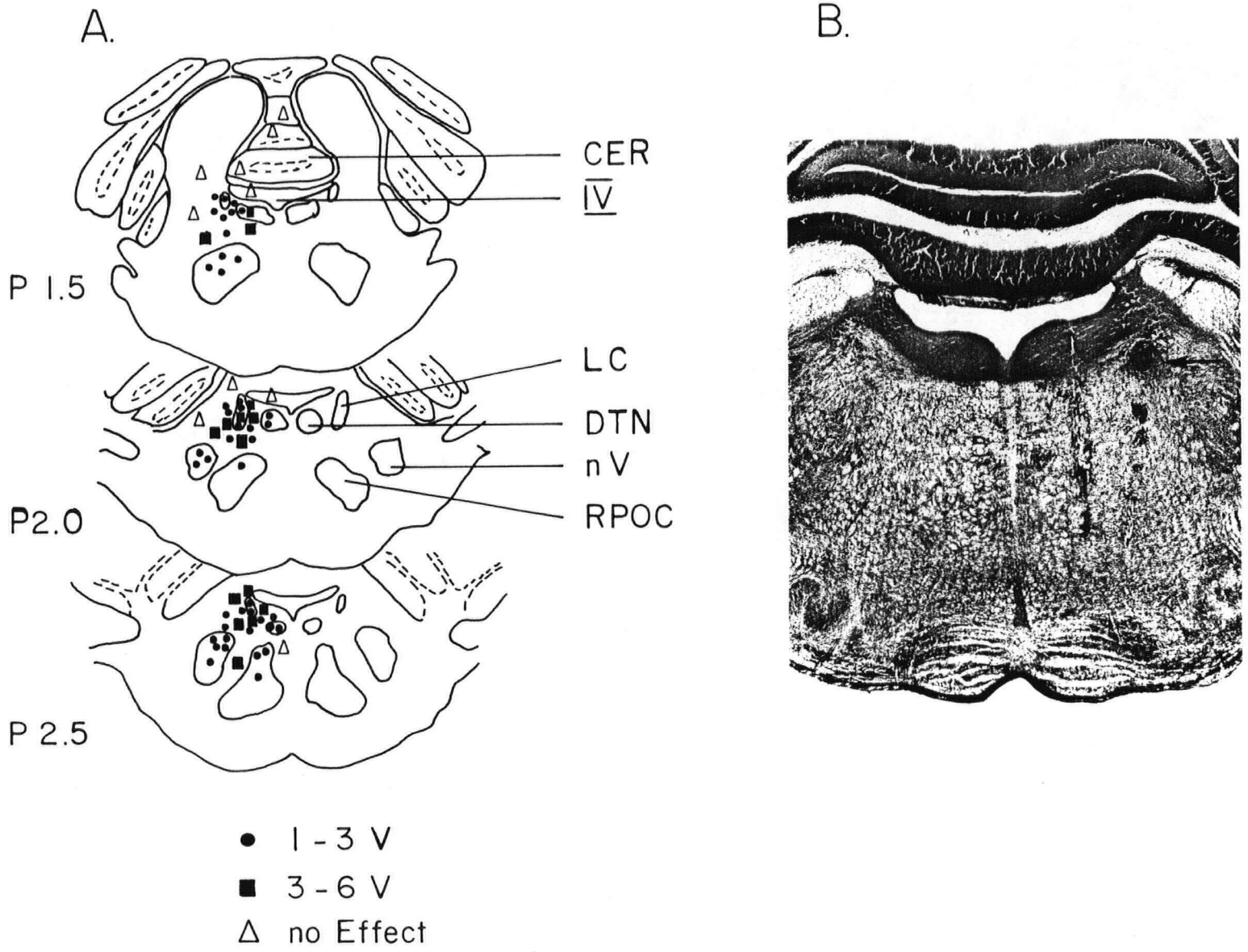
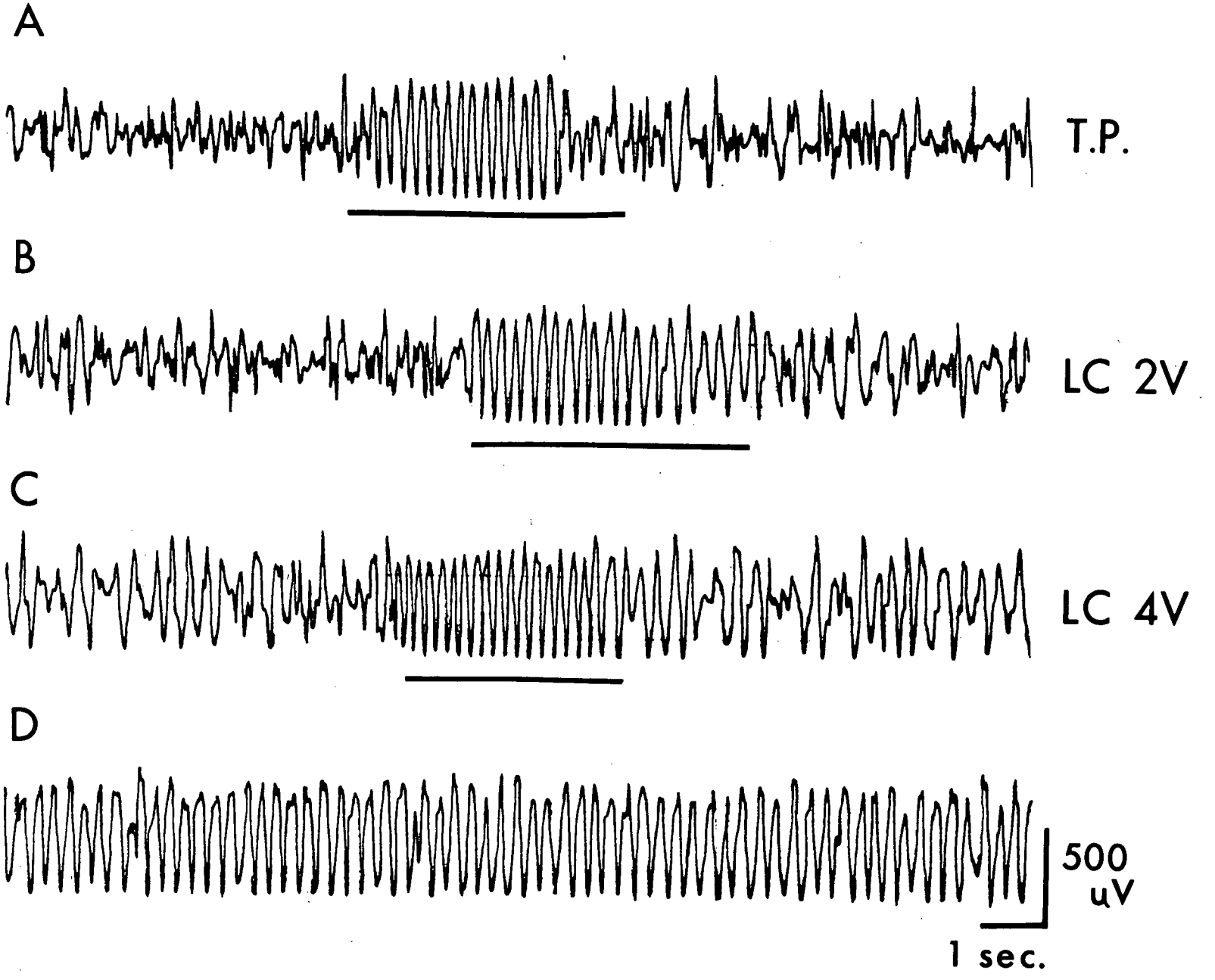

A.
B.
CER
IV
LC
DTN
nV
RPOC
P 1.5
P2.0
P 2.5
• 1 - 3 V
■ 3 - 6 V
Δ no Effect

FIG. 5- 5: Electrical Activity Recorded In The Dentate Gyrus Of A 6-OHDA Treated Rat

Rhythmical activity was evoked by tail pinch (T.P.) and stimulation in the region of LC with the indicated voltages (B and C). D shows continous RSA recorded 5 minutes following eserine injection.


A
T.P.
B
LC 2V
C
LC 4V
D
500 uV
1 sec.

Effects Of Amphetamine

In control rats the intravenous injection of D-amphetamine (2-5 mg/kg) evoked RSA in a dose-dependent manner. This response (Fig. 5-7) had an onset latency of 2-5 minutes and persisted for 2-4 hrs. The frequency of amphetamine induced RSA ranged between 5-7 Hz and was remarkably constant for each animal. The injection did not result in any overt movement, although the rate of ventilation often increased during the first 5 min post injection.

Amphetamine also elicited RSA in rats which had an electrolytic lesion of the LC or a 6-OHDA injection into the dorsal bundle. The latency to onset and the frequency of RSA produced by amphetamine did not differ significantly between groups.

Effects Of Atropine On Spontaneous And Evoked Activity

It has been suggested that under some conditions RSA is blocked by atropine whereas in cases such as those related to concurrent movement RSA is resistant to blockage by atropine (Vanderwolf, 1975). In the present series of experiments, using urethane anaesthetized rats, RSA was always blocked by 2.0-5.0 mg/kg atropine independent of whether it was spontaneously occurring, elicited by a tail pinch or initiated by stimulation in the region of LC. In addition, atropine pretreatment 2-5 minutes prior to

FIG. 5- 6: Frequency Of RSA As A Function Of LC Stimulus Intensity In Control And 6-OHDA Lesioned Rats

The vertical bars represent standard errors of the mean.

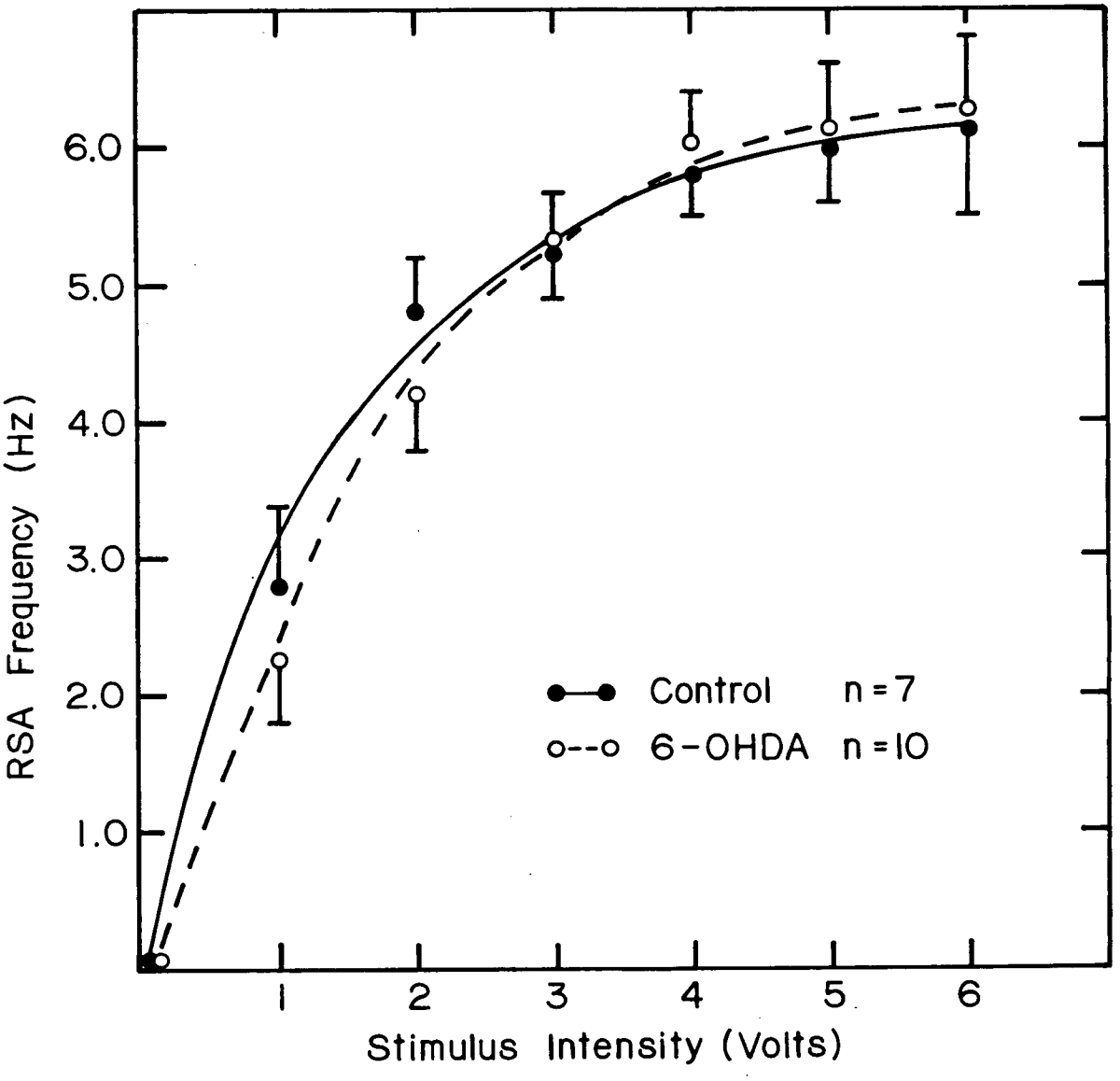
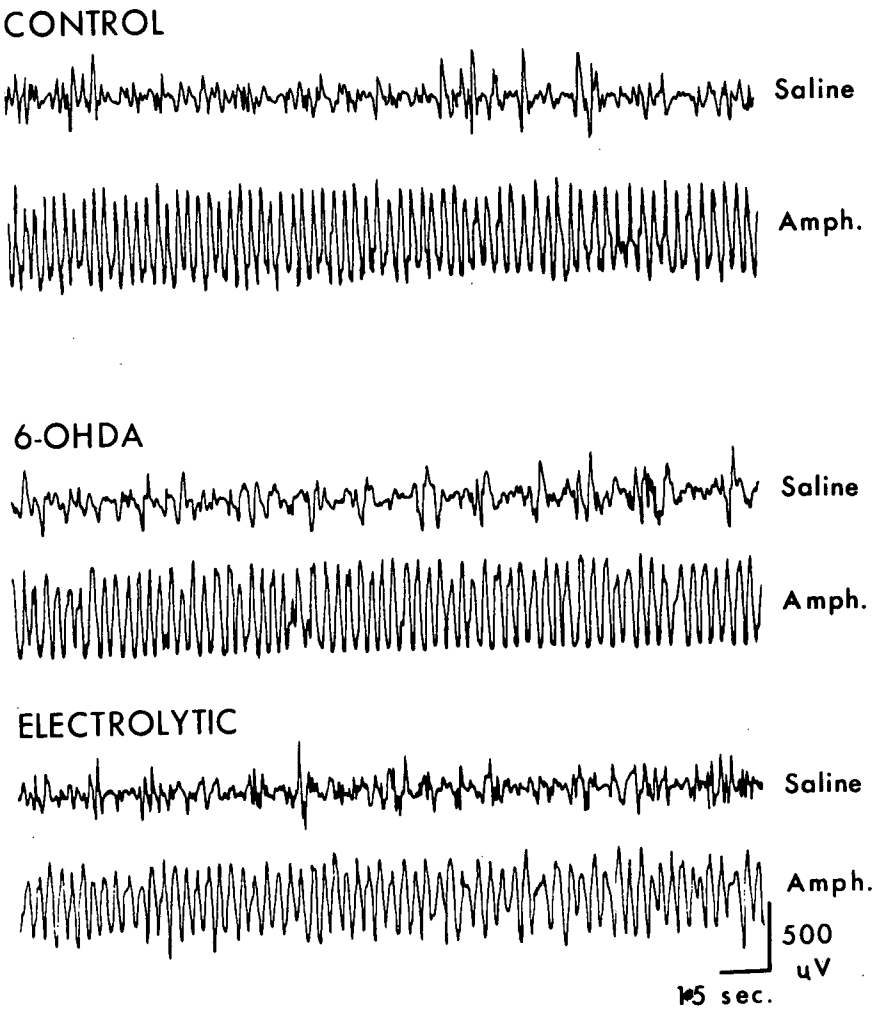

RSA Frequency (Hz)
6.0
5.0
4.0
3.0
2.0
1.0
1
2
3
4
5
6
Stimulus Intensity (Volts)
Control n=7
6-OHDA n=10

FIG. 5- 7: Effects Of Amphetamine On Electrical Activity Of The Dentate Gyrus

The top sweep of each record shows the ongoing electrical activity 5.0 minutes following a saline vehicle injection and the lower sweep shows the activity 5.0 minutes following the intravenous injection of amphetamine (2.0 mg/Kg). Note that amphetamine initiated rhythmical electrical activity in all three cases.


CONTROL
Saline
Amph.
6-OHDA
Saline
Amph.
ELECTROLYTIC
Saline
Amph.
500
uV
1.5 sec.

amphetamine injection was found to completely prevent RSA for periods of up to 2.0 hrs.

5.5 Discussion

Does The Dorsal NA Bundle Underly RSA ?

The results of the present chapter demonstrate that the dorsal noradrenergic bundle orginating in the locus coeruleus does not play an essential role in the generation of RSA recorded in urethane anaesthetized rats. If the dorsal noradrenergic bundle were critical for the generation of rhythmical activity one would expect that 1) destroying this projection would abolishe rhythmical activity and 2) direct electrical stimulation of the cell bodies of origin would initiate rhythmical activity at threshold intensities lower than those required at adjacent sites. However, 6-OHDA lesions of the dorsal bundle which resulted in 90-95% depletion of NA in the hippocampus did not alter the spontaneous or sensory-evoked patterns of hippocampal electrical activity. In addition 6-OHDA lesions did not increase the stimulus intensity required to initiate RSA from electrode placements localized to LC proper relative to placements situated in adjacent areas.

These results support the simultaneous findings of Robinson et al (1977) which show that the dorsal NA bundle is not essential for the generation of RSA in

freely moving rats. These researchers did not find any low threshold sites within the LC for the initiation of RSA and were also unable to block RSA following depletion of hippocampal NA.

The results of the present chapter and those of Robinson et al (1977) do not eliminate the possibility that NA containing axons other than those coursing through the dorsal bundle play a role in the generation of RSA. It is possible that NA containing cell groups caudal to LC (i.e. A2 and A4 according to Dahlstrom and Fuxe, 1964) may influence extra-hippocampal regions such as the septal area which are associated with the generation of RSA. The recent observation of Moore (1978) that 6-OHDA lesions of dorsal NA bundle totally deplete NA in the hippocampus but only partially (48%) lower septal NA content suggest that caudal groups may indirectly influence RSA.

The failure to abolish RSA by making specific lesions taken together with the observation that diffuse areas in brainstem initiate RSA suggests that multiple ascending systems modulate RSA. The septal area may be the site of interactions between these various systems and may thus be the only critical region for the generation of RSA.

Action Of Amphetamine

The intravenous injection of amphetamine was shown to produce RSA in rats which had electrolytic destruction of LC, 6-OHDA lesion of the dorsal bundle and control rats. The mechanisms mediating this action of amphetamine are not known and may be related to enhanced synaptic release of the catecholamines dopamine and noradrenaline (reviewed by Moore, 1977). The effect of amphetamine is unlikely to be mediated by enhanced NA release from the terminals of LC axons since RSA was demonstrated following electrolytic lesions of LC. Amphetamine could enhance the activity of the mesolimbic DA system which has been shown to innervate the lateral septal area (Lindvall, 1975; Assaf and Miller, 1977). The neurones of the lateral septum have been implicated in the generation of rhythmical burst discharge of medial septal neurones (McLennan and Miller, 1974a, 1976).

It is not known to what extent the actions of amphetamine are dependent on septal-hippocampal cholinergic neurones. The preliminary observations that in urethane anaesthetized rats the action of amphetamine can be blocked by atropine emphasize the role of cholinergic systems. There is also the possibility that amphetamine injections enhance the release of acetycholine (Moore, 1977). However, there is no direct evidence that Ach in the septal area

is enhanced by amphetamine.

Possible Contribution Of Urethane Anaesthesia

The results of the present chapter were obtained in urethane anaesthetized rats and the possibility arises that these conditions mask the potential influence of LC on RSA. As pointed out in chapter 3, urethane narrows the range of RSA from 3-12 Hz in freely moving rats down to 3-7 Hz and any contribution of LC in driving frequencies above 7.0 Hz will not be seen. The results of Gray, McNaughton, James and Kelly (1975) do in fact suggest that NA lowers the threshold for 'septal driving' of RSA at an optimal frequency of 7.7 Hz. However, septal driving has not yet been related to physiological occurrence of RSA and the results of Robinson et al (1977) in freely moving rats suggest that the full frequency range of RSA (i.e. 3-12 Hz) occurs in the absence of hippocampal NA. However the evidence that the full frequency band occurs in the absence of LC noradrenergic system does not eliminate the possibility that this system modulates RSA by lowering the threshold for certain frequencies.

5.5 Summary

The results of the present chapter eliminate the possibility that the LC noradrenergic system plays plays an essential role in generation of RSA. It is proposed that amphetamine elicits RSA by mechanisms other than enhanced NA release in the hippocampus.

CHAPTER 6: CHARACTERIZATION OF THE PERFORANT PATH-DENTATE PROJECTION

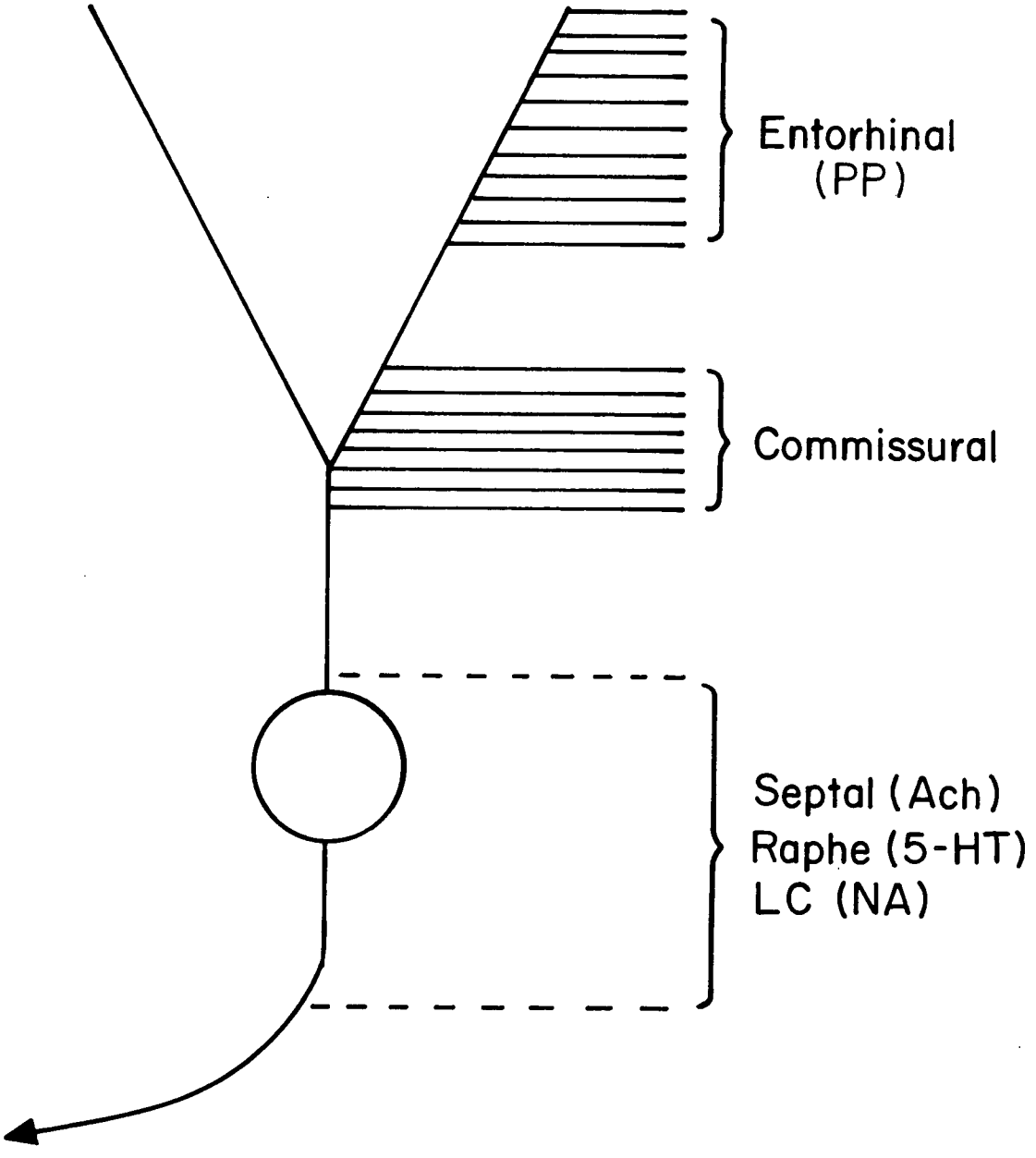
6.1 Introduction

As reviewed in chapter 1, histological studies of the hippocampal formation reveal two major cell types, the pyramidal cells of the hippocampus proper and the granule cells (G-cells) of the dentate gyrus. The G-cells form a curved sheet of cell bodies with apically oriented dendrites reaching the obliterated hippocampal fissure (chapter 1, Fig. 1). In addition to this clear segregation of dendrites and cell bodies, there is a highly organized laminar distribution of the termination of the major afferent fibres. As shown in Fig. 6-1, afferents from the ipsilateral entorhinal cortex terminate in the outer molecular layer onto distal dendrites wheras commissural and associational afferents terminate more proximal to the cell bodies in the inner molecular layer. While the precise organization of other extrinsic afferents is not yet known, histochemical studies suggest that monoamine-containing systems terminate in the vicinity of the G-cell bodies and the dentate hilus (reviewed in section 1.1.1)

The most massive of the above inputs is that from the entorhinal cortex which projects via the perforant path (PP) to the outer two-thirds of the dendritic

FIG. 6- 1: Laminar Organization Of Afferents To The Dentate.

Afferents from the lateral and medial regions of the entorhinal cortex project via the perforant path onto the outer two-thirds of of the dentate molecular layer which contains the dendrites of G-cells. Commissural and associational (not shown here) fibres from the contralateral and ipsilateral CA3, respectively, terminate in the inner molecular layer. Cholinergic and monoamine-containing fibers project to more diffuse regions in the vicinity of the cell body layer and dentate hilus.


Entorhinal
(PP)
Commissural
Septal (Ach)
Raphe (5-HT)
LC (NA)

tree. According to Nafstad (1967), the small diameter axons of the PP make en-passage synaptic contact with many spines of the profusely branching dendrites of G-cells. Electrophysiological analysis of this strictly laminated system revealed that stimulation of PP results in extracellularly recorded field potentials having a topographical organization (Gloor et al, 1964; Andersen and Lomo, 1966; Lomo, 1971a; McNaughton and Barnes, 1977)

Electrodes situated in the outer molecular layer of the dentate record a negative extracellular field which reflects the synaptic current generated by dendrites and is termed the population EPSP (Lomo, 1971a). At the cell body layer the evoked response consists of a positive wave with a superimposed negative spike representing the synchronous firing of neuronal action potentials and is therefore referred to as the population spike (Lomo, 1971a).

An important feature of the above projection system is the plasticity of synaptic transmission which is observed when the PP is stimulated at particular frequencies. Relatively brief tetanic stimulation results in an enhancement of both the population EPSP and spike response which persists for hours and on occassions for several days (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973; Douglas and Goddard, 1975). In contrast, a single conditioning pulse to the

PP results only in short term (less then 1 second) potentiation of the response to a subsequent test pulse (Lomo, 1971b; Steward et al, 1976). The first mentioned paradigm, commonly referred to as post-tetanic potentiation (PTP) as well as the latter, known as paired pulse facilitation, have also been confirmed in invitro hippocampal slices (Dudek et al, 1976; Miller, unpublished observations). The mechanisms which mediate the above forms of functional plasticity are not known. Andersen (1975) suggests that they may reflect the ability of the presynaptic terminal to release more transmitter. However, the possibility of an altered responsiveness of the postsynaptic elements is just as likely (Dunwiddie et al, 1978).

In order to evaluate these possibilities, this part of the thesis examines the role of extrinsic afferents to the dentate in modifying the response of G-cells to stimulation of the perforant path. Such analysis is essential for determining whether changes in the efficacy of the cortical input via the PP occur as a result of the activity of afferents to the postsynaptic cell in addition to those postulated to occur in the presynaptic elements of the test pathway. The purpose of the present chapter is to characterize the entorhinal-dentate projection in the urethane anaesthetized rat and analyze paired pulse facilitation of the PP-evoked responses. The following chapters examine the effects of stimulating extrinsic afferents

originating in the contralateral hippocampus (chapter 7), median raphe nucleus (chapter 8) and the locus coeruleus (chapter 9) on these response.

6.2 Experimental Procedures

Extracellular recording experiments were performed on a total of 94 urethane anaesthetized rats. Concentric bipolar stimulating electrodes were positioned in the angular bundle (8.1 mm posterior to bregma; 4.2-4.4 mm lateral; 3.0-3.5 below cortical surface) to activate the perforant path as it leaves the ipsilateral entorhinal cortex or in the subiculum adjacent to the hippocampal fissure where the PP enters the dentate gyrus. Extracellular potentials were recorded from the dorsal hippocampus and the dentate gyrus using either glass micropipettes filled with 4M NaCl (2-5Mohm resistance) or sharpened tungsten microelectrodes (9-12 Mohm). In some cases more than one recording electrode were used to record simultaneously from both the synaptic region in the molecular layer and from the cell body layer . The signals were amplified and analyzed as described in chapter 2. Typically the average of 20-30 stimulus presentations (ISI 2.5-4.0 seconds) were plotted. The effects of conditioning stimulation of the PP on a subsequent test volley delivered through the same electrode was analyzed using the paired-pulse paradigm. This consisted of comparing the amplitudes and rates of

rise of the response evoked by the first (conditioning) pulse to that evoked by the second (test) pulse. The interval between conditioning and test pulses (C-T interval) ranged between 0-1500 msec.

6.3 Results

Fig. 6-2 shows the extracellular field potentials evoked by low intensity (1-2V) stimulation of the PP and recorded at the indicated depths in the dentate gyrus. The location of the electrode at each recording site can be approximated by the corresponding schematic of the upper and lower blades of the dentate. As shown by Lomo (1971a) in the rabbit and Gloor et al (1964) in the cat, a short latency negative wave is recorded in middle molecular layer with a maximum at the level of the PP synapses (Nafstad, 1967). At the cell body layer the response reverses to a positive wave on which the discharges of individual G-cells are superimposed. The mirror images of these responses occur in the lower blade of the dentate.

As shown in Fig. 6-3, increasing the stimulus intensity enhances the amplitude of the dendritic negative field and significantly increases the number of spikes recorded on the positive cell layer field. At higher stimulus intensities (3-7 V) the units discharge synchronously giving rise to a large negative spike which for the reasons outlined below is referred to as

FIG. 6- 2: Depth Profiles Of The Field Potentials Evoked In The Dentate Gyrus By A Weak Perforant Path Volley

A: Extracellular field potentials recorded along a vertical track at the indicated depths below the cortical surface.

B: Depth profile of the evoked potentials measured 1.0-1.5 msec after their onset (indicated by vertical bars in A).

C: Schematic representation of the approximate positions of the upper and lower blades of the dentate gyrus.

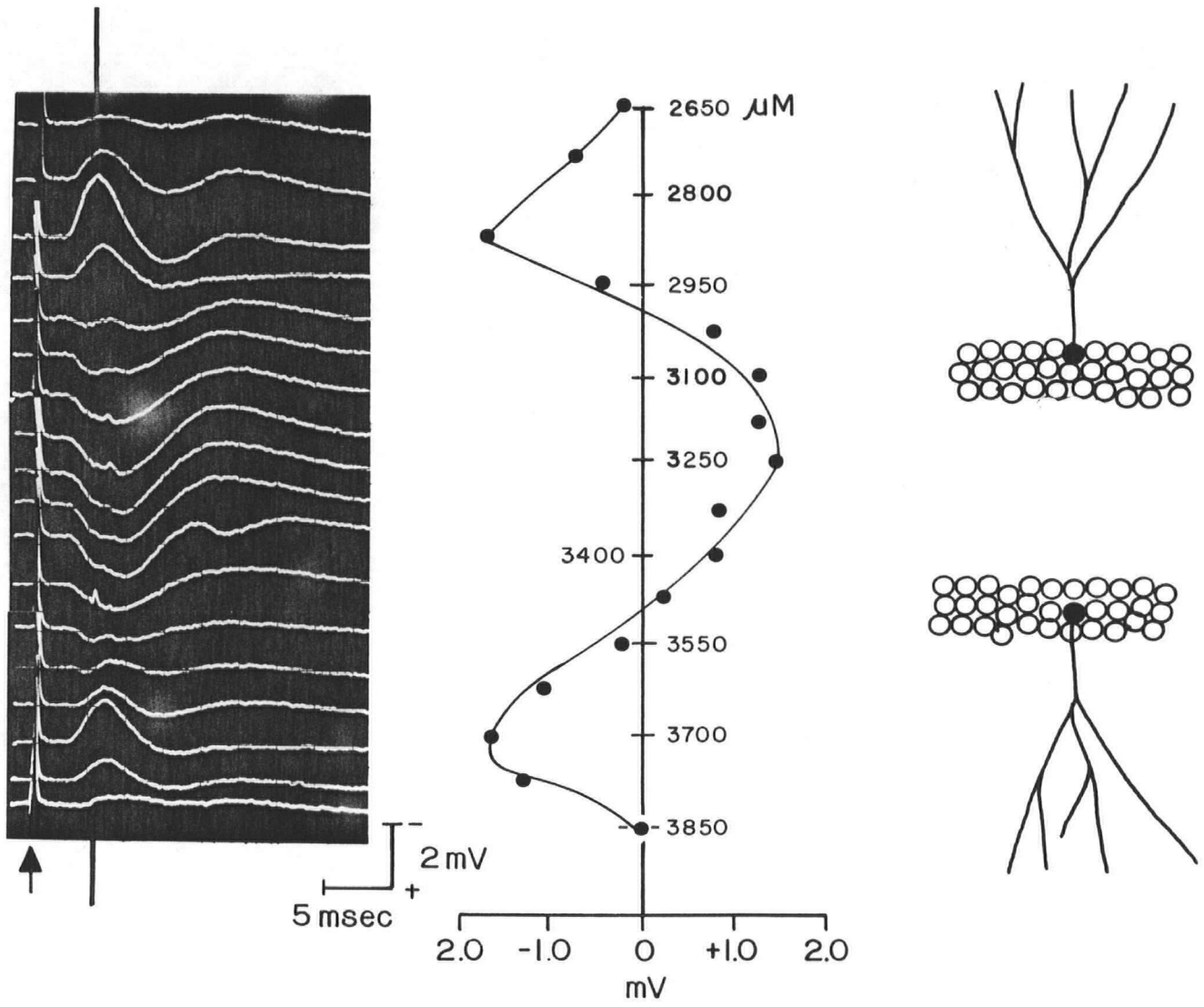
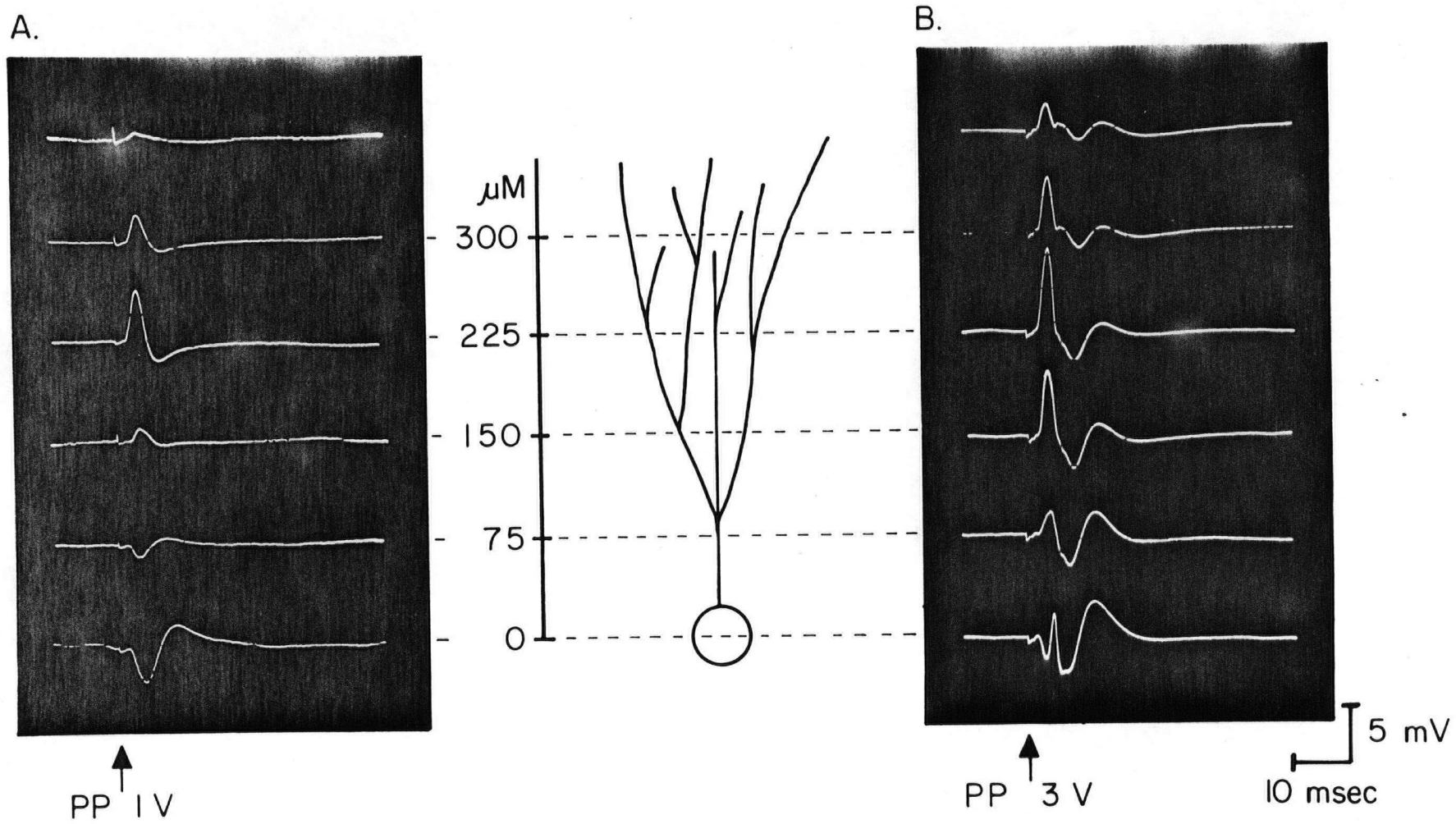

2650 µM
2800
2950
3100
3250
3400
3550
3700
3850
2 mV
5 msec
2.0
-1.0
0
+1.0
2.0
mV

FIG. 6- 3: Field Potentials Evoked In The Dentate By PP Stimulation At Various Intensities

A: Field potentials recorded at the indicated distances above the granule cell body layer following a weak PP volley.

B: Depth profile as in A with a stronger PP volley.


A.
B.
μM
300
225
150
75
0
PP 1 V
PP 3 V
5 mV
10 msec

the population spike response of dentate granule cells.

Identification Of Dentate Responses.

A. Molecular Layer : As shown in Fig. 6-4, a large negative response is recorded by an electrode situated in the middle molecular layer of the dentate. This negative response (N2 in Fig. 6-4B) is maximal 150-190 um from G-cell body layer in the region of the distal dendrites where the PP synapses terminate (Blackstad, 1958; Nafstad, 1967). The onset of this negative response occurs at 2-4 msec following stimulation of the PP and reaches a peak at 4-6 msec. The latency to the onset of this response and its rise time are directly related to stimulus intensity (Fig. 6-4B,C). The time from the onset of this field to the earliest discharge of G-cells ranged between 1.5-2.5 msec. The above negative response was evoked monosynaptically since it followed moderate (up to 50 Hz) but not high frequency (100-200 Hz) stimulation of the perforant path (Fig.6-4D). In addition, its onset latency was relatively consistent and thus unlikely to result from polysynaptic activation (Fig. 6-4A).

A similar negative response was previously recorded in the dentate molecular layer of cats (Gloor et al, 1964) and rabbits (Lomo, 1971a). Lomo (1971a) observed that the onset of the extracellular negative wave coincided with the onset of the intracellular

FIG. 6- 4: Identification Of The Field Potentials Recorded In The Dentate Molecular Layer.

A: 5 superimposed oscilloscope sweeps showing the small initial deflection (N1) and the prominent negative response (N2).

B: effects of increasing the stimulus intensity on the above responses.

C: expanded plots showing the size of N1 and rate of rise of N2 at various stimulus intensities.

D: inability of the large negative field (N2) to follow high frequency stimulation suggesting that it is produced synaptically.

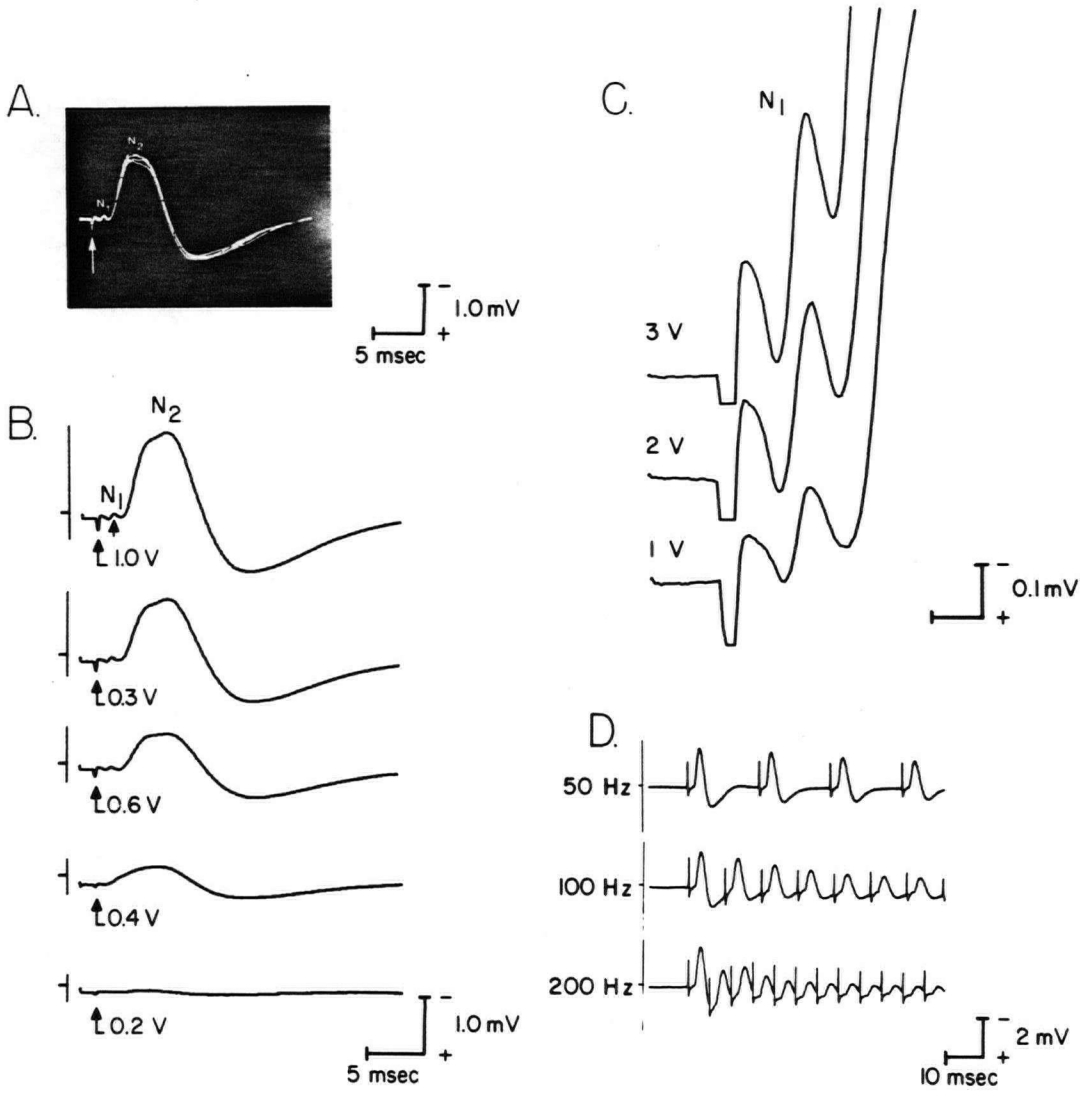
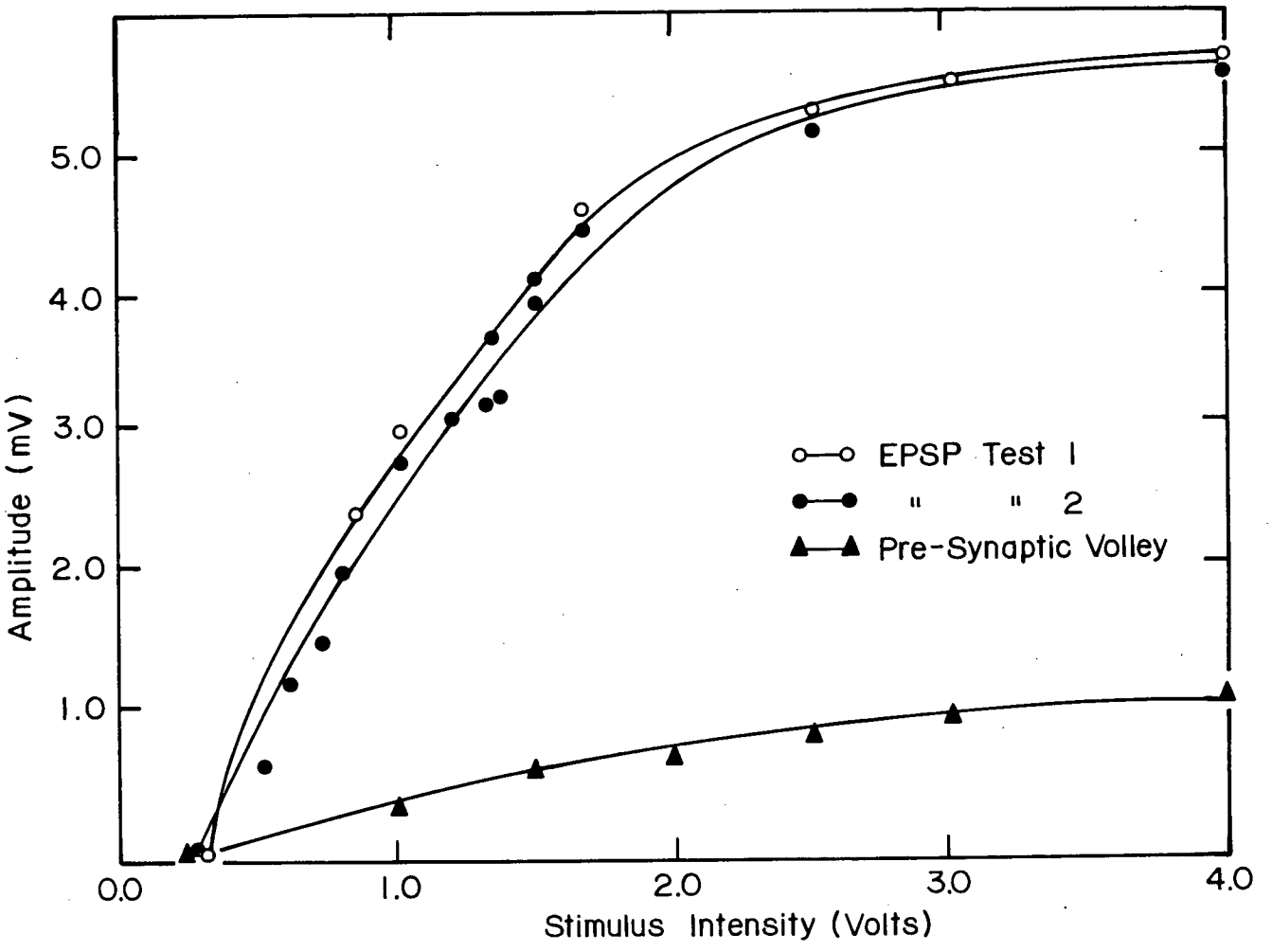

A.
N1
N2
1.0 mV
5 msec
B.
N1
N2
1.0 V
0.3 V
0.6 V
0.4 V
0.2 V
1.0 mV
5 msec
C.
N1
3 V
2 V
1 V
0.1 mV
D.
50 Hz
100 Hz
200 Hz
2 mV
10 msec

FIG. 6- 5: Relationship Between PP Stimulus Intensity And Amplitude Of Responses Recorded In The Dendritic Region Of The Dentate

Each point represents the average oftwenty responses recorded in the dendritic region of the dentate gyrus.

The amplitude of the EPSP was measured while increasing (0) and decreasing (.) stimulus intensity to evaluate the variability of the response.


5.0
4.0
3.0
2.0
1.0
Amplitude (mV)
0.0
1.0
2.0
3.0
4.0
Stimulus Intensity (Volts)
EPSP Test 1
" " 2
Pre-Synaptic Volley

EPSP. Since this negative monosynaptic wave is maximal in the layer of the perforant path synapses, it will be called the extracellular EPSP. This interpretation is in accord with that of Gloor et al (1964) and Lomo (1971a).

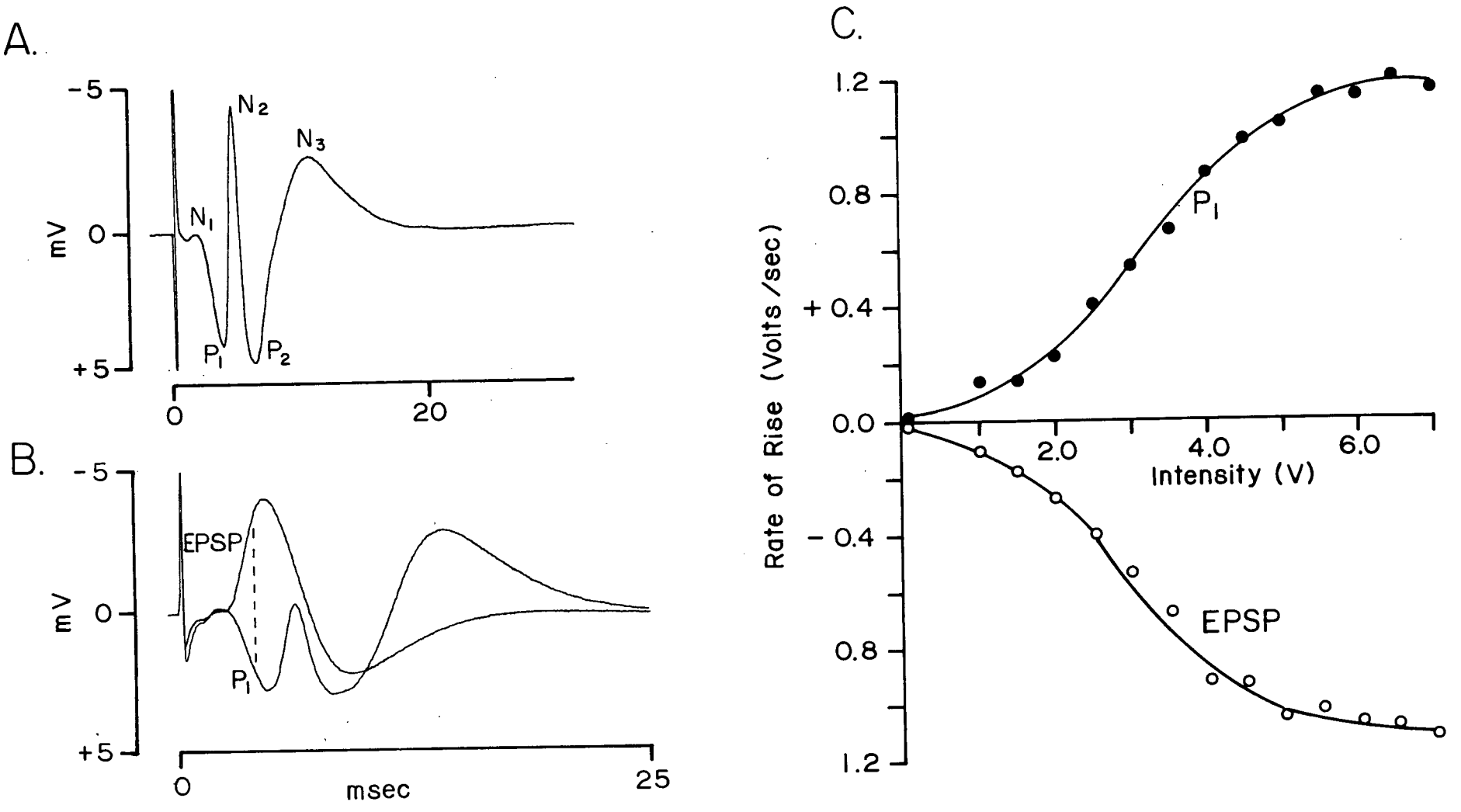
In some extracellular records obtained in the molecular layer a small negative deflection (N1 in Fig. 6-4C) precedes the onset of the EPSP by 0.4-0.6 msec. This small deflection is only recorded in a restricted zone in the vicinity of the PP synapse and is linearly related to stimulus intensity (Fig. 6-5). Unlike the EPSP, this early component is not attenuated by high frequency stimulation (Fig. 6-4D) and is actually enhanced at a short pulse intervals when the EPSP is refractory. It is, therefore, concluded that the early negative component is the PP fibre volley and thus does not show characteristics of the synaptic response described above. A similar interpretation of this response was previously proposed by Lomo (1971a). In addition, Lomo (1971a) observed that the presynaptic volley was not as resistant to anoxia as the synaptic response.

FIG. 6- 6: Identification Of The Evoked Potential Recorded At G-cell Body Layer.

A: Components of the response recorded at G-cell body layer following stimulation of PP with a strong (10 V) volley. The plot is an average of 20 trials and peaks are labelled according to their polarity and latency.

B: Relationship between the evoked EPSP recorded in the dendritic layer and the mirror image positive wave recorded at cell body layer.

C: Rate of rise of P1 response and dendritic EPSP as a function of stimulus intensity.


A.
-5
0
+5
mV
N2
N3
N1
P1
P2
0
20
B.
-5
0
+5
mV
EPSP
P1
0
msec
25
C.
1.2
0.8
+ 0.4
0.0
- 0.4
0.8
1.2
Rate of Rise (Volts /sec)
P1
EPSP
2.0
4.0
6.0
Intensity (V)

B. Cell Layer : Fig. 6-6A shows the components of the extracellular field potential evoked by a strong perforant path volley (10V) and recorded at the cell body layer. These components are labelled according to their polarity and latency to onset. P1 is recorded simultaneously with the rise of the negative dendritic EPSP and appears as its mirror image (Fig. 6-6B). Discharge of G-cells rarely occurs at the start of P1 but rather 1.5-2.0 msec after its onset. The rate of rise of the P1 is similar to that of the EPSP (Fig. 6-6D) and is most likely the extracellular reflection of the dendritic EPSP recorded at the cell body layer. The observation that it is the opposite polarity to the EPSP and is not caused by the discharge of G-cells suggests that it acts as a source providing current to the dendritic EPSP sink (Lomo, 1971a).

Component N2 is maximal in the region of G-cell bodies and is recorded only following stimulation of the perforant path with higher intensity. Only a positive wave is recorded by the same electrode following low stimulus intensities (0.5-1.0 V) sufficient to evoke the dendritic EPSP. As the stimulating intensity is gradually increased (1.0-2.0 V) single units are recorded on the positive wave. The number of units increase with higher stimulus intensities (Fig. 6-7) until a prominent N2 component is recorded. The latency to the peak of N2 corresponds to the mean latency to discharge of individual G-cells

FIG. 6- 7: Development Of The Dentate Population Spike.

Responses recorded in the G-cell layer following PP stimulation with the indicated voltages. Note recruitment of single unit discharge to form a compound spike at higher stimulus intensity.

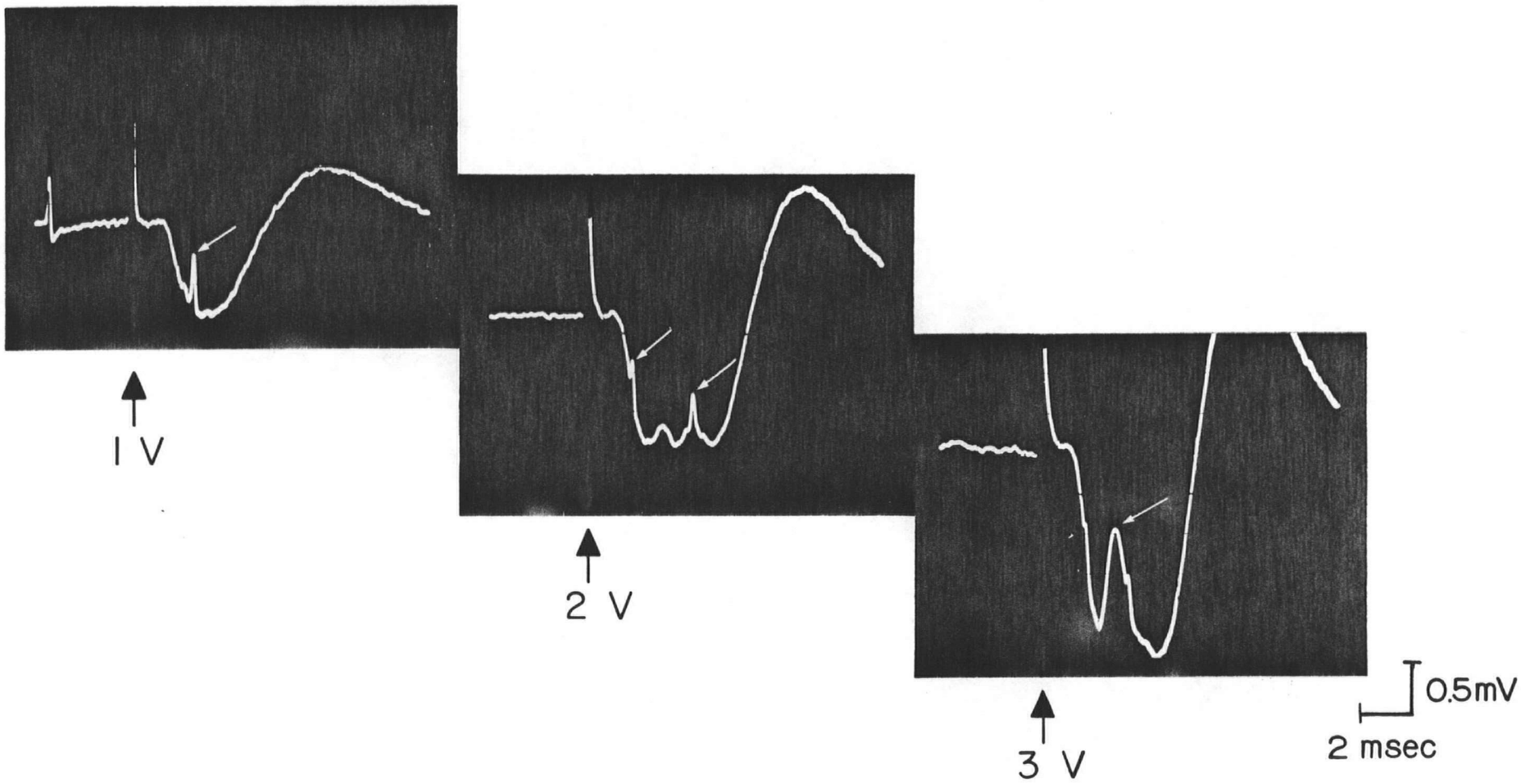
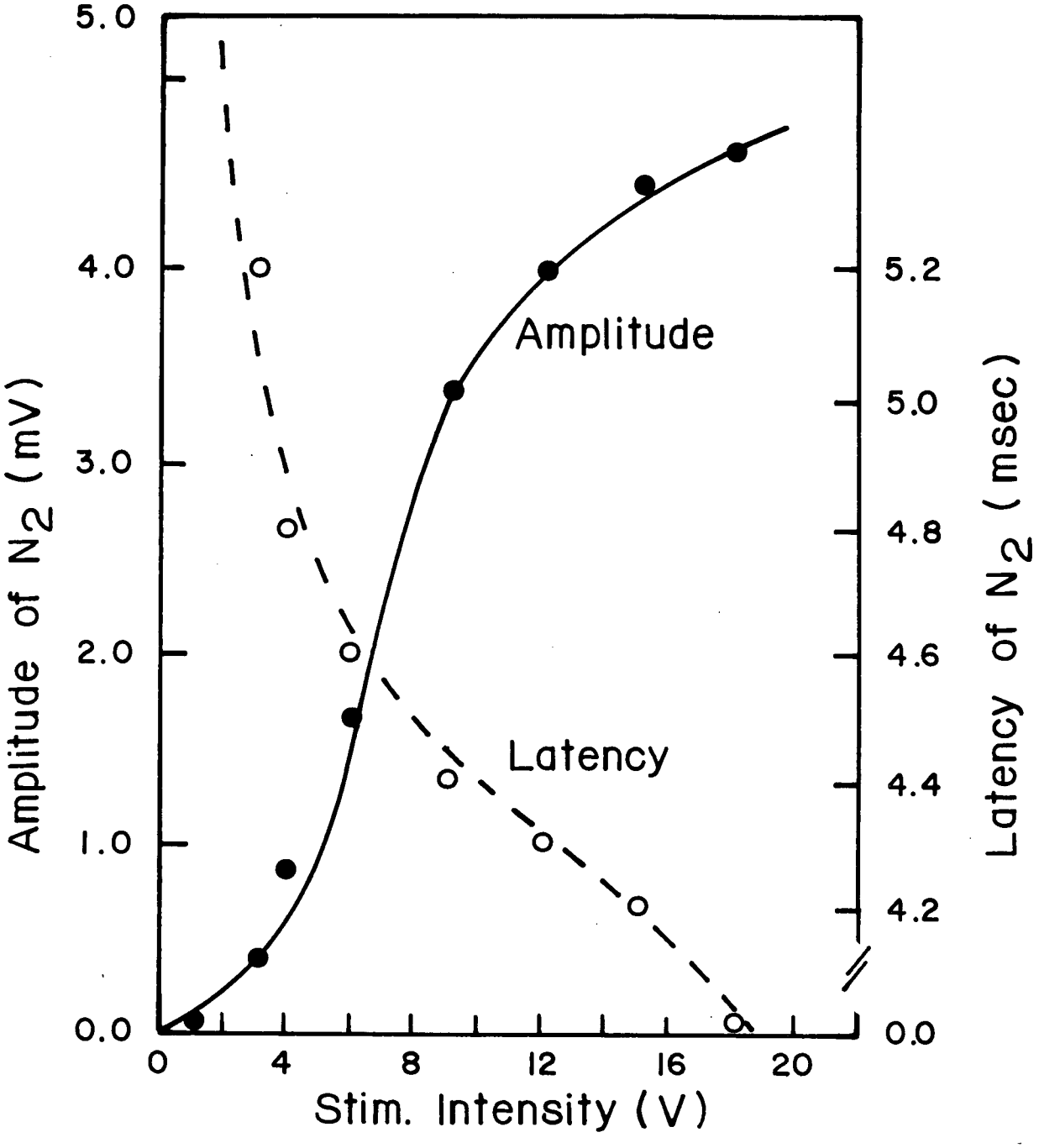

1 V
2 V
3 V
0.5mV
2 msec

FIG. 6- 8: Effects Of Stimulus Intensity On Amplitude And Latency Of The G-cell Layer response

The peak latency and amplitude of the N2 component of the cell layer response is plotted as a function of stimulus intensity.


5.0
4.0
3.0
2.0
1.0
0.0
Amplitude of N2 (mV)
Amplitude
Latency
5.2
5.0
4.8
4.6
4.4
4.2
0.0
Latency of N2 (msec)
0
4
8
12
16
20
Stim. Intensity (V)

(Andersen, Skrede and Bliss, 1971) and is inversely related to stimulus intensity (Fig. 6-8).

Since the N2 component is maximal in the G-cell layer (Fig. 6-9) and is related to the discharge of G-cells, it most likely represents the monosynaptic activation of a population of G-cells (Gloor et al, 1964; Lomo, 1971a). Further support of the synaptic nature of N2 is that it does not follow high frequency (>100 Hz) stimulation and shows latency shifts characteristic of monosynaptic responses (Fig. 6-10).

Additional evidence that the N2 component is caused by G-cell discharge was obtained by antidromically activating the same units following stimulation of the mossy fibre (MF) pathway. As shown in Fig. 6-11, low intensity stimulation of the MF pathway results in a sharp negative spike localized to the G-cell body layer. Unlike the synaptically evoked N2 wave, this spike is not influenced by high frequency stimulation (>150 Hz) and does not show an appreciable shift in latency (Fig. 6-11D). In addition the amplitude of the synaptic N2 component could be enhanced by low frequency (5-7 Hz) stimulation whereas the antidromic spike could not be. The N2 component may thus represent the monosynaptic activation of a number of dentate G-cells and in the terminology of Lomo (1971a) will be hereafter referred to as the population spike.

FIG. 6- 9: The Cell Layer Response Recorded At Various Depths In The Dentate.

Each point and corresponding potentials are the averages of 20 responses recorded along the same dorsal-ventral electrode track.

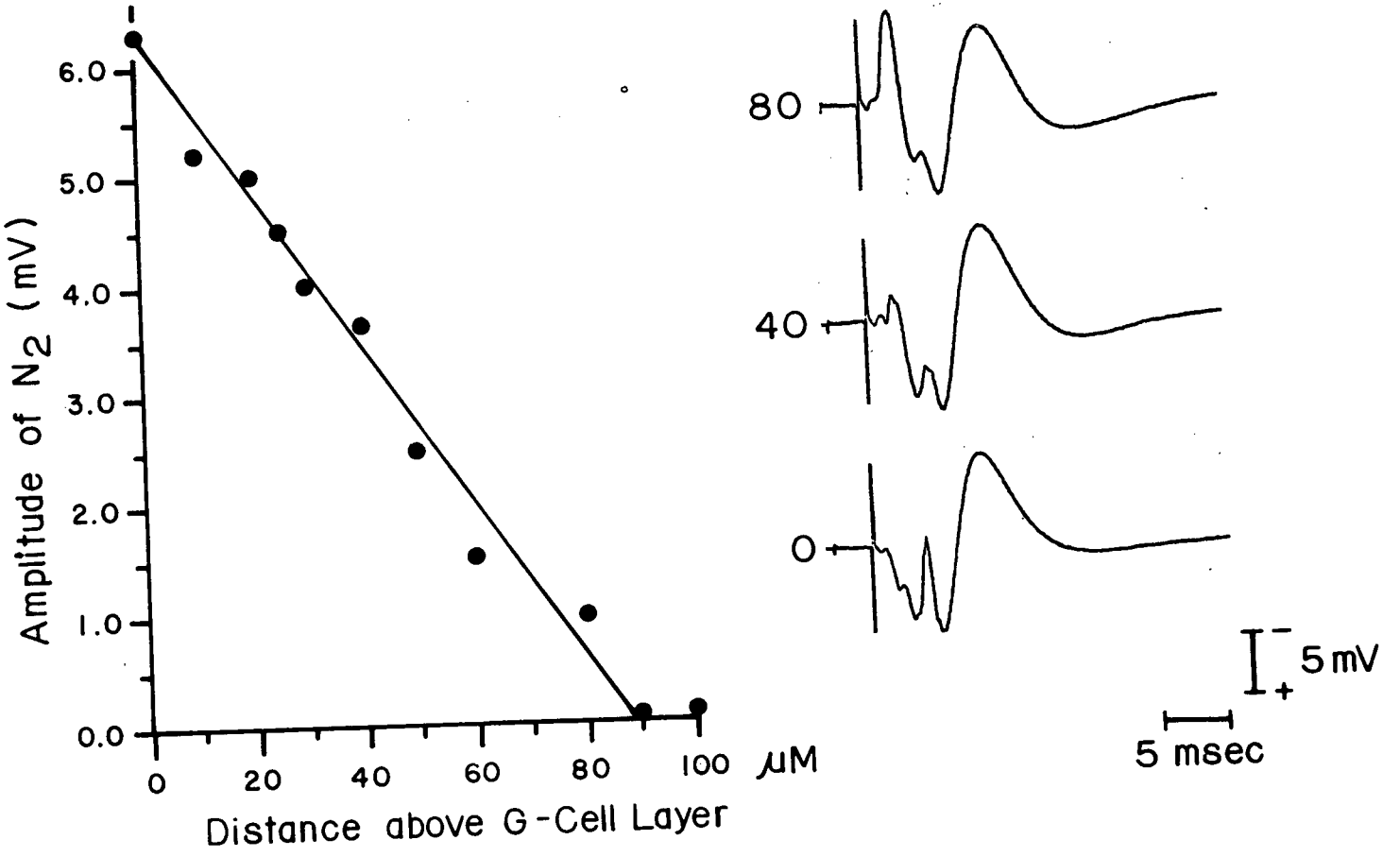

Amplitude of N_2 (mV)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
20
40
60
80
100
μM
Distance above G-Cell Layer
80
40
0
5 mV
5 msec

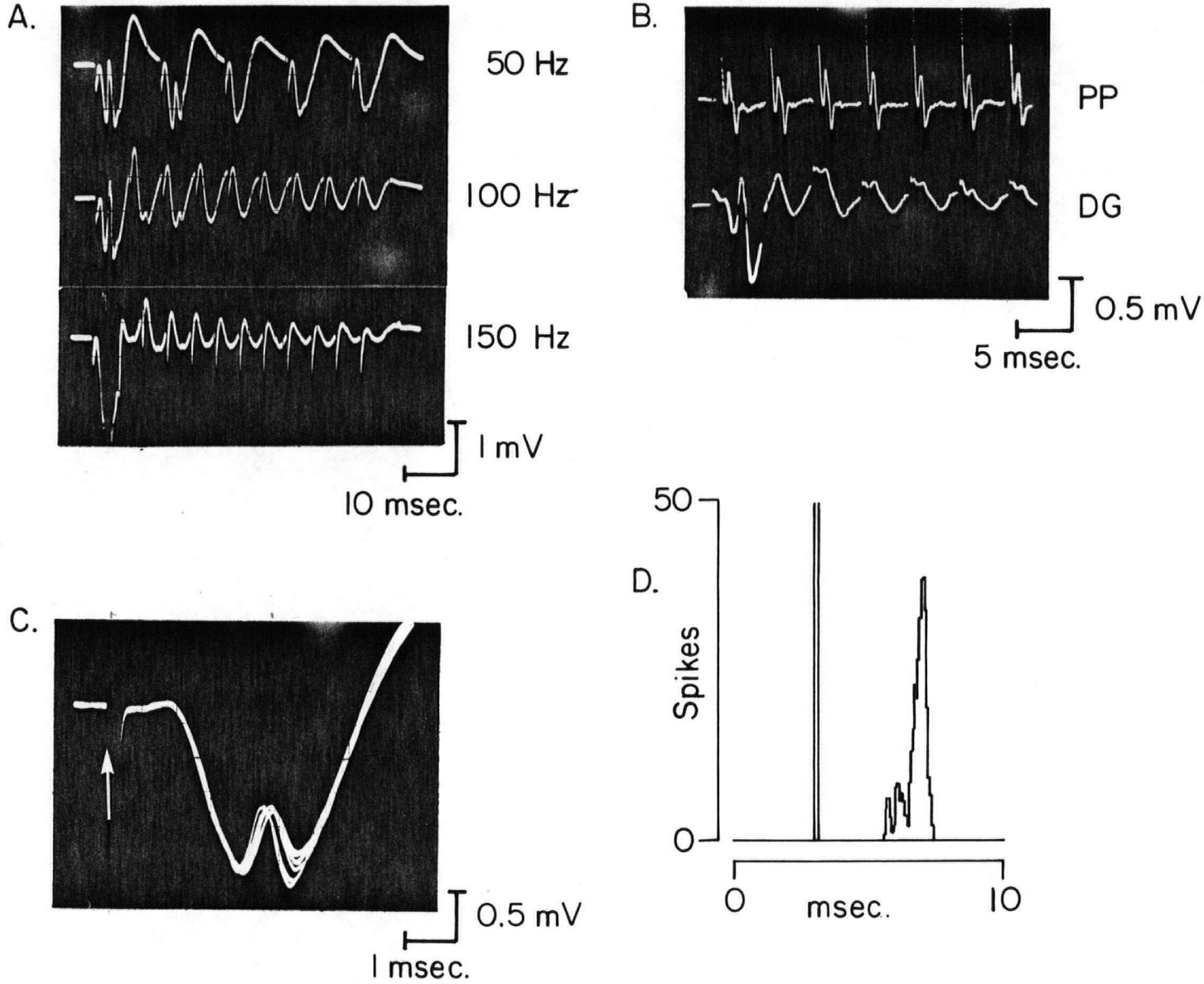
FIG. 6-10: Presynaptic And Postsynaptic Responses Following Stimulation Of The Perforant Path.

A: effect of increasing stimulus frequency on PP evoked population spike response.

B: fibre volley recorded from the PP (top sweep) and the G-cell layer response (lower sweep) following stimulation of the entorhinal cortex at 175 Hz. Note that, unlike the population spike, the PP volley is not attenuated by high frequency stimulation.

C: 5 superimposed oscilloscope sweeps indicating variation of the population spike latency characteristic of a monosynaptic response.

D: histogram showing the distribution of peak latencies following PP stimulation (ISI=2.5 seconds). The first vertical bar indicates constancy of the stimulus artifact.


A.
50 Hz
100 Hz
150 Hz
1 mV
10 msec.
B.
PP
DG
0.5 mV
5 msec.
C.
0.5 mV
1 msec.
D.
50
Spikes
0
0
msec.
10

Component P2 is recorded at the G-cell body layer at threshold intensities for the production of a dendritic EPSP. Since P2 is always recorded with the P1 component it may also be interpreted as an image of the dendritic EPSP. However P2 is not always symmetrical with P1 and may have neurones discharging anywhere along its length (Fig. 6-12). Since some of those evoked neurones which appear on the P2 component are not antidromically evoked but are synaptically activated by MF stimulation (Fig. 6-12), they are unlikely to represent G-cells. The neurones that discharge on the P2 component are frequently recorded in the dentate hilus and may represent interneurones that were activated by G-cell axon collaterals. In this regard, Lomo (1968) has suggested that P2 component signifies the start of IPSP's on granule cells which are initiated by inhibitory interneurones. On the basis of the above data it may be suggested that P2 may be in part the reflection of dendritic EPSP and in part the start of inhibitory mechanisms.

The late negative component (N3) has not yet been identified. It is recorded in the region of the cell body layer and is maximal in the region of the dentate hilus at approximately 2.0 mm from the midline. Stimulating intensities below threshold for the production of a population spike produce a reliable N3 component and as the stimulating intensity is increased

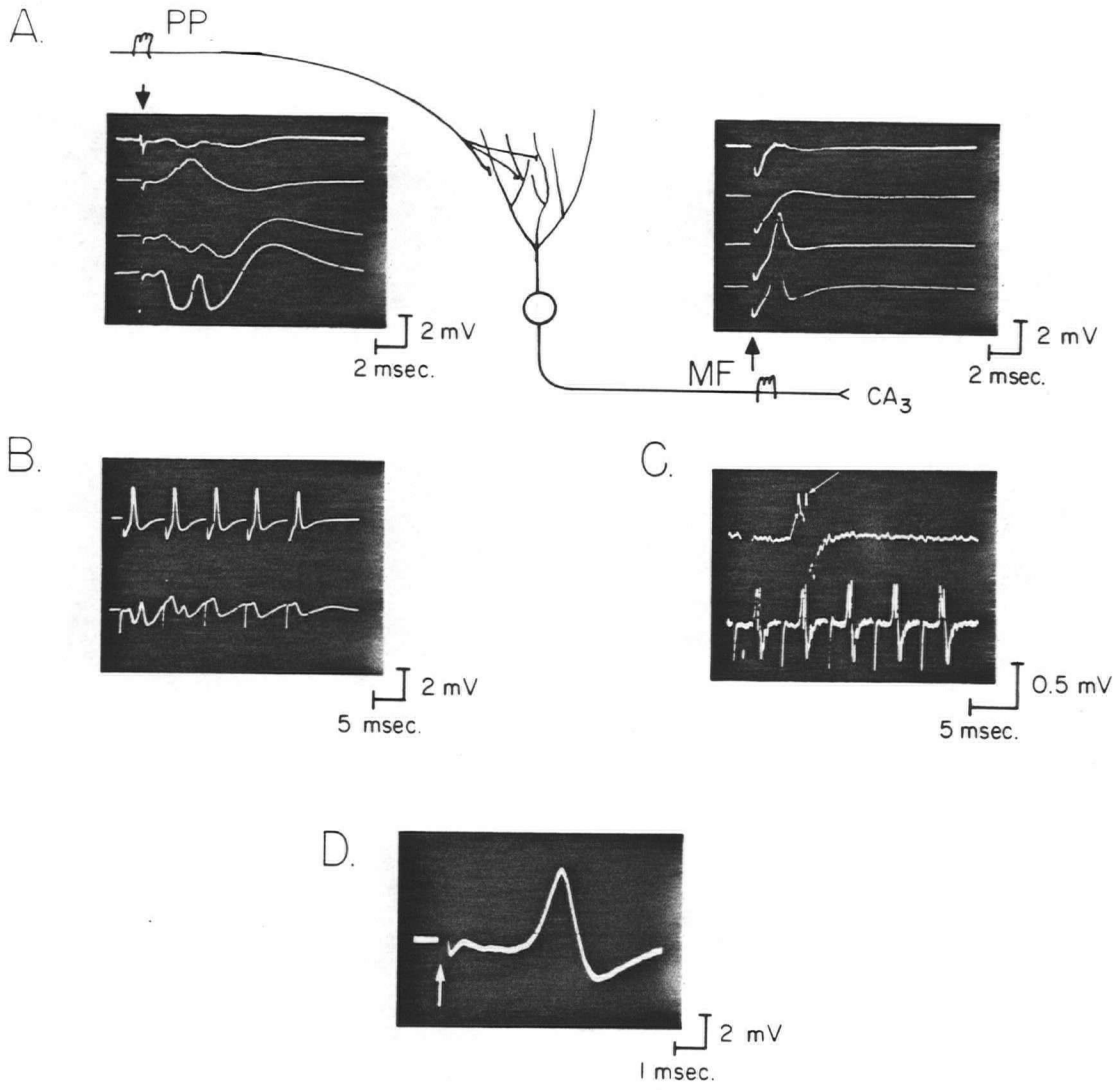
FIG. 6-11: Antidromic And Orthodromic Activation Of Dentate Granule Cells.

A: depth profiles indicating a relationship between the PP population spike (left) and a similar negative response evoked by mossy fibre (MF) stimulation (right).

B: the effects of repetitive stimulation (100 Hz) on the MF (top trace) and PP(bottom trace) evoked responses recorded at the G-cell layer. Note that the amplitude of response evoked by MF stimulation is not attenuated and thus may be antidromic.

C: antidromic activation of G-cell following MF stimulation. The top trace indicates relationship between antidromically activated cell (oblique arrow) and field. Bottom trace indicates that both resonses followed 200 Hz stimulation of MF.

D: MF evoked response at fast sweep speed indicating the consistent latency and amplitude characteristic of antidromic responses (ISI = 1.0 sec).


A.
PP
2 mV
2 msec.
MF
CA3
2 mV
2 msec.
B.
2 mV
5 msec.
C.
0.5 mV
5 msec.
D.
2 mV
1 msec.

the amplitude of N3 also increases. The amplitude of N3 was dependent on the position of the stimulating electrode but no stimulating positions were found that evoked a population spike without the N3 component.

The evoked discharges of neurones were not usually superimposed on the N3 component, although in some cases small spikes were recorded near the peak of this late negative wave (Fig. 6-12). The latency to the onset of these spikes was 9-15 msec and was always longer than that for the monosynaptic population spike. However, it is difficult to assess whether these individual spikes were related to P2 or N3. The same neurones that are evoked near N3 are synaptically activated by mossy fibre stimulation (Fig. 6-12) suggesting that they are not G-cells. Furthermore the amplitude of N3 was significantly reduced by low frequency stimulation (<20 Hz) which potentiated the population spike response and often resulted in the complete disappearance of N3 and the appearance of new negative spikes resembling the N2 component (Fig. 6-12D).

The long latency of the N3 suggests that it may have resulted from stimulating commissural projections coursing through the angular bundle to terminate in the entorhinal cortex. However, a complete transection of the commissural projections in 6 rats (see chapter 7) 10-20 days before the recording experiments did not

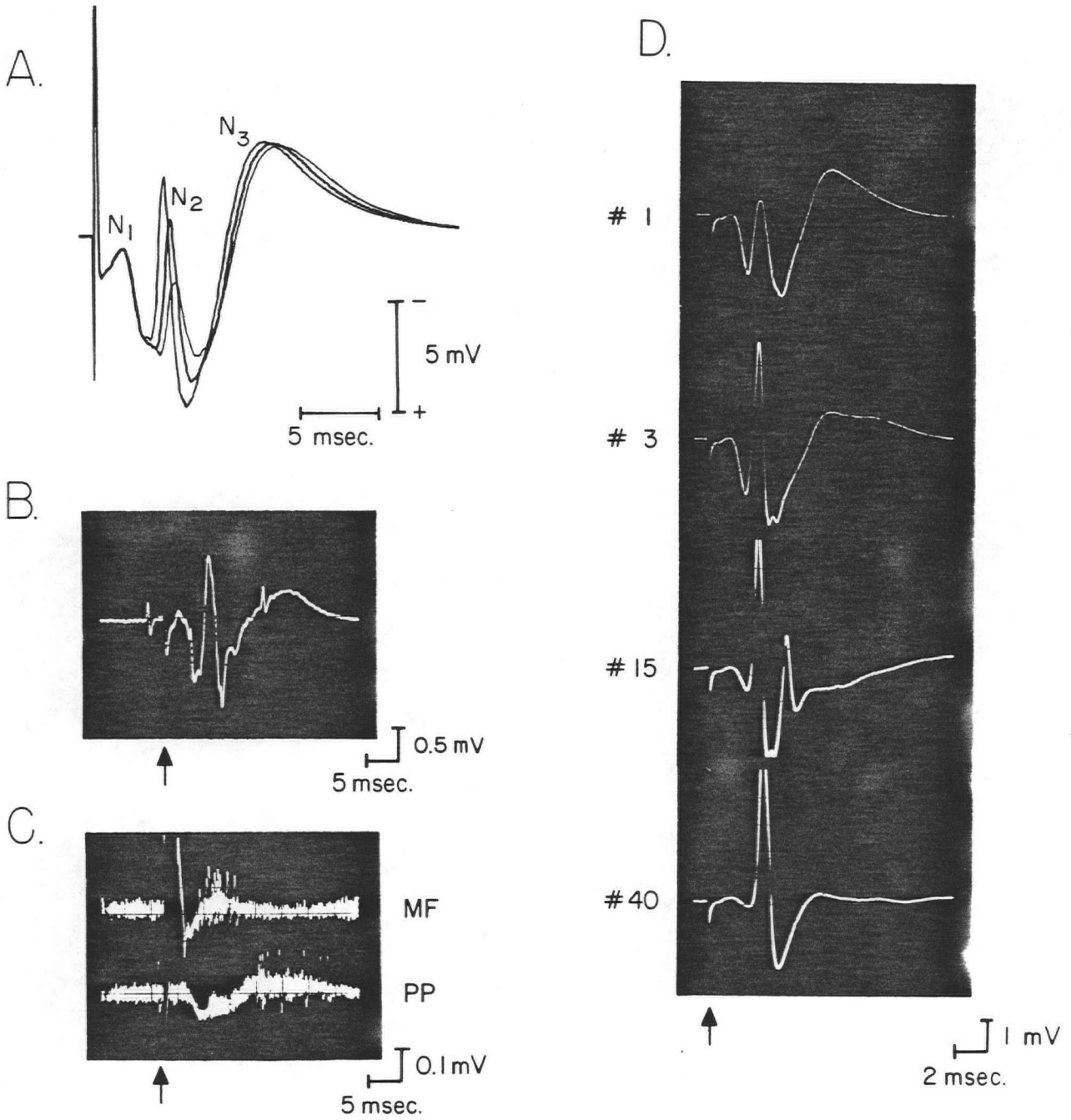
FIG. 6-12: Identification Of The Late Negative Component (N3) Recorded In The Dentate Following Stimulation Of The Perforant Path.

A: relationship between the latency of the population spike (N2) and the later negative component (N3). Note also that the population spike amplitude is more variable than the presynaptic volley (N1).

B: a single unit evoked in the vicinity of N3 following stimulation of PP. This unit was firing spontaneously during the experiment.

C: evoked discharge of a neurone recorded in the dentate hilus following stimulation of PP (2 V) and MF (2.5 V). Note that this neurone is synaptically activated by MF stimulation indicating that it is not a G-cell.

D: effects of frequency stimulation (10 Hz) on the PP-evoked responses indicating fractionation of late negative component and enhancement of the population spike response. The numbers of pulses giving rise to the corresponding response are indicated on the left.


A.
N1
N2
N3
5 mV
5 msec.
B.
0.5 mV
5 msec.
C.
MF
PP
0.1 mV
5 msec.
D.
1
3
15
40
1 mV
2 msec.

eliminate the N3 component.

On the basis of the above data, it is proposed that N3 represents the polysynaptic activation of neurones in the dentate hilus. The observations that it had a longer latency than the population spike and is maximal in the region of the hilus support this suggestion.

The least conspicuous component of the response recorded at the G-cell body layer is the earliest negative component (N1 in Fig. 6-4). Unlike all the components described above, N1 is not always observed. When present,the N1 component was recorded 0.4-0.8 msec before the onset of P1 component and followed stimulation frequencies greater than 200 Hz which blocked the monosynaptic activation of G-cells (Fig. 6-10D). It is likely that N1 component is the extracellular reflection of the perforant path volley recorded in the region of the G-cell layer. This interpretation is in accord with the terminology of Lomo (1971a) and Steward et al (1976).

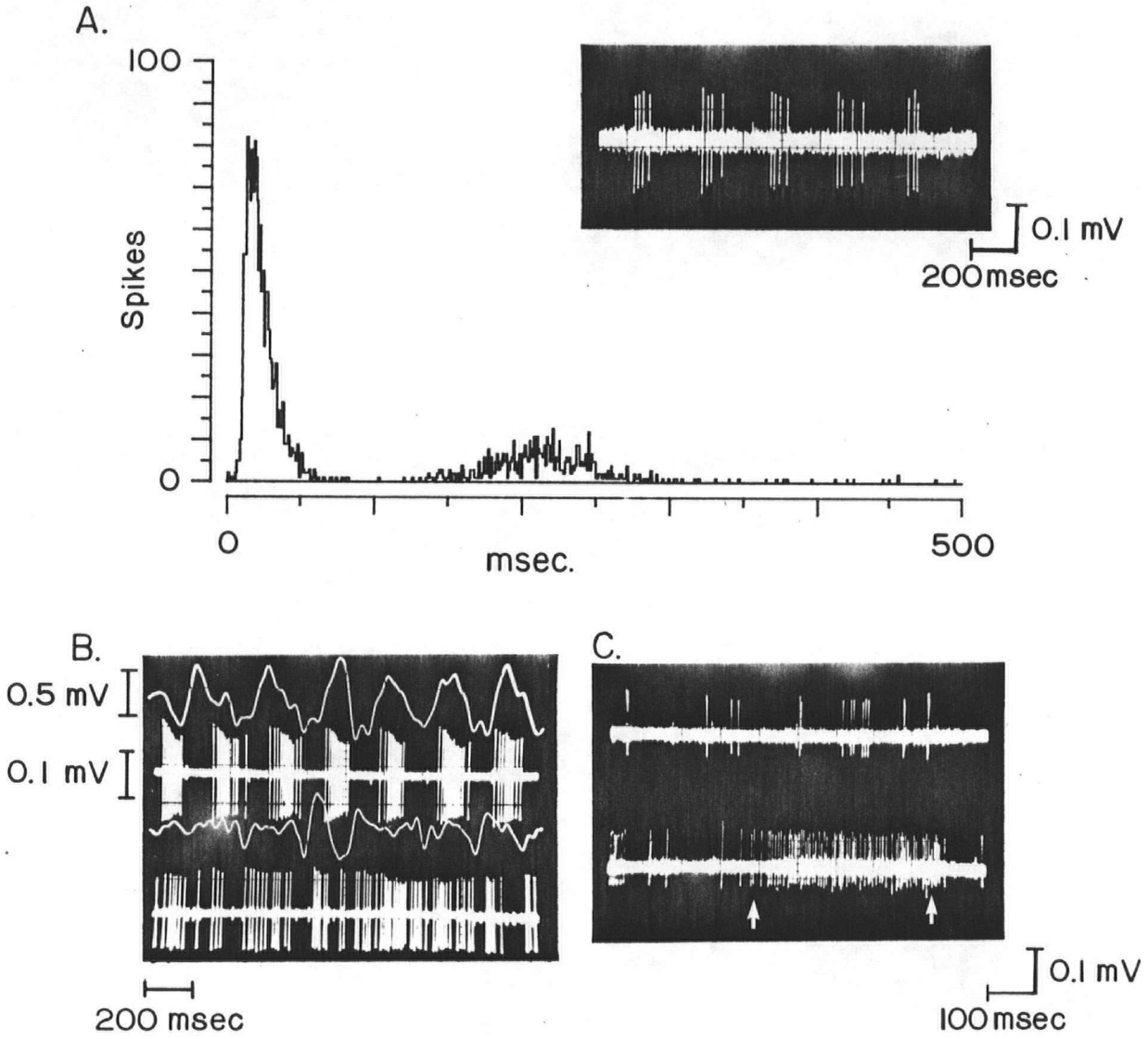
The extracellular field responses recorded in the dentate following stimulation of the PP are summarized in Fig. 6-2. Stimulation of the angular bundle produces a PP fibre volley in the presynaptic terminals. This is followed by the depolarization of G-cell dendrites which is reflected by a large negative field referred to as the population EPSP. The depolarization

FIG. 6-13: Discharge Pattern Of Dentate Granule Cells.

A: interval histogram and corresponding oscilloscope sweep indicating bursting discharge pattern of a unit recorded in the G-cell layer.

B: relationship between the discharge pattern of a dentate cell and slow electrical activity recorded in the vicinity of the microelectrode. The same cell is recorded during RSA (top sweeps) and irregular activity (lower sweeps).

C: a non-bursting dentate unit recorded during sensory stimulation (between the two arrows).


A.
100
Spikes
0
0
msec.
500
0.1 mV
200msec
B.
0.5 mV
0.1 mV
200 msec
C.
0.1 mV
100msec

discharges a proportion of the granule cells causing the negative population spike. Following G-cell discharges neurones in the dentate hilus are activated by mossy fibre axon collaterals giving rise to the late components of the PP-evoked response. The G-cells project via MF on to pyramidal cells of CA3.

C. Single Unit Discharge

Dentate granule cells (G-cells) recorded in urethane anaesthetized rats fire spontaneously in either an irregular or bursting pattern at rates between 2 and 90 Hz. Somatosensory stimulation such as a tail pinch increased the firing rate and evoked a bursting discharge pattern in a proportion (approx.20%) of G-cells (Fig. 6-13). This bursting discharge pattern was related to rhythmical slow activity recorded simultaneously from the vicinity of the microelectrode or from bipolar electrodes in the overlying CA1 region of the hippocampus (chapter 3).

The response of spontaneously firing G-cells consists of single spike activation at a latency of 3-7 msec following stimulation of the perforant path (Fig. 6-14). This short latency activation did not follow high frequency (>100 Hz) stimulation and could vary approximately 0.5-1.0 msec between consecutive stimuli at threshold intensity (ISI=1.0 sec). These monosynaptically activated neurones were identified as

FIG. 6-14: The Response Of An Identified Dentate Granule Cell To Stimulation Of The Perforant Path.

A: top trace indicates spontaneous discharge and bottom trace shows activation-inhibition sequence following single pulse stimulation of PP.

B: fast sweep speed showing single spike activation which preceded the inhibition.

C: corresponding PST histograms of the above unit following stimulation of PP at two different intensities (bin width = 5.0 msec; 50 stimulus presentations in each case).

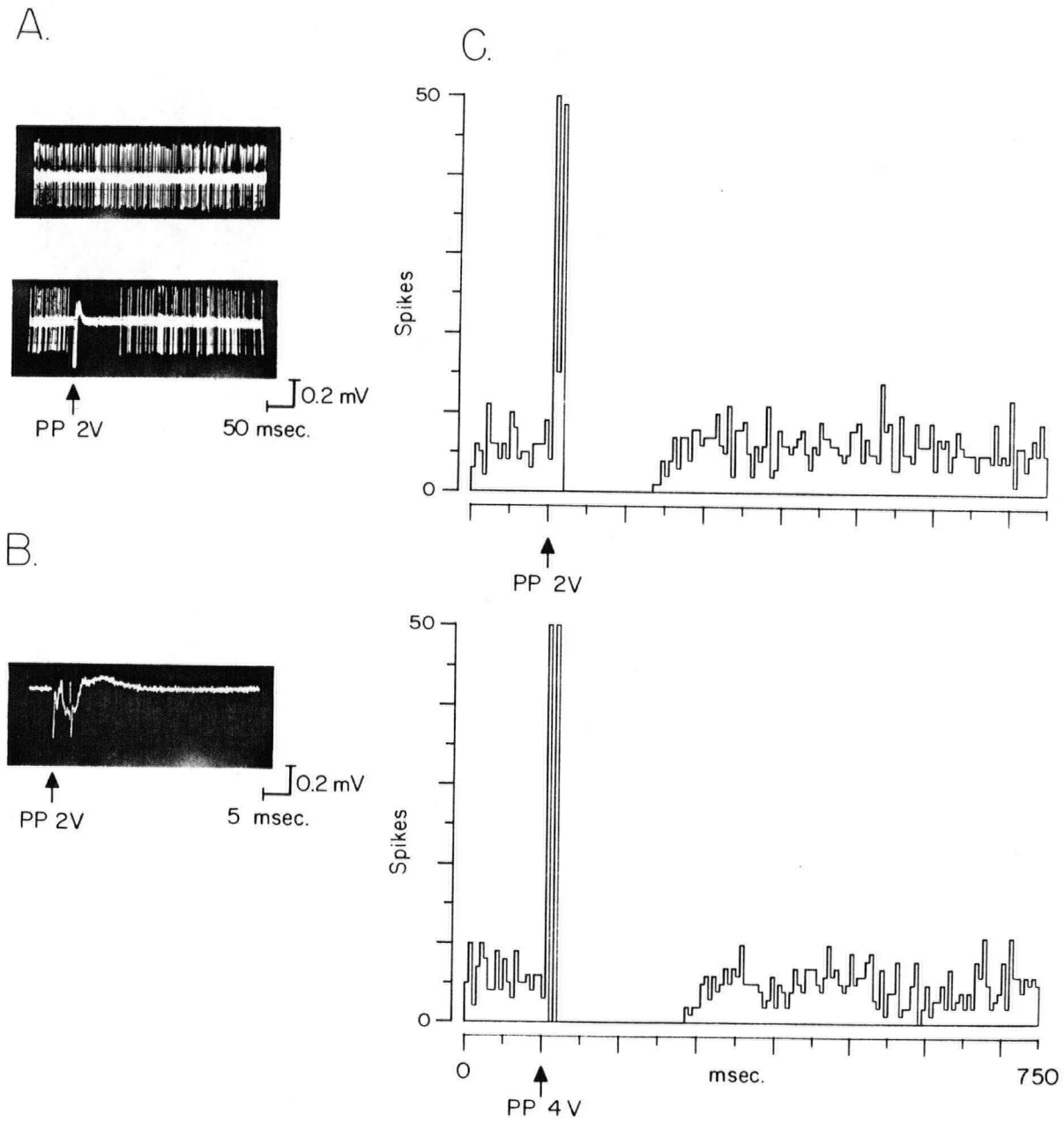

A.
0.2 mV
PP 2V
50 msec.
B.
0.2 mV
PP 2V
5 msec.
C.
50
Spikes
0
PP 2V
50
Spikes
0
0
msec.
750
PP 4V

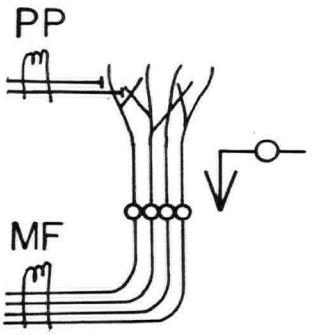
FIG. 6-15: Identification Of Dentate Neurones.

A: schematic arrangement of recording electrode in the G-cell layer and stimulating electrodes in PP and G-cell axons (MF).

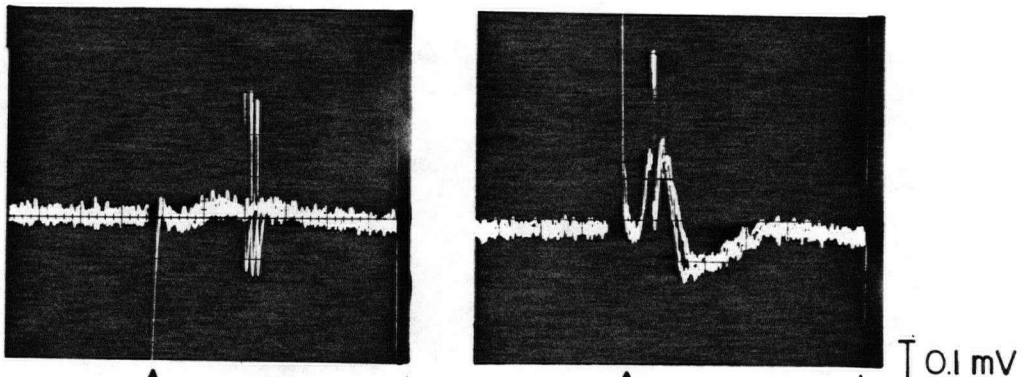
B: response of a dentate cell to stimulation of the PP (left) and MF (right). Note that the response to PP had a variable latency indicating an interposed synapse, whereas, there is minimal latency shift following MF stimulation indicating that it is antidromic. Each record consists of 5 superimposed sweeps.

C: the left photo shows a dentate cell recorded in the same experiment which was synaptically evoked by MF stimulation indicating that this particular unit is not a G-cell. The right photo shows a G-cell which is evoked by MF stimulation on the antidromic field and follows high frequency (200 Hz) stimulation.

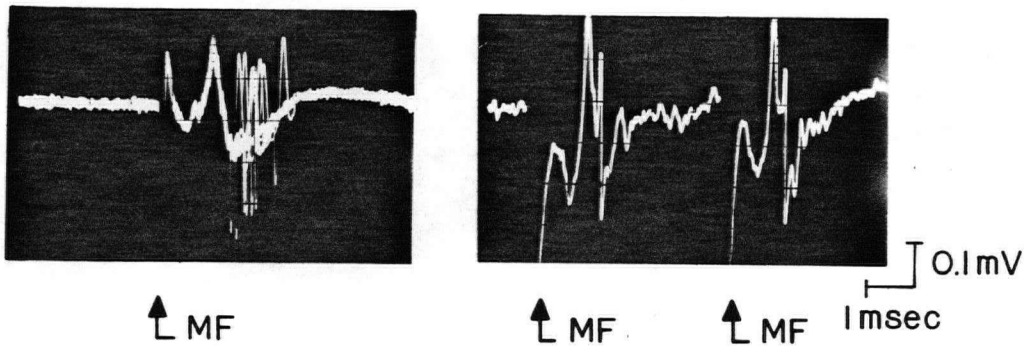
A.



B.



C.



G-cells since 1) they were recorded in the G-cell layer, 2) they discharged at the same latency as the population spike and 3) they were often antidromically activated by MF stimulation (Fig. 6-15). Cells which were not evoked near the population spike and were synaptically activated by MF stimulation were tentatively identified as hilar neurones (Fig. 6-16). The short latency activation of spontaneously firing G-cells was always followed by a period of inhibition lasting 50-300 msec (Fig. 6-14). Individual G-cells which were not activated by low intensity stimulation were still inhibited. In all cases duration of the inhibition could be lengthened considerably (up to 700 msec) by suprathreshold stimulation of the perforant path (Fig. 6-14). Antidromic activation of G-cells was also followed by inhibition having a similar duration. The inhibition of G-cells is postulated to occur as a result of collateral activation of inhibitory interneurones (Gloor, 1963; Andersen et al, 1964).

Multiple discharge of single G-cells in response to PP stimulation was rarely encountered. However, in 3 of 94 experiments the same granule cells were observed to fire twice on the positive field. In these cases low intensity (1-3 V) stimulation evoked a single spike at 3-7 msec latency whereas higher intensity stimulation resulted in the appearance of a second spike 2-3 msec following the first spike. The occurrence of a second

FIG. 6-16: Response Of Hilar Neurones Following Stimulation Of The Perforant Path And The Mossy Fibres.

A: stimulation of either PP or MF synaptically activate the same neurone recorded in the hilar region. The 4 superimposed oscilloscope sweeps in each case indicate that the unit is evoked after the primary response (N2) and shows a variable latency.

B: a hilar neurone which is evoked by PP stimulation and appears on the P2 component of the positive wave. The lower trace at slow sweep speed indicates inhibition of the spontaneous discharge following the initial activation response.

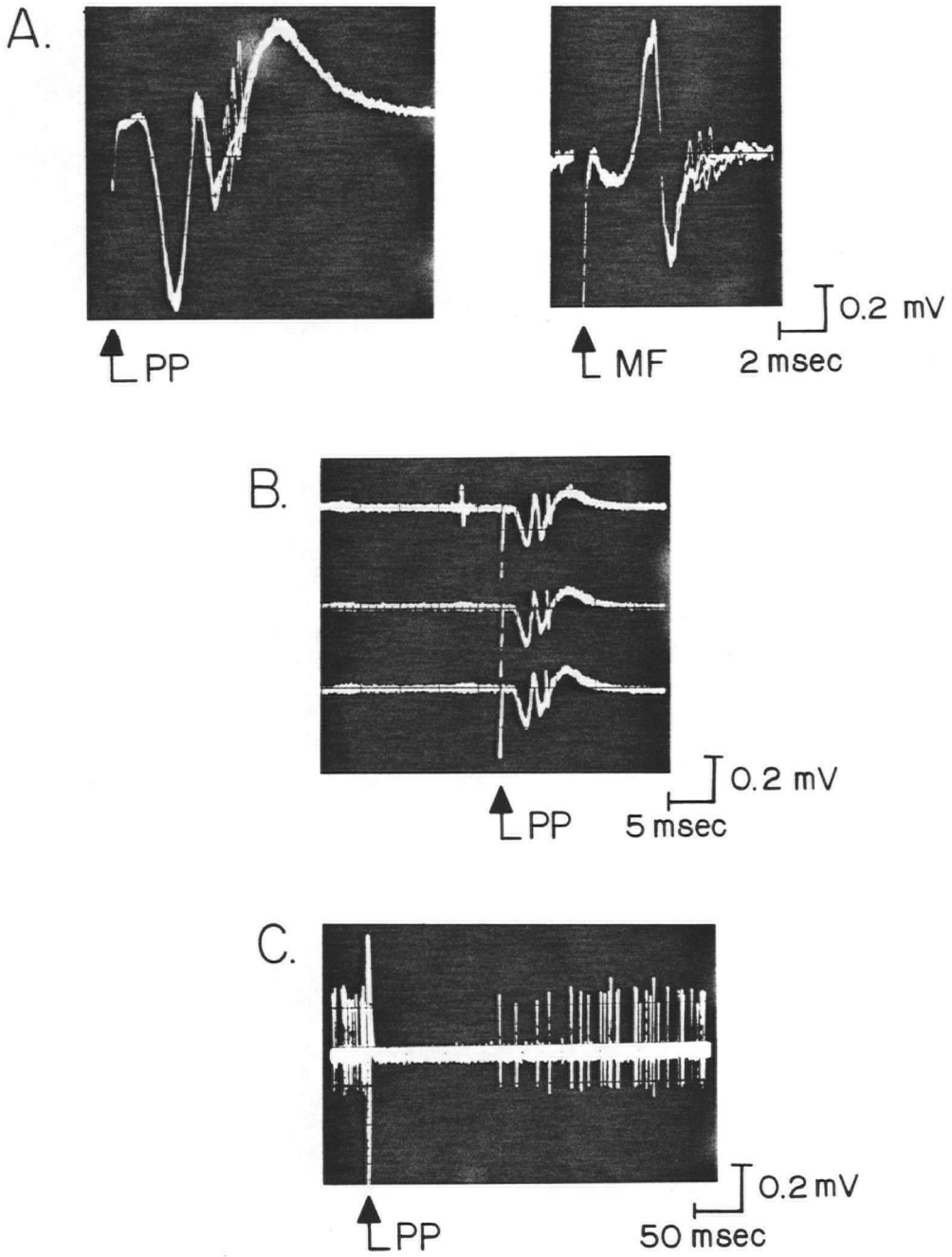
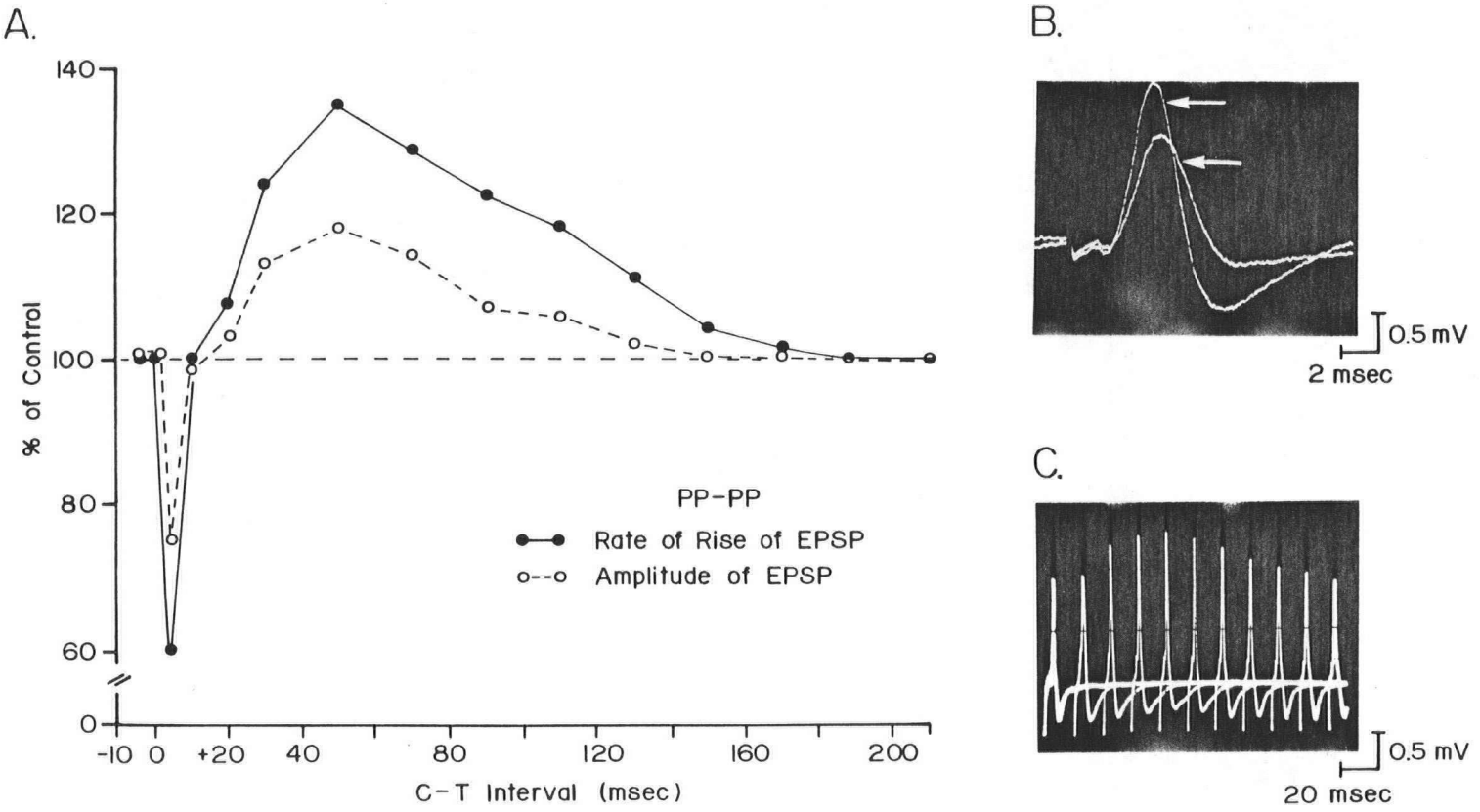

A.
PP
MF
0.2 mV
2 msec
B.
PP
0.2 mV
5 msec
C.
PP
0.2 mV
50 msec

FIG. 6-17: Paired Pulse Potentiation Of The Extracellular EPSP

A: the peak amplitudes (open circles) and rate of rise of the test response measured 1.0 msec after its onset (filled circles) are plotted as a percentage of control responses at the indicated C-T intervals.

B: the negative EPSP recorded in the dendritic layer following conditioning (lower arrow) and test (top arrow) stimulation of the PP at an interval of 35 msec.

C: superimposed oscilloscope sweeps of the condition (first deflection) and the test EPSP's at intervals of 10-100 msec.


A.
140
120
100
80
60
0
% of Control
-10 0 +20 40 80 120 160 200
C-T Interval (msec)
PP-PP
Rate of Rise of EPSP
Amplitude of EPSP
B.
0.5 mV
2 msec
C.
0.5 mV
20 msec

spike was dependent on the position of the stimulating electrode in the angular bundle. Multiple discharge of G-cells may thus be a result of activation of separate afferents from the medial and lateral entorhinal cortex (McNaughton and Barnes, 1977).

Potentiation Of Dentate Extracellular Responses

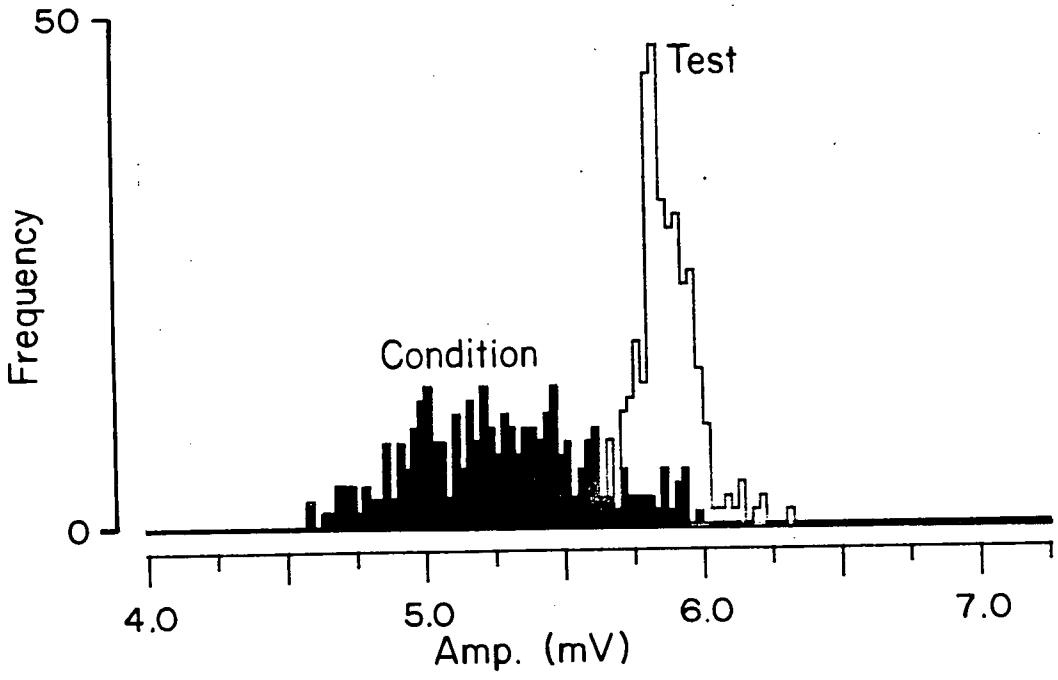
A. Population EPSP

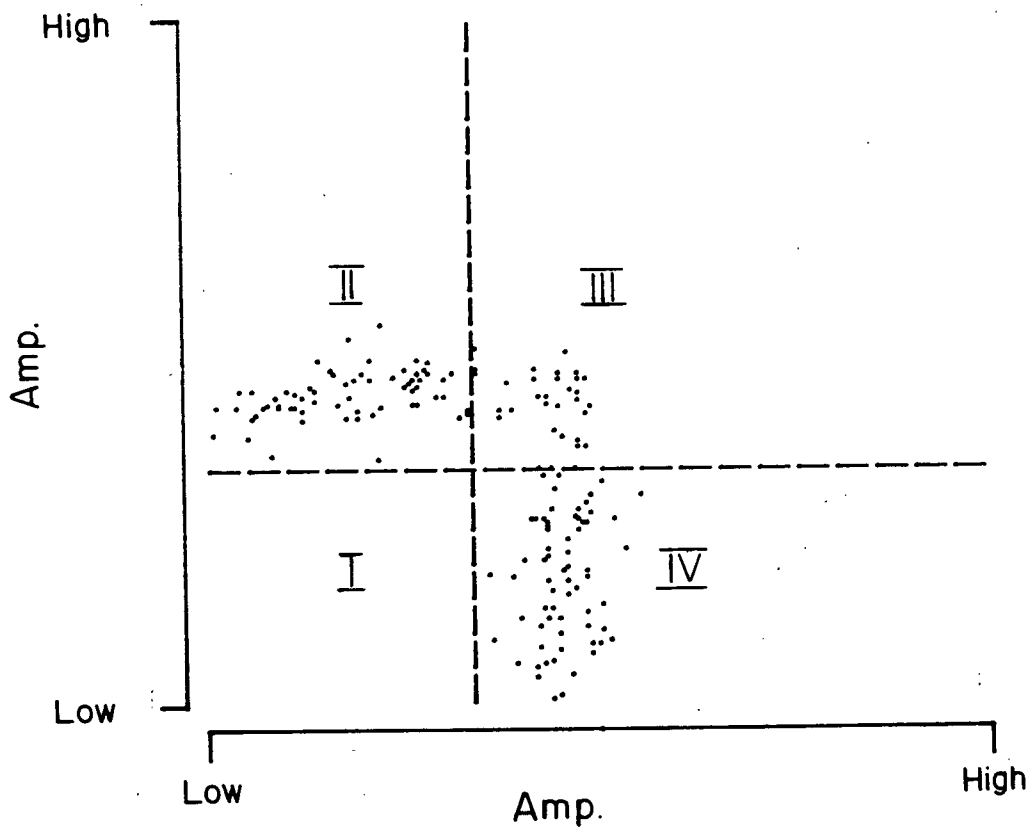
Fig. 6-17 shows the effects of a conditioning PP volley on the amplitude and rate of rise of the population EPSP produced by a subsequent test stimulus to the same pathway. Measurements of the EPSP were made 1.0 msec after its onset to eliminate contamination associated with G-cell discharges (see chapter 2). The response to a single test pulse was compared to that elicited by a conditioning pulse at the indicated C-T intervals. Potentiation of the test response was observed at C-T intervals of 20-200 msec with the maximum increase of approximately 150% of control was obtained at intervals of 30-50 msec. At short C-T intervals (less than 5.0 msec) the size of the test EPSP was much lower than control. This attenuation may be due to refractoriness of the postsynaptic element. It is interesting to note that at the shortest C-T intervals the test presynaptic volley is actually enhanced at a time when the test EPSP is depressed. An enhanced presynaptic volley at these short intervals is

FIG. 6-18: Statistical Analysis Of EPSP Potentiation.

A: distribution of EPSP amplitudes evoked by conditioning and test perforant path volleys. Note that there is less variance in the amplitude of the test EPSP and that the modal amplitude of the test EPSP is greater than the control EPSP.

B: joint amplitude density plot of EPSP amplitudes evoked by conditioning and test perforant path volleys. Note that there are no responses in the first quadrant indicating that the test EPSP was always larger than the control EPSP. See Chapter 2 for description of the analysis.


50
0
Frequency
Test
Condition
4.0
5.0
6.0
7.0
Amp. (mV)


High
Low
Amp.
II
III
I
IV
Low
High
Amp.

most likely due to temporal summation of stimulating currents.

As shown in Fig. 6-18, at a given C-T interval the conditioning and test EPSP exhibited unique amplitude distributions. The fact that at optimal C-T intervals (35 msec in Fig. 6-18) the test EPSP is reliably larger than the condition EPSP is further demonstrated in the joint amplitude density plot (described in Chapter 2) shown in Fig. 6-18B. The 4th quadrant contains the number of trials (76 out of 84) on which a test EPSP was larger than a condition EPSP. In contrast the first quadrant shows that there were no occasions in which a conditioning EPSP was larger than a test EPSP. In two separate experiments in which the variance of amplitudes and latencies of EPSP's evoked by 500 condition and test PP volleys were measured, more variance was observed in the amplitude and latency of the conditioning volleys. The modal amplitude was greater and the latency of EPSP was shorter (by 0.3-0.5 msec) following the test volley.

Leaves 234-235 do not exist.

B. Population Spike

Fig. 6-19 shows the time course of potentiation of the EPSP recorded by one barrel of an electrode which is situated in the synaptic layer and the population spike simultaneously recorded by a second barrel located in G-cell body layer. At short C-T intervals (0-20 msec) the amplitude of the test population spike is depressed whereas at longer intervals it was potentiated 2-3 times control value. It is important to note that the amplitude of the test spike was depressed at short intervals (10-20 msec) when the test EPSP is potentiated. In most experiments the population spike was potentiated for much longer C-T intervals (200-400 msec) while the test EPSP was not significantly different at these periods.

The magnitudes of depression and potentiation of the population spike were dependent on the intensity of the conditioning PP volley. Paired pulse stimulation with low intensity did not give rise to the initial period of depression but produced marked potentiation (Fig. 6-20). In some cases conditioning stimulation did not evoke a population spike response whereas a test volley of the same intensity resulted in a large population spike.

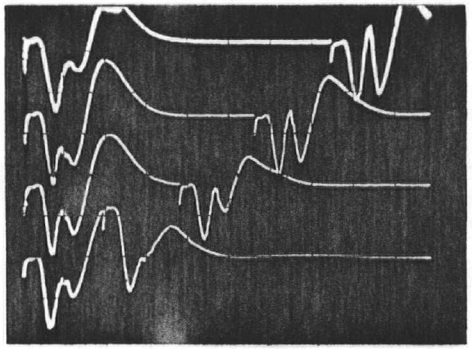
The magnitude of potentiation of the population spike was up to 4 times greater than that of the population EPSP. This difference in the magnitude of

FIG. 6-19: Paired-pulse Potentiation Of The EPSP And The Population Spike Response.

A: the population spike response evoked by a conditioning (first response in each sweep) and test (second response) PP pulses. Note depression of of the test population spike at short C-T intervals (< 20 mSec) and potentiation at longer intervals.

B: amplitude of the test pop spike (filled circles) and rate of rise of EPSP (open circles) plotted as function of C-T interval. Each point is the average of 20 trials.

A.


1 mV
5 msec

B.

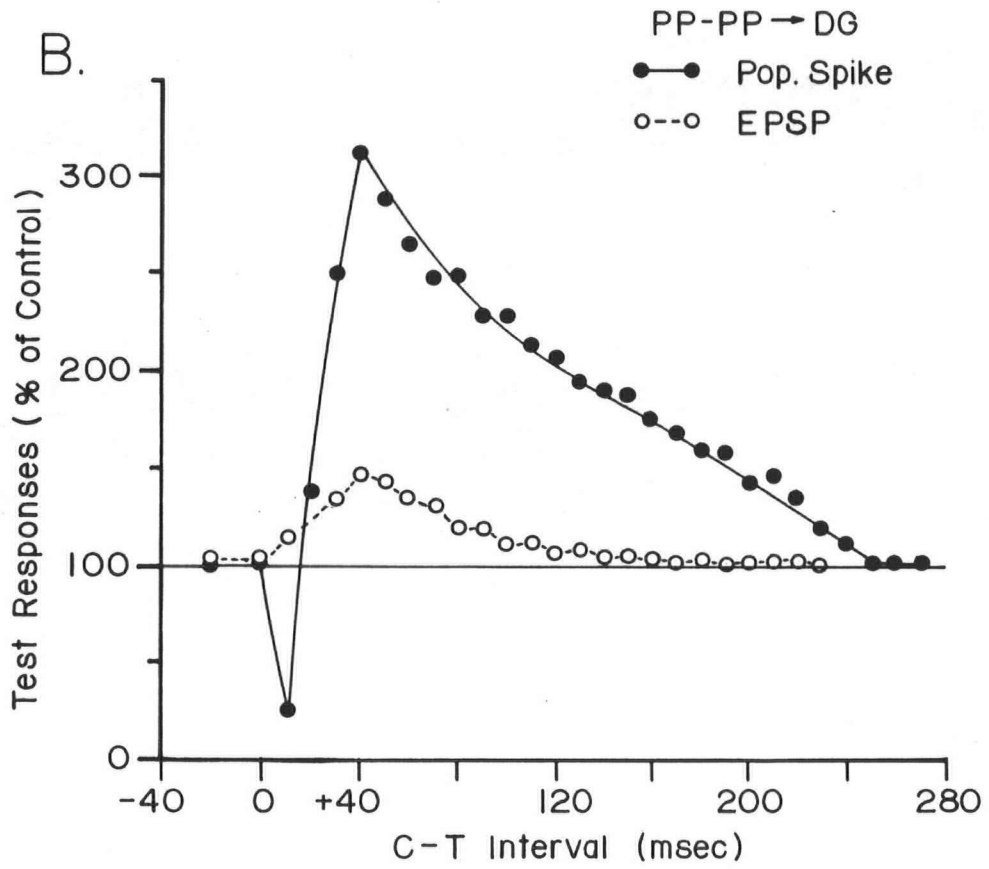
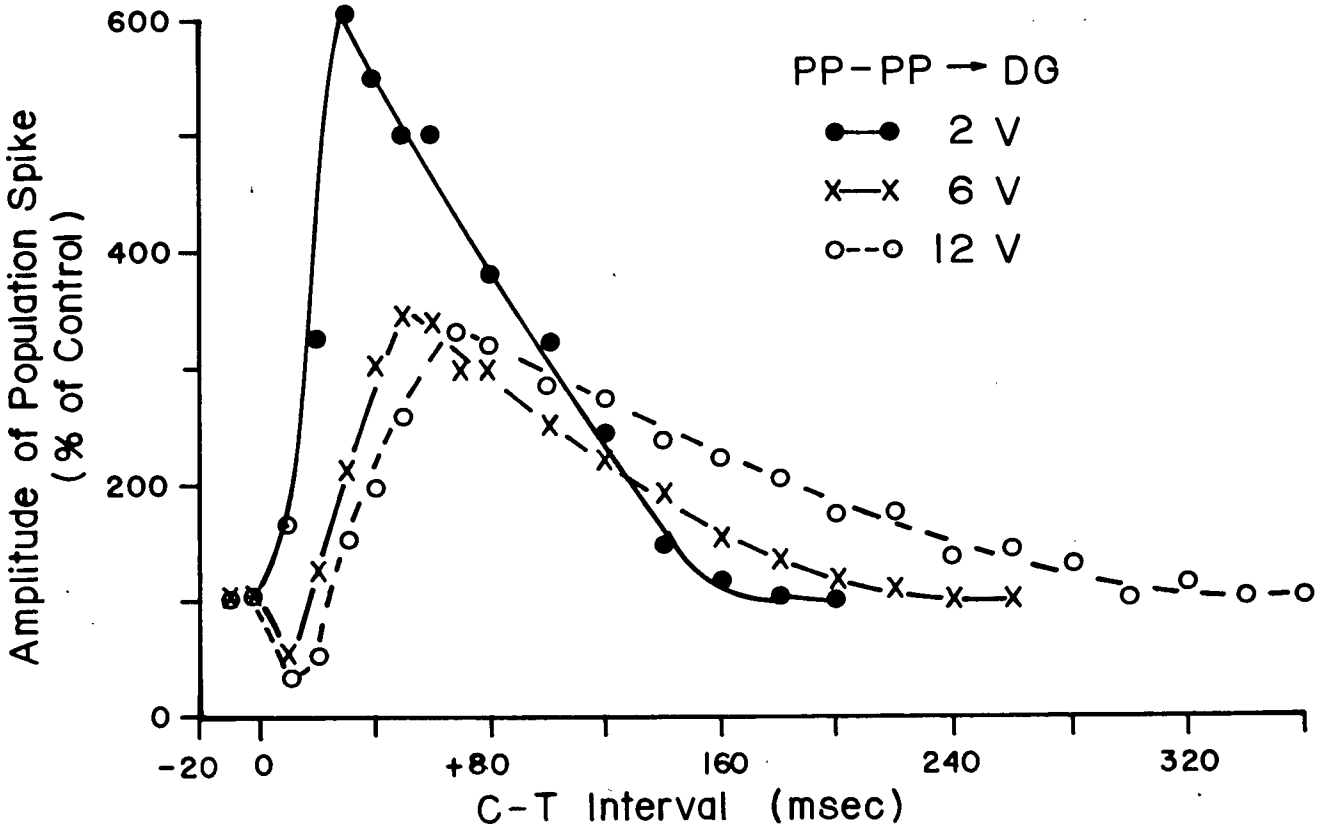

PP-PP → DG
Pop. Spike
EPSP
Test Responses (% of Control)
300
200
100
0
-40
0
+40
120
200
280
C-T Interval (msec)

FIG. 6-20: Effects Of Stimulus Intensity On Paired-pulse Potentiation Of The Population Spike.

Amplitude of the test population spike is plotted as a function of condition-test (C-T) interval. The intensity of both the conditioning and test pulse was increased.


PP-PP → DG
2 V
6 V
12 V
Amplitude of Population Spike
(% of Control)
600
400
200
0
-20
0
+80
160
240
320
C-T Interval (msec)

potentiation was not due to reaching a maximal EPSP amplitude since reducing the intensity of the conditioning and test PP volleys also did not result in potentiation of the EPSP to the same degree as that of the population spike. Furthermore, a larger EPSP than that evoked by the test pulse could be evoked by increasing the intensity of the test volley. Therefore it is unlikely that the lesser degree of potentiation of the EPSP relative to the population spike is due to maximal activation of the postsynaptic elements.

C. Single Unit Discharge

As shown in Fig. 6-21, paired pulse stimulation of the perforant path also resulted in potentiation of single spike discharge. This potentiation occurred either as an increase in the number of neurones elicited by the test volley or a reduced latency to discharge of individual neurones. As shown in the middle trace of Fig. 6-21, spontaneously firing neurones which were not evoked by a conditioning volley of low intensity appear on the positive field produced by the test pulse.

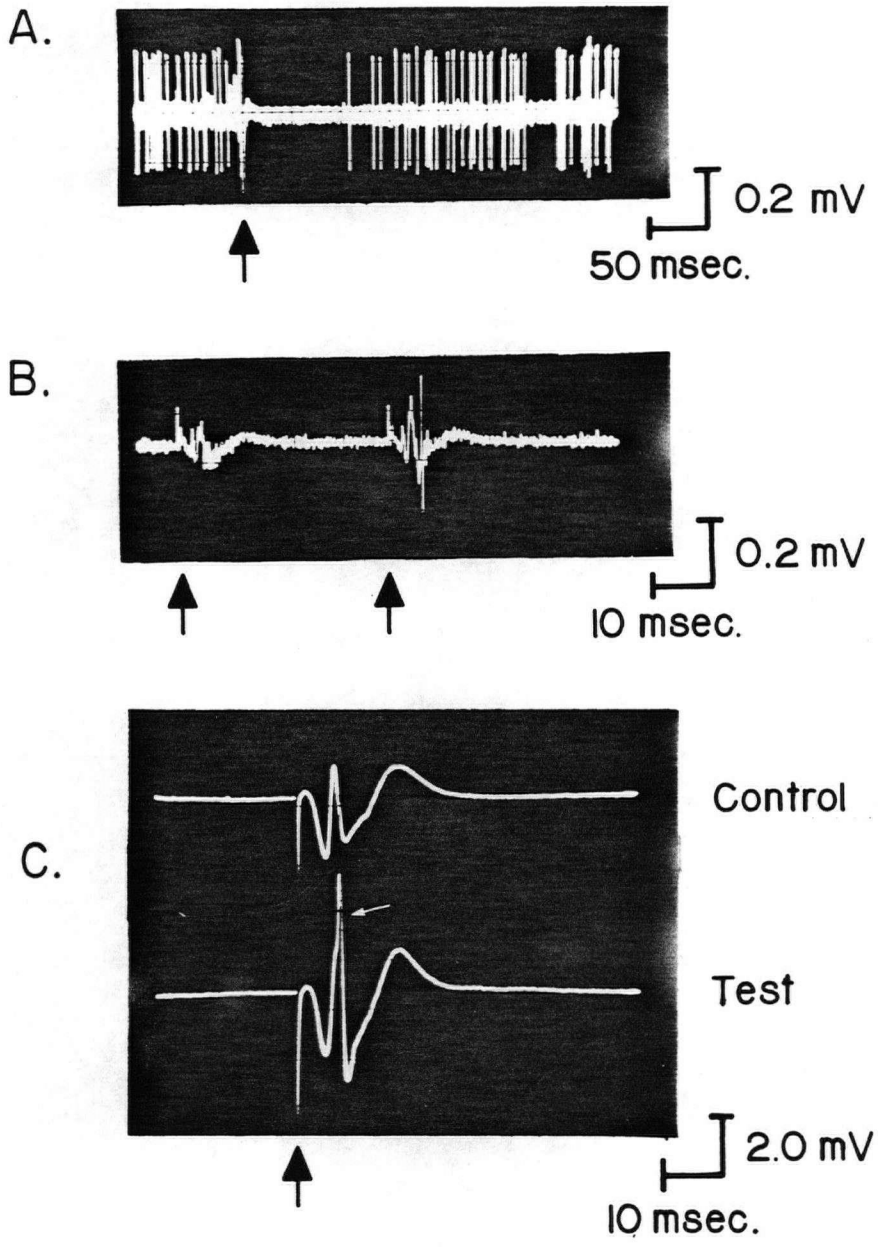
As Fig. 6-21 shows potentiation of individual spike discharge occurred at a time when the population spike was also potentiated inasmuch as a G-cell that was not evoked on the control population spike was evoked coincidently with the potentiated population

FIG. 6-21: Potentiation Of G-cell Discharge By Paired Pulse Stimulation Of The Perforant Path.

A: spontaneously firing G-cell which is inhibited but not excited by a single conditioning PP volley.

B: response of the above neurone to double pulse stimulation indicating that it is evoked by the test and not the conditioning volley.

C: correlation between potentiation of the population spike and single unit discharge (arrow). The unit was not evoked on the conditioning population spike (top sweep) but appears on the test population spike (lower sweep).


A.
0.2 mV
50 msec.
B.
0.2 mV
10 msec.
C.
Control
Test
2.0 mV
10 msec.

spike. As in the case of the population spike response, strong conditioning volleys which evoked a single spike resulted in inhibition of the test spike at short (5-20 msec) C-T intervals. It is noteworthy that at C-T intervals longer than 20 msec potentiation of single spike discharge occurs at a time when the spontaneous discharge of the same G-cell is completely inhibited by the conditioning volley. This suggests that mechanisms underlying potentiation of G-cell discharge can overcome the simultaneously occurring inhibition of G-cells. However at short C-T intervals the potentiation is not sufficient to overcome the potent inhibition.

6.4 Discussion

Field Analysis Of The Perforant Path Input To The Dentate Gyrus

Studying field potentials in an area that has a simple laminar organization allows a comparison between anatomically verified terminal areas and electrophysiological responses. Electrical stimulation of PP results in an extracellular negative field which is maximal in the middle molecular layer. The same zone contains the densest terminal degeneration following entorhinal lesions as observed in light (Blackstad, 1956) and electron (Nafstad, 1967) microscopic investigations in the rat. This extracellular negative field (shown in Fig. 6-22) may represent the summed

EPSP's resulting from the direct action of PP terminals on the dendrites of G-cells since 1) it follows the presynaptic volley by 0.4-0.8 msec which is within one synaptic delay, 2) it follows moderate but not high frequency stimulation and 3) it has been recorded simultaneously with intracellular EPSP's of individual G-cells (Lomo, 1971a). This extracellular EPSP may be due to an active sink restricted to the level of the PP synapses drawing current from passive sources in the area of the cell body. Previous studies have also interpreted the negative response recorded in the molecular layer as synaptic current reflecting the direct action of PP transmitter on the dendrites of G-cells (Lomo, 1971a; Steward et al, 1976).

In the region of the granule cell body PP stimulation evokes a positive waveform which, in part, appears as the mirror image of the extracellular EPSP for which it is a source. Individual G-cells are evoked on the positive field and increased stimulating current synchronously discharges a large number of G-cells giving rise to a large negative spike which is referred to as the population spike response. The observations that the units giving rise to the population spike are G-cells identified by antidromic activation and histological localization of the recording electrode supports the suggestion that the N2 component shown in Fig. 6-4 is the population spike of G-cells. These conclusions are supported by previously published

experiments in rabbits and cats (Gloor et al, 1964; Lomo, 1971a).

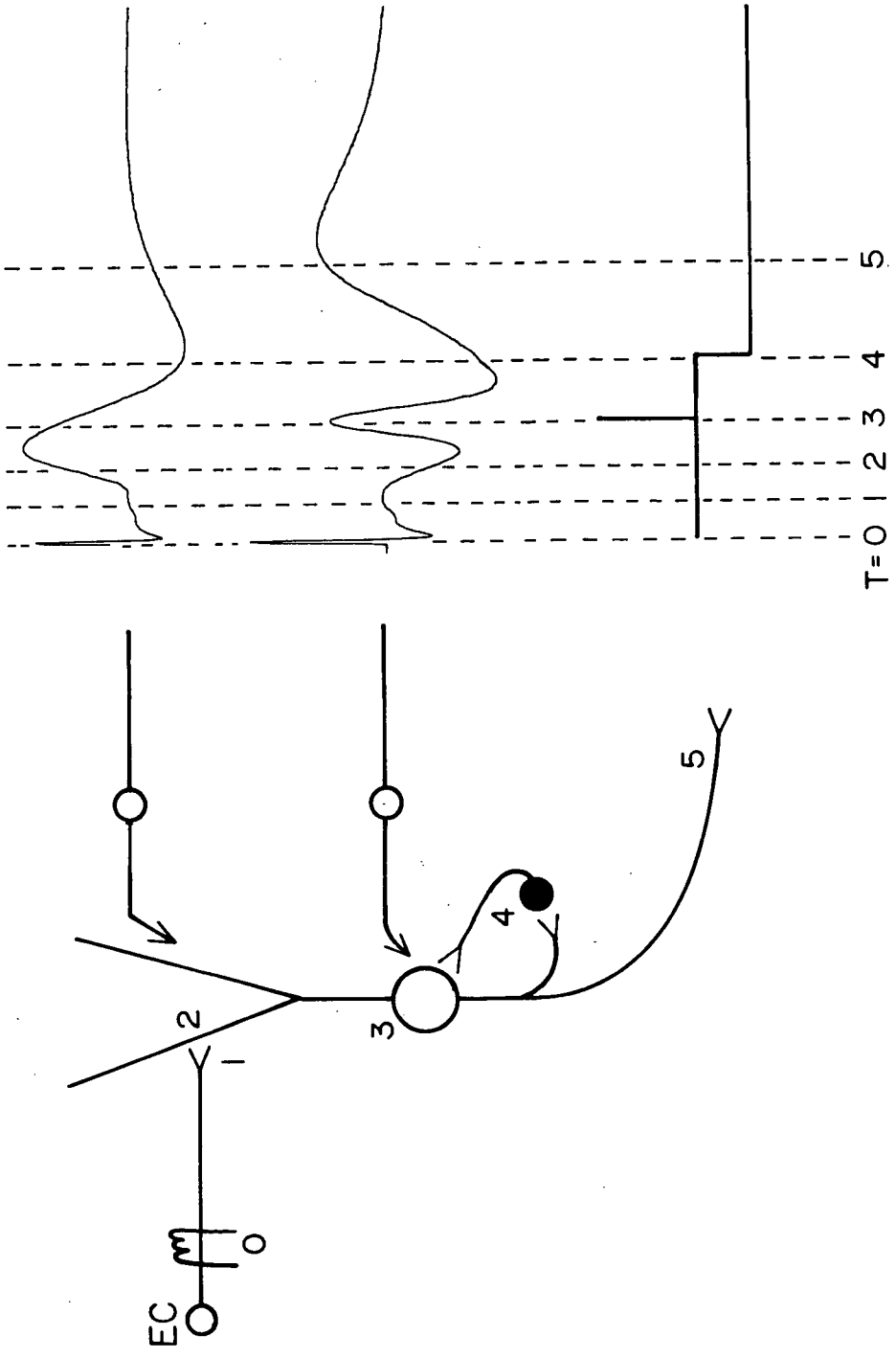
In addition to the monosynaptic activation of G-cells, polysynaptic activation of interneurones via axon collaterals of G-cells give rise to the late components of the field recorded in the area of the cell body layer (N3). The observation that similar late components are recorded following activation of mossy fibres or stimulation of the commissural pathway (see chapter 7), suggests that the late components are not a unique feature of the PP input onto G-cells but may reflect the activity of neurones in the area of the dentate hilus. This may explain the failure to find stimulating sites in the angular bundle which were not associated with late responses and the fact that the late negative component (N3 in Fig. 6-4) was not abolished by lesioning commissural pathways.

Response Of Spontaneously Firing G-cells.

The spontaneous discharge pattern of G-cells consists of both irregular and bursting discharge patterns. The bursting discharge pattern may underly slow rhythmical electrical activity recorded in the dentate (See chapter 3, Bland et al, 1976). Single pulse stimulation of the PP results in single spike activation of G-cells at short latency (3-7 msec) which corresponds to the population spike response. This

FIG. 6-22: Interpretation Of The Responses Recorded In The Dentate Following PP Stimulation.

A stimulating electrode in the PP activates axons originating in the entorhinal cortex (EC) and results in a stimulus artifact recorded in the dentate at time (T)=0. The stimulus propogates to PP terminals and results in a small fibre volley (T=1). The release of PP transmitter causes EPSP's on G-cell dendrites and the associated inward current results in a negative extracellular potential (T=2) in the molecular layer (top profile) and a positive potential at the cell body layer (middle profile). The subsequent discharge of G-cells (bottom schematic) results in a negative population spike (T=3). Activation of interneurones (T=4) by G-cell axon collaterals inhibits the discharge of G-cells and gives rise to the long latency components of the field responses (T=5).


5
4
3
2
1
T=0
5
4
3
2
1
0
EC

activation is followed by prolonged inhibition which may be caused by recruitment of inhibitory interneurones located immediately below the G-cell layer (Andersen et al, 1964). The observation that some cells which are not activated by the PP are still inhibited suggests that these inhibitory interneurones project to neighbouring cells. This is supported by Golgi studies in which the fusiform cells below the granule cells were observed to have long axons which make contact with G-cells up to 1.0 mm away (Cajal, 1911). This potent inhibition which in post-stimulus histograms appears from 0-5 msec following the activation of G-cells may prevent the multiple discharge of G-cells in response to a perforant path volley. The few cases in which the same cell was observed to fire twice on the positive field may be a result of activating two separate afferents coursing through the angular bundle. McNaughton and Barnes (1977) have recently shown that afferents from the lateral and the medial entorhinal cortex which project to the outer and middle molecular layer, respectively, may be activated by angular bundle stimulation. The second spike which occurred 2-3 seconds after the initial spike may be the result of EPSP's generated on the most distal dendrites and would thus reach the soma after more proximal synaptic currents (Rall and Rinzel, 1973).

The Discharge Of Neurones Other Than G-cells

The discharge of neurones other than G-cells may have been recorded in the area of the G-cell body layer and adjacent hilar zone. Unfortunately, recordings were hard to obtain from these neighbouring neurones because they generate small amplitude spikes and may be obscured by the G-cell population responses. In a few cases when stable recordings were obtained from those neurones, converging excitatory inputs from PP and mossy fibres at latencies longer than those of G-cells were readily demonstrated. Those neurones may thus be activated by G-cell axon collaterals. Since Golgi studies reveal a heterogeneous population of cell types in the region of the hilus these neurones are unlikely to produce synchronous population spikes similar to those generated by the uniform discharge of G-cells. It is possible that the cells which were activated by both PP and MF stimulation correspond to the anatomically described fusiform cells which project to the G-cell bodies. These cells would thus be the inhibitory interneurones postulated by Andersen et al (1966) and schematically shown in Fig. 6-22.

Potentiation Of The Extracellular EPSP

The above discussion has presented evidence that the first 1-1.5 msec of the negative dendritic response reflects the synaptic current generated by the perforant path. An increase in the amplitude and rate of rise of this response occurs 5-200 msec following a conditioning pulse to the same pathway. Such potentiation could occur either as a result of an increase in the size of the afferent test volley or due to an increase in efficiency of synaptic transmission between the perforant path and the G-cell dendrites. The first mentioned possibility of presynaptic involvement is unlikely since the amplitude of the PP volley which reflects presynaptic current was not potentiated in this and previous experiments (Lomo, 1971b). However, this argument is based on the assumption that the amplitude of the presynaptic volley reflects the activity of presynaptic terminals in control and potentiated states. The data presented in Fig. 6-4 shows that this is the case in control situations since an increase in stimulus intensity enhances the amplitude of the presynaptic volley and the EPSP. Perhaps in the potentiated state terminals other than those which are activated in control states and which do not give rise to the presynaptic volley are activated. On the basis of the present data one cannot eliminate this possibility.

An increase in the effectiveness of transmission between the perforant path terminals and the dendrites of G-cells may be mediated by an augmented release of transmitter and/or changes in the postsynaptic element which substantially increase the synaptic current generated by the same amount of transmitter. The mechanisms that could give rise to an increase in extracellular synaptic current without involving changes in transmitter release include enhanced sensitivity of the postsynaptic membrane and morphological changes in dendritic spines which affect their current generating characteristics (Rall, 1970). However, the above changes in the postsynaptic elements must be specific to the area adjacent to PP synapses since conditioning stimulation of commissural afferents which terminate adjacent to the PP synapses does not alter the rate of rise of a test EPSP evoked by the PP (Steward et al, 1977; See also Chapter 7).

Potentiation Of The Population Spike

On the basis of the present data and that of Andersen et al (1971) the amplitude of the population spike response is related to the number of individual G-cells which are activated following stimulation of the perforant path. Double pulse stimulation of PP results in a marked increase in the amplitude of the PP spike evoked by the test volley relative to that evoked by the conditioning stimulus. Such potentiation could

occur either as a result of an increase in synaptic current thus producing a greater depolarization of G-cells or by an enhanced probability that a given G-cell is evoked by the test volley. The latter could be due to a lowering of the threshold for the generation of an action potential by an individual G-cell or due to an increase in the effectiveness of dendritic depolarizations reaching the soma.

As discussed in the previous section, there is an increase in the synaptic current generated by the test PP volley which could in part account for potentiation of the population spike response. However, the magnitude of potentiation of the EPSP (less than 150%) was usually much less that the degree of potentiation of the population spike (up to 700%). Furthermore in some animals potentiation of the population spike response was observed in the absence of apparent changes in the rate of rise of the EPSP (See also Lomo, 1971b).

A comparison between the time course of potentiation of the EPSP and the population spike (Fig. 6-19) reveals that the amplitude of test population spike is greater than control at long C-T intervals (over 200 msec) when the test EPSP is not different from control. It is also noteworthy that at short C-T intervals (0-20 msec) the amplitude of the test PP spike is depressed at a time when the EPSP is

potentiated. Depression of the population spike amplitude at short C-T interval may be due to potent recurrent inhibition initiated by the conditioning volley (Lomo, 1971b; Steward et al, 1976). Perhaps potentiation of the population spike at long C-T intervals is due to the action of polysynaptic mechanisms on G-cells.

The relationship between paired-pulse potentiation discussed above and long-lasting potentiation resulting from tetanic stimulation of the PP is not clear. Bliss and Lomo (1973) have shown that the rate of rise of the EPSP and amplitude of the population spike could be potentiated for periods ranging from 30 minutes to 10 hours following a brief train (3-4 sec) of high frequency (100 Hz) stimulation. Bliss, Gardner-Medwin (1973) reported that long-lasting potentiation was not produced on all occasions when there was paired pulse stimulation. Furthermore, these studies (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973) observed increases in the amplitude of the population spike with no change in the synaptic current. This observation and the fact that the magnitude and time course of potentiation of the population spike differ from those of the EPSP suggest that at least a component of long-lasting potentiation may be due to changes in tonic influences acting on the G-cells.

The above suggestions imply that potentiation of

the population spike could occur in absence of changes in transmitter release and may be dependent on the activity of the postsynaptic elements. In order to examine this possibility, the effects of stimulating extrinsic afferents to the dentate on PP-evoked responses will be studied in the remaining chapters.

CHAPTER 7. NEURONAL TRANSMISSION IN THE DENTATE GYRUS: ROLE OF THE COMMISSURAL INPUT

7.1 Introduction

The previous chapter characterized the field responses recorded in the dentate following stimulation of the perforant path (PP). Homosynaptic potentiation of this cortical input involved both an increase in synaptic current and amplitude of the population spike response. It was suggested that the increased efficacy of the PP input may could be a result of changes in the postsynaptic cell in addition to those postulated to occur in the presynaptic elements of this pathway.

Since the termination of the commissural (COMM) input to the dentate gyrus is restricted to a zone extending 50-100 um above the G-cell layer and just below the PP synapses (Blackstad, 1956; Gottlieb and Cowan, 1973), activation of the COMM input may generate synaptic current at sites adjacent to PP synapses. This arrangement allows an examination of the possibility that conditioning COMM stimulation potentiates PP-evoked responses.

The aim of the present chapter is to: 1) determine the laminar profile of synaptic currents recorded in the dentate following stimulation of the COMM input. 2) examine whether conditioning stimulation of the COMM input alters the field responses evoked by a test PP

volley.

7.2 Experimental Procedures.

Extracellular recordings were performed on a total of 15 urethane anaesthetized rats. Concentric bipolar electrodes were positioned in the angular bundle to activate the perforant path and in the contralateral CA3-CA4 area of the hippocampus to activate the commissural projection at its origin. In some experiments an additional bipolar electrode was positioned in the commissural pathway as it courses through the ventral psalterium (1.5 mm posterior to bregma; 0.5-2.0 mm lateral; 3.0 mm below the cortical surface). Single unit activity and field potentials were recorded from the dentate as described in the previous chapters. The responses to 20-30 stimulus presentations (ISI 2.5-4.0 seconds) were averaged and plotted at predetermined depths to allow a comparison between the field responses evoked by stimulation of PP and COMM pathways.

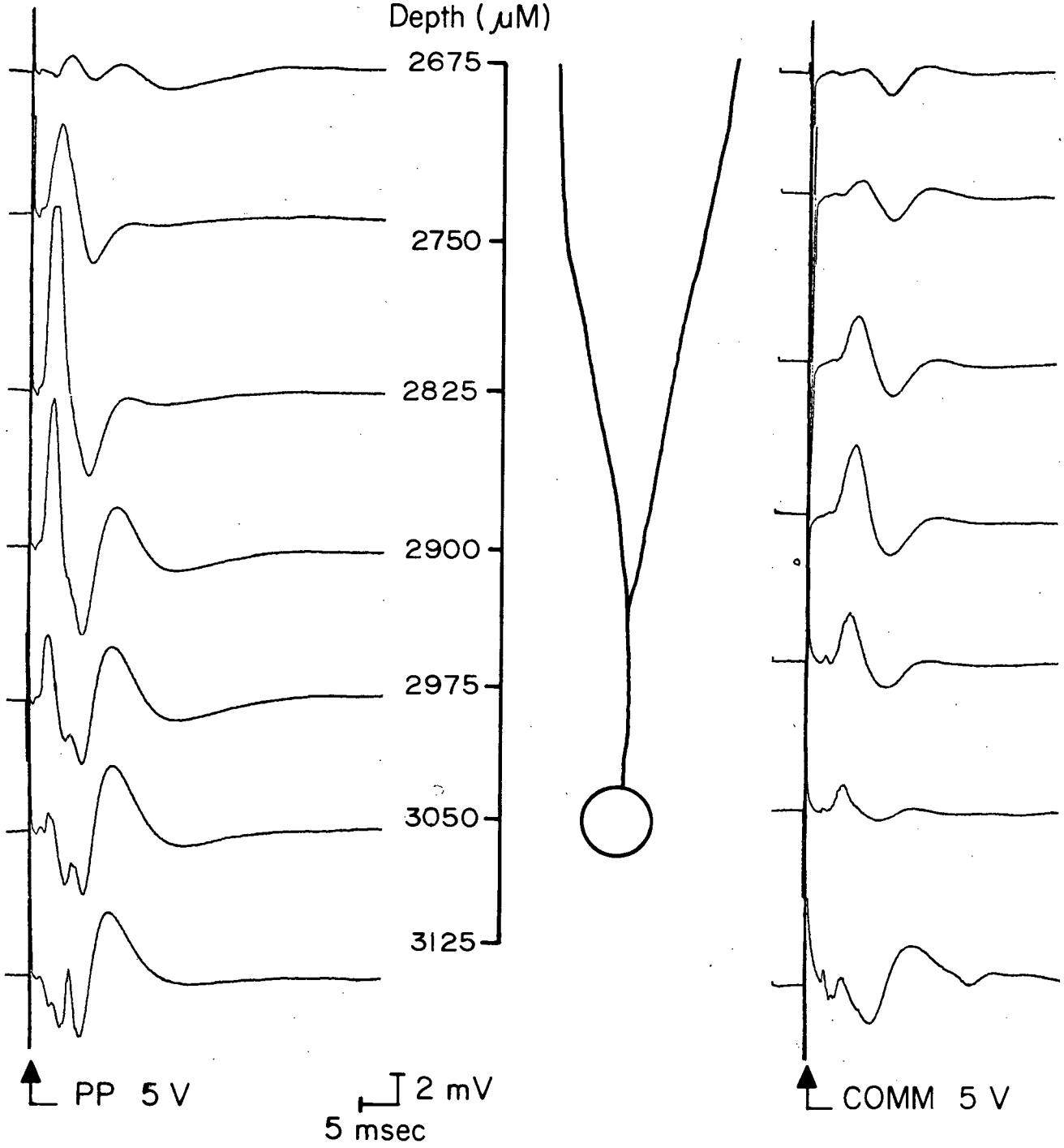
7.3 Results

Fig. 7-1 shows the extracellular field potentials recorded at indicated depths in the dentate following stimulation (5 V) of the PP and Comm pathways. As shown in the previous chapter, PP stimulation results in a short latency negative wave which is maximal 175-225 um above the G-cell layer and a positive wave with a superimposed population spike recorded at cell body layer. COMM stimulation also results in a short latency (4-7 msec at its peak) field response in the dentate. When the recording electrode is situated in the most distal dendrites a low amplitude positive waveform is recorded. This positive wave reverses to a negative wave at the level of the PP synapse and reaches a maximum negativity 100 um above the G-cell layer. As the recording electrode is advanced further towards the G-cell layer the extracellular negativity reverses to a positive wave at approximately 25 um above the cell bodies.

FIG. 7- 1: Laminar Profiles Of The Field Potentials Evoked In The Dentate By Stimulation Of The Perforant Path And Commissural Pathway.

Field potentials evoked by PP (left) an COMM (right) volleys recorded in the upper blade of DG along a vertical track at the indicated depths below the surface of the cortex.

Each record is the average of 20 trials and the arrows indicate the stimulus artifacts.


Depth (μM)
2675
2750
2825
2900
2975
3050
3125
PP 5 V
2 mV
5 msec
COMM 5 V

Identification Of The Dentate Responses

A. Molecular Layer

Fig. 7-1 shows that the most prominent response in the molecular layer is a negative field which is maximal in the inner molecular layer at a distance of 75-125uM above the G-cell layer. The onset of this field occurs 3-5 msec after stimulation of the contralateral CA3 or 2-4 msec following direct activation of the COMM pathway in the ventral psalterium (1.5 mm from the midline). In the same animal the onset and peak latencies of this negative wave are consistent and latency shifts greater than 0.5 msec were rarely encountered. This negative response followed moderate (1-75 Hz) but not high (>200 Hz) frequency stimulation.

Since this negative wave is maximal in the inner molecular layer at the same level as the termination of the COMM pathway (Gottlieb and Cowan, 1973; Hjorth-Simonsen and Laurberg, 1978), it may be the extracellular reflection of a monosynaptic EPSP reflecting the depolarization of apically oriented dendrites. Although intracellular data is lacking, the similarity between this negative dipole and that produced by the PP (described in chapter 6) supports this suggestion. Since the maximal negativity is 75-125 um above the G-cell layer, the depolarizing

synaptic current may be generated by the proximal dendrites of G-cells. A similiar interpretation was previously proposed by Deadwyler et al (1975). However, the possibility that these currents are generated by other structures such as the distal dendrites of neurones whose cell bodies are below the G-cell layer can not eliminated.

Long Latency Responses

As shown in Fig. 7-2, the above short latency responses were sometimes (6 of 15 animals) followed by secondary responses having onset latencies between 15 and 20 mSec. Since these late responses display a greater shift in latency than the earlier components and do not follow 20-50 Hz stimulation (Fig. 7-3B) they may be evoked polysynaptically. The laminar profile of the late component was not similar to the earlier responses evoked by COMM stimulation but was related to the PP-evoked profile (Fig. 7-2B). However, similar late components were never observed following stimulation of the PP itself.

The late components were completely eliminated by a transection of the ipsilateral perforant path. As shown in Fig. 7-3C, if the knife cut is made between the entorhinal cortex (EC) and the PP stimulating electrode, the late COMM response disappears whereas the PP response is normal. Hjorth-Simonsen (1973) has

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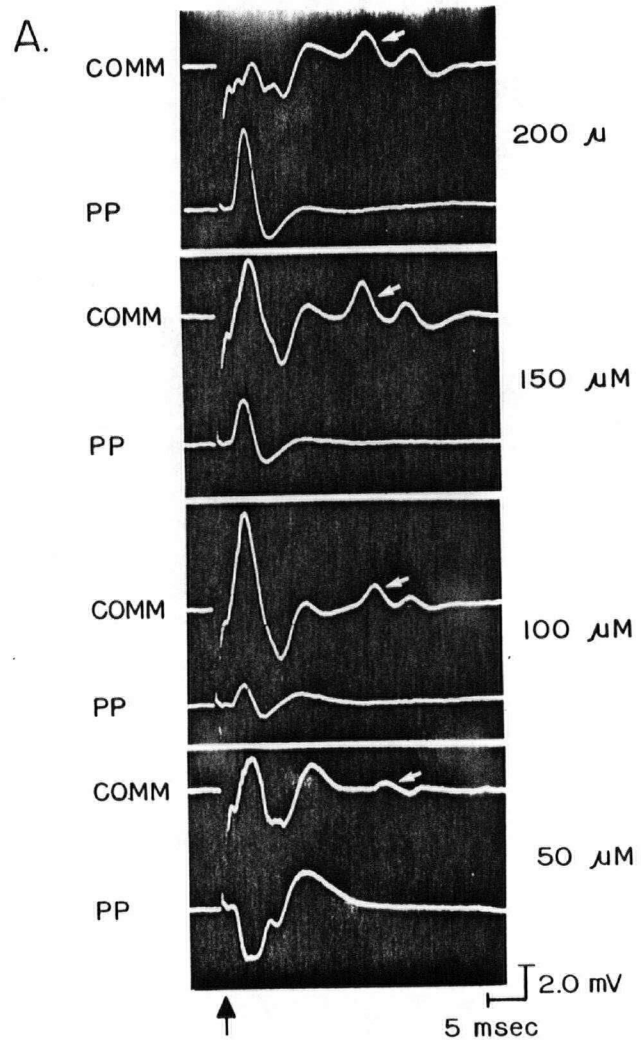
FIG. 7-2: Laminar Profiles Of Primary And Secondary Field Potentials Recorded In The Dentate Following Stimulation Of The CGMM Pathway.

A: depth profiles recorded at the indicated distance above the granule cell layer following stimulation of the PP (lower oscilloscope sweeps) and COMM pathway in the ventral psalterium (upper oscilloscope sweeps). Note the appearance of secondary responses (oblique arrows) following COMM stimulation.

B: profile of the amplitude of the early (COMM1) and late (COMM2) evoked potentials measured 1 msec after their onset. Profile of PP evoked potentials is included for comparison. Note that the late COMM2 response parallels the PP profile.


A.
COMM
PP
200 μ
150 μM
100 μM
50 μM
2.0 mV
5 msec

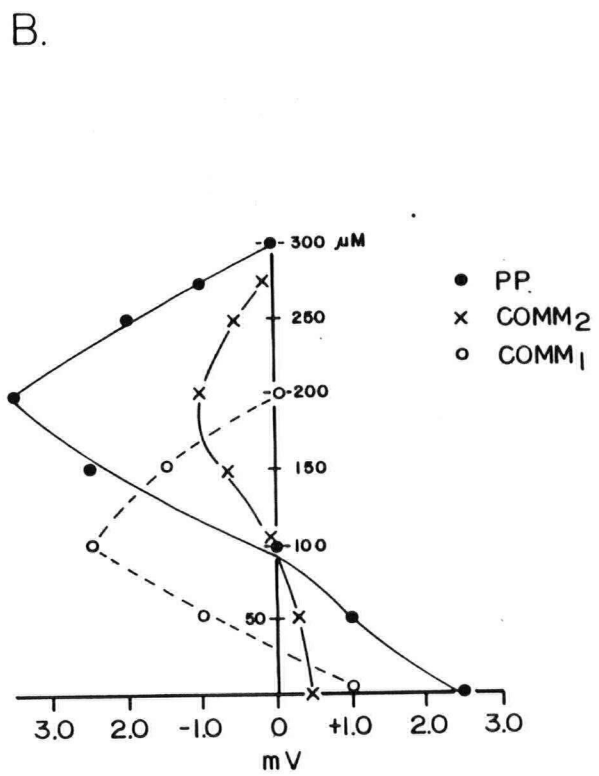
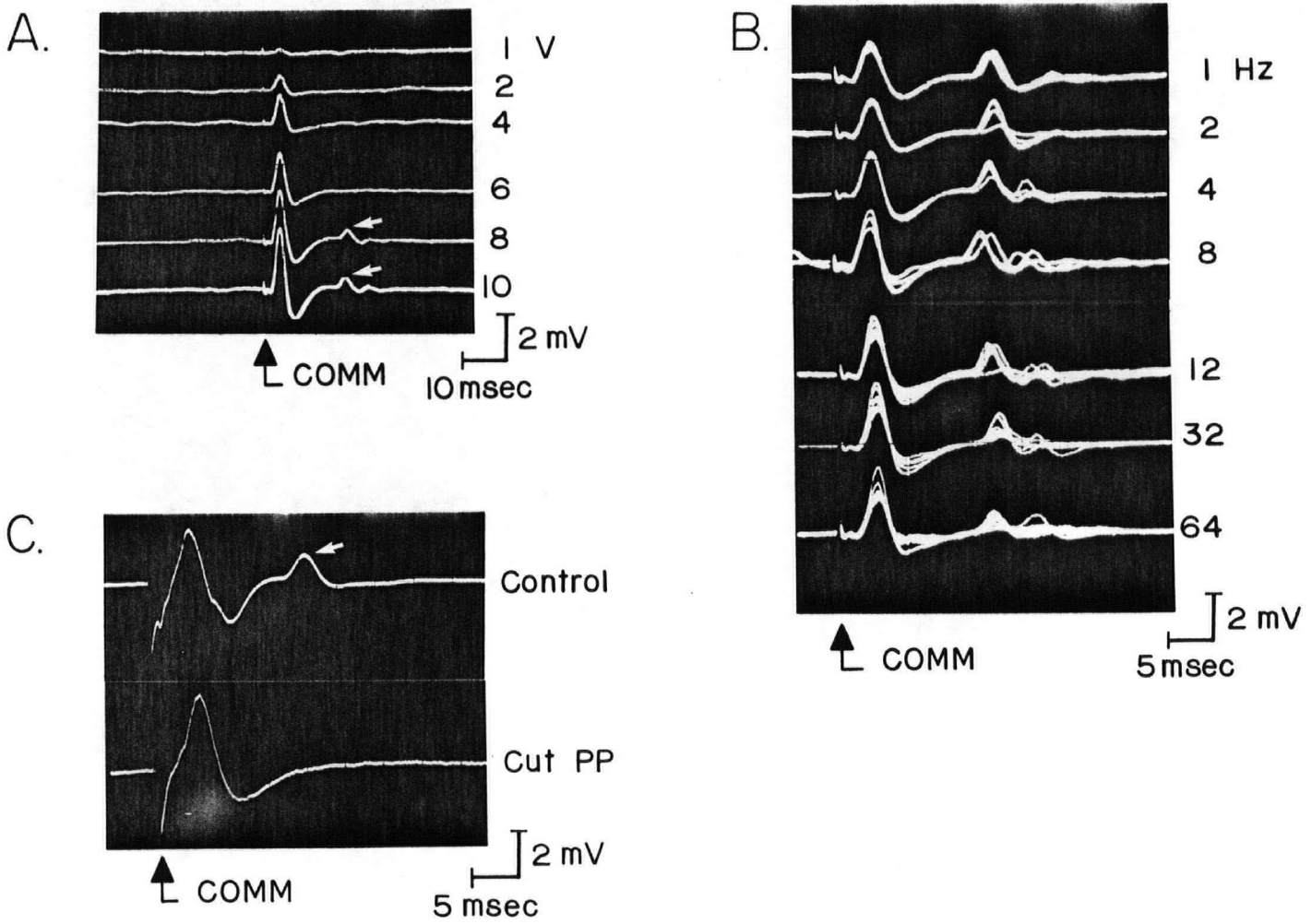

B.
300 μM
250
200
150
100
50
PP
COMM2
COMM1
3.0
2.0
-1.0
0
+1.0
2.0
3.0
mV

FIG. 7- 3: Characteristics Of Early And Late Field Responses Recorded In The Dentate Following Stimulation Of The Commissural Pathway.

A: effects of stimulus intensity on the amplitude of responses recorded in the molecular layer. Each record is 5 superimposed sweeps at the indicated stimulus intensity. Note that the late components are only produced at higher voltages.

B: effect of frequency stimulation on the early and late responses indicating the polysynaptic nature of the late response since it does not follow moderate (40-80 Hz) stimulus frequencies.

C: elimination of the late component but not the early response following transection of the PP suggesting that the late response is polysynaptically relayed via the entorhinal cortex.


A.
1 V
2
4
6
8
10
2 mV
COMM
10 msec
B.
1 Hz
2
4
8
12
32
64
2 mV
COMM
5 msec
C.
Control
Cut PP
2 mV
COMM
5 msec

Leaves 267-270 do not exist.

provided anatomical evidence for a direct projection from CA3 to the ipsilateral EC which gives rise to the PP. Thus the late responses could result from activation of the entorhinal cortex and subsequent depolarization of G-cell dendrites. This possibility is strengthened by the observations of Deadwyler et al (1975) that stimulation of CA3 evokes unit discharges in EC which precede the late dentate responses by 1.5 msec. Although no single EC units were recorded in the present investigation field responses which precede the dentate late component by 5-7 msec were recorded in the EC of 1 animal. Although Deadwyler et al (1975) suggest that the ipsilateral entorhinal cortex was activated directly by contralateral hippocampus, it is also possible that antidromic activation of axon collaterals of the ipsilateral CA3 results in activation of the EC on the same side. The latter possibility is more likely since there is no direct anatomical evidence for a contalateral projection.

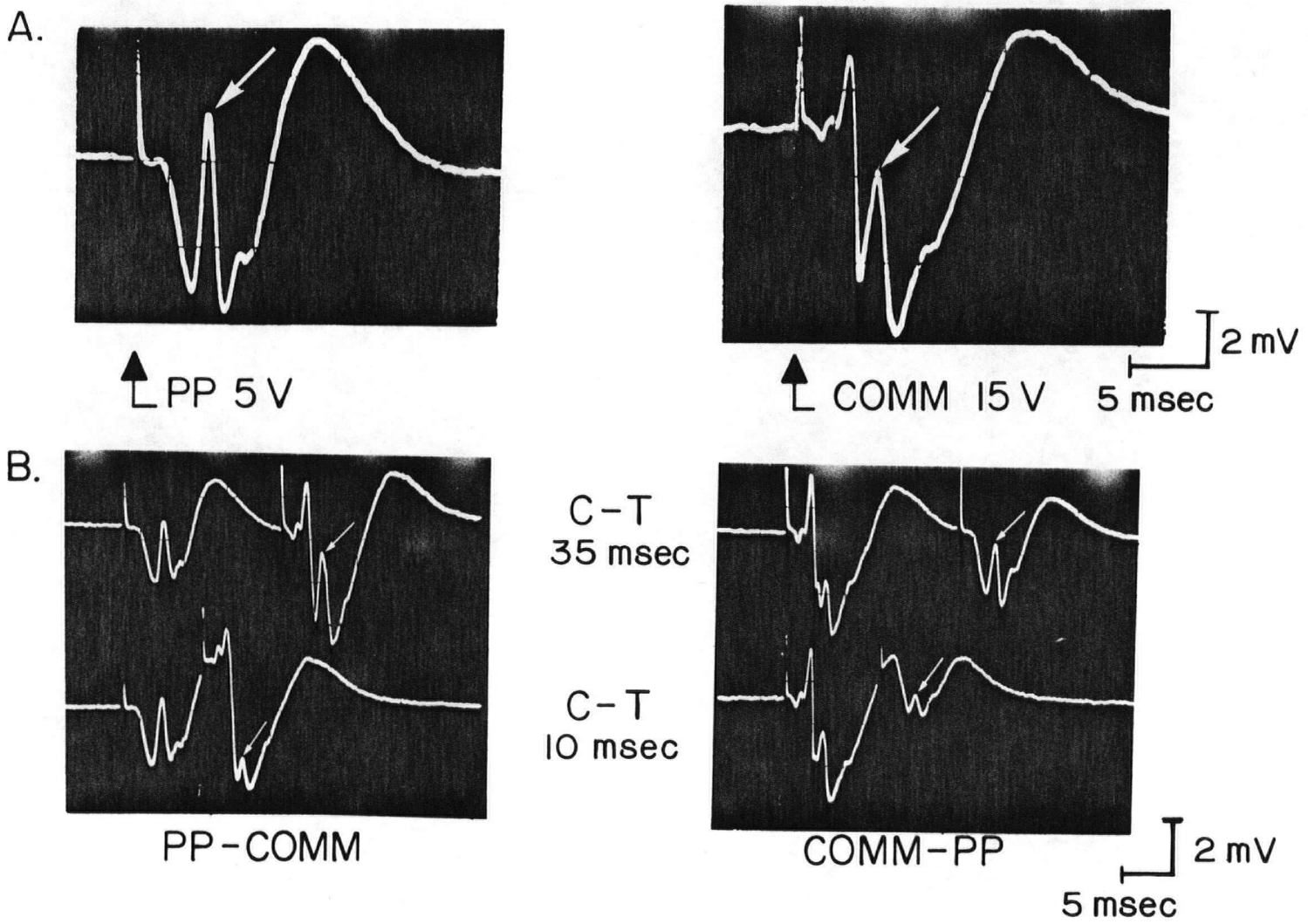
B. G-cell Layer

Fig. 7-4 shows the extracellular field potentials evoked by COMM and PP stimulation and recorded in the cell body layer. An early negative deflection is recorded at the same latency as the presynaptic volley which was observed in the molecular layer and 0.5-0.8 msec before the onset of the earliest cell discharge or compound spikes (indicated by oblique

FIG. 7- 4: Field Potentials Recorded In The Granule Cell Layer Following Stimulation Of The Perforant Path And Commissural Pathways.

A: comparison between the responses evoked by PP and COMM stimulation at the indicated voltages. In each case a negative population spike is indicated by the arrow.

B: interaction between PP and COMM evoked responses at the indicated C-T intervals. Note depression of the negative spike at the shorter C-T interval suggesting convergence of the two inputs on the units giving rise to this response.


A.
PP 5 V
COMM 15 V
2 mV
5 msec
B.
C-T
35 msec
C-T
10 msec
PP-COMM
COMM-PP
2 mV
5 msec

arrows in Fig. 7-4). It may thus represent the presynaptic volley of the COMM pathway. The reason that it is more prominent than the PP fibre volley recorded from the same site may be related to the observations that COMM fibres pass through the G-cell layer en route to the inner molecular layer. In 2 of 15 animals a prominent spike was recorded at the same latency (2.5 mSec) as the fibre volley. This spike was less than 0.2 msec in duration and may thus be caused by a single axon.

Following the fibre volley a large compound spike (oblique arrow) is sometimes (in 4 of 15 animals) recorded in response to COMM stimulation. However, the stimulating voltages required to produce a compound spike was 3 times higher than those required to evoke a prominent dendritic potential. The amplitude of the compound spike was maximal at the same depth as the PP-evoked population spike in the G-cell layer. The COMM-evoked spike was markedly depressed when preceded (1-20 mSec) by a PP-evoked population spike. On the other hand, if a COMM volley precedes PP stimulation the PP-evoked population spike is depressed. The compound spikes recored following commissural stimulation may reflect the synchronous discharge of a population of dentate neurones and will thereby be referred to as the COMM-evoked population spike. It is noteworthy that the COMM evoked population spike always occurs early on the descending limb of the positive wave, whereas, the PP

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Leaves 275-276 do not exist.

evoked population spike may occur late on the descending limb or on the ascending limb. This may be due to a shorter conducting distance between COMM synapses on proximal dendrites than PP synapses on distal dendrites.

The last negative component has not yet been identified but its similarity to the N3 component following PP stimulation suggests that it also represents the polysynaptic activation of neurones in the dentate hilus.

C. Single Unit Discharge

The response of 60 spontaneously firing dentate cells was studied following stimulation of both the PP and COMM pathway. Of those, 11 (18%) were activated 4-8 msec following stimulation of the COMM pathway (5-10 V) and 21 (35%) were activated by the perforant path (3-5 V). The PP was always stimulated with lower intensity in order to avoid population spikes which even in a filtered record obscured the discharge of individual neurones. As shown in Fig. 7-5, convergence of COMM and PP inputs on 9 neurones was demonstrated by pairing the two inputs at sub and suprathreshold intensities and a range of condition-test intervals. Two subthreshold stimuli paired at 1-5 msec intervals resulted in a spike following the second pulse (Fig. 7.5B, bottom trace) suggesting that

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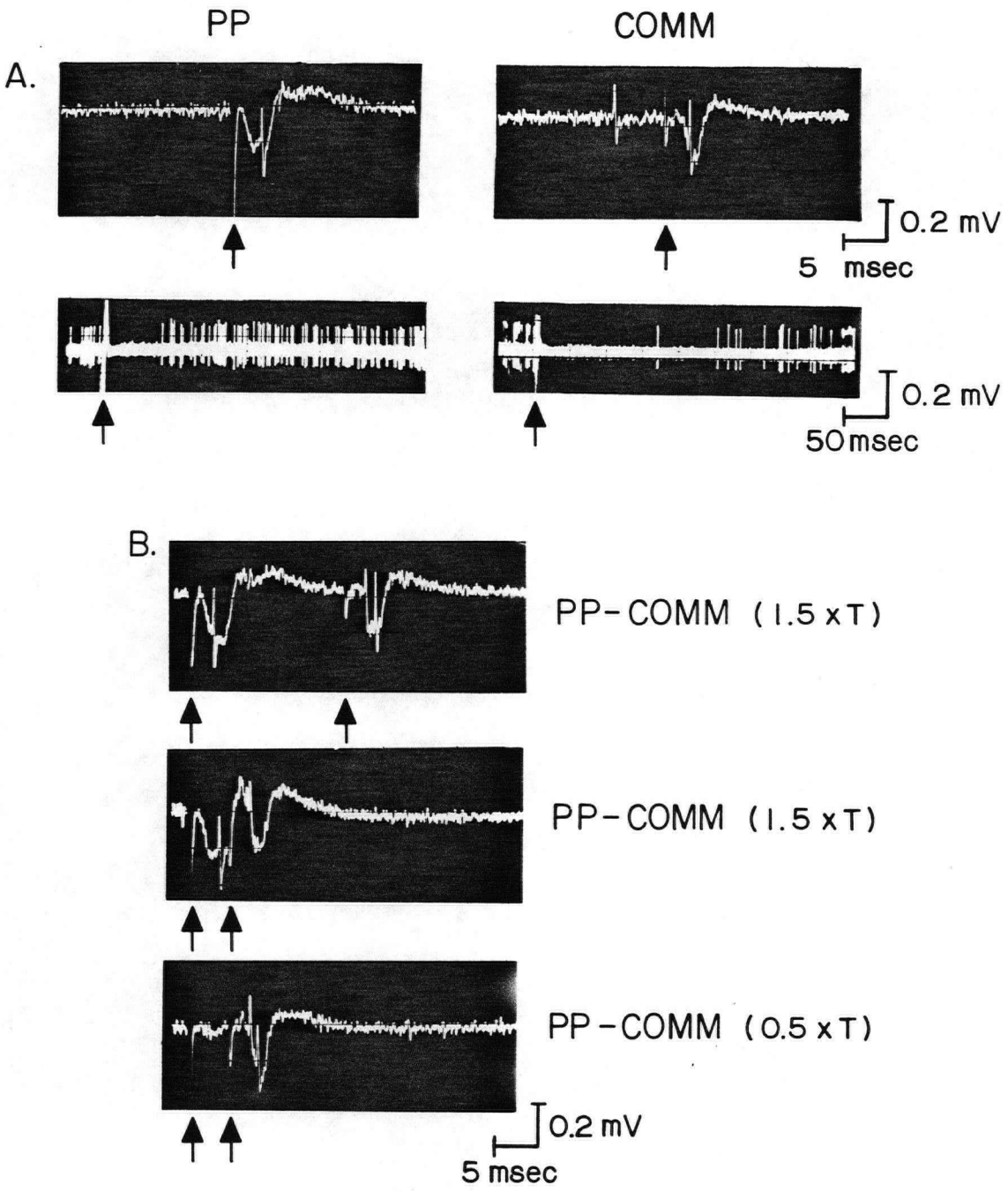
FIG. 7- 5: Response Of Spontaneously Firing Dentate Neurones Following Stimulation Of The Perforant Path And Commissural Input.

A: top: fast sweep speed indicating single spike activation of the same neurone following stimulation of PP (left arrows) and COMM (right arrows). bottom: same cell at slow sweep speed indicating inhibition following the activation. Note that COMM stimulation produced longer inhibition.

B: interaction between PP and COMM stimulation indicating possible convergence of the two inputs on the same neurone shown in A. Note that both pulses at suprathreshold intensity elicit a spike (top) but when the C-T interval is shortened the response to the second pulse is occluded (middle). In contrast, a spike is only elicited following the second of two subthreshold pulses (bottom).


PP
COMM
A.
0.2 mV
5 msec
0.2 mV
50 msec
B.
PP-COMM (1.5 x T)
PP-COMM (1.5 x T)
PP-COMM (0.5 x T)
0.2 mV
5 msec

LEAVES 280 AND 281 OMITTED IN PAGE NUMBERING.

temporal/spatial summation had occurred. However, pairing two suprathreshold pulses at the same critical interval resulted in a spike following the first but not the second pulse (Fig. 7-5; middle trace) due to either refractoriness of the neurone or recurrent inhibition which is described below.

The short latency activation of spontaneously firing neurones elicited by COMM stimulation was always followed by a prolonged period of inhibition lasting 50-800 msec. Spontaneous units (49 out of 60) that were not activated by COMM stimulation were still inhibited by low intensity stimulation (1-3 V) of the COMM pathway. The length of inhibition following stimulation of the COMM pathway at 1.5T intensity required to activate a neurone was usually 2-3 times longer that elicited by stimulation of the PP at the same relative intensity (1.5 X threshold).

Origin Of The Commissural Pathway.

Histologically verified sites for evoking the responses described above were located in the CA3-CA4 subfield of the contralateral dorsal hippocampus . Responses having the shortest latency and maximum amplitude were obtained following stimulation sites which were homotopic to the recording electrode. If the stimulating electrode was moved 0.5-1.0 mm from this optimal site a positive-going wave was recorded in the

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Leaves 283-286 do not exist.

dentate independent of the depth of the recording electrode. Placements which were 2.5-3.0 mm from the midline were more effective than temporal placements in the region of the fimbria. Electrode placements in the ventral psalterium were more effective in evoking dentate field responses than those situated in the hippocampus proper.

Effects Of Commissural Stimulation On PP-evoked Responses

Fig. 7-6 shows the effects of low intensity (3-5 V) conditioning COMM volley on the amplitude of the population spike and rate of rise of the EPSP evoked by a test PP volley. Whereas the amplitude and rise time of the test EPSP were not altered, the test population spike was markedly potentiated at long C-T intervals (20-400 msec) and depressed at shorter intervals (5-20 msec). As shown in table II, maximum potentiation of the PP-evoked population spike (290±33%) was observed 50-70 msec after conditioning stimulation of the COMM pathway. It is noteworthy that potentiation of the population spike was observed in every animal (n=9) as long as the amplitude of the population spike evoked by a control PP volley was submaximal. If the PP was stimulated with high intensity (20-30 V) the population spike was not markedly potentiated (i.e. Less than 125% of control) by a preceding COMM pulse. The conditioning stimulus intensity was not as critical since

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FIG. 7- 6: Effects Of Commissural Stimulation On Dentate Field Responses Evoked By A Test Perforant Path Volley.

A: amplitude of the population spike and rate of rise of EPSP evoked by a test PP volley preceded by conditioning stimulation of COMM pathway (lower graph) or the PP itself (upper graph). Note the heterosynaptic potentiation of the population spike but not the EPSP.

B: averages of 20 population spike responses evoked by stimulating the perforant path (arrows) alone and following conditioning stimulation of the perforant path itself or commissural pathway.

C: EPSP evoked by stimulating PP alone (trace #2) and following conditioning stimulation of PP (trace #3) or COMM (trace #1) at the same C-T interval (35 msec) as in B showing that the rate of rise of the EPSP is altered only after double pulse stimulation of the perforant path (i.e. PP-PP).

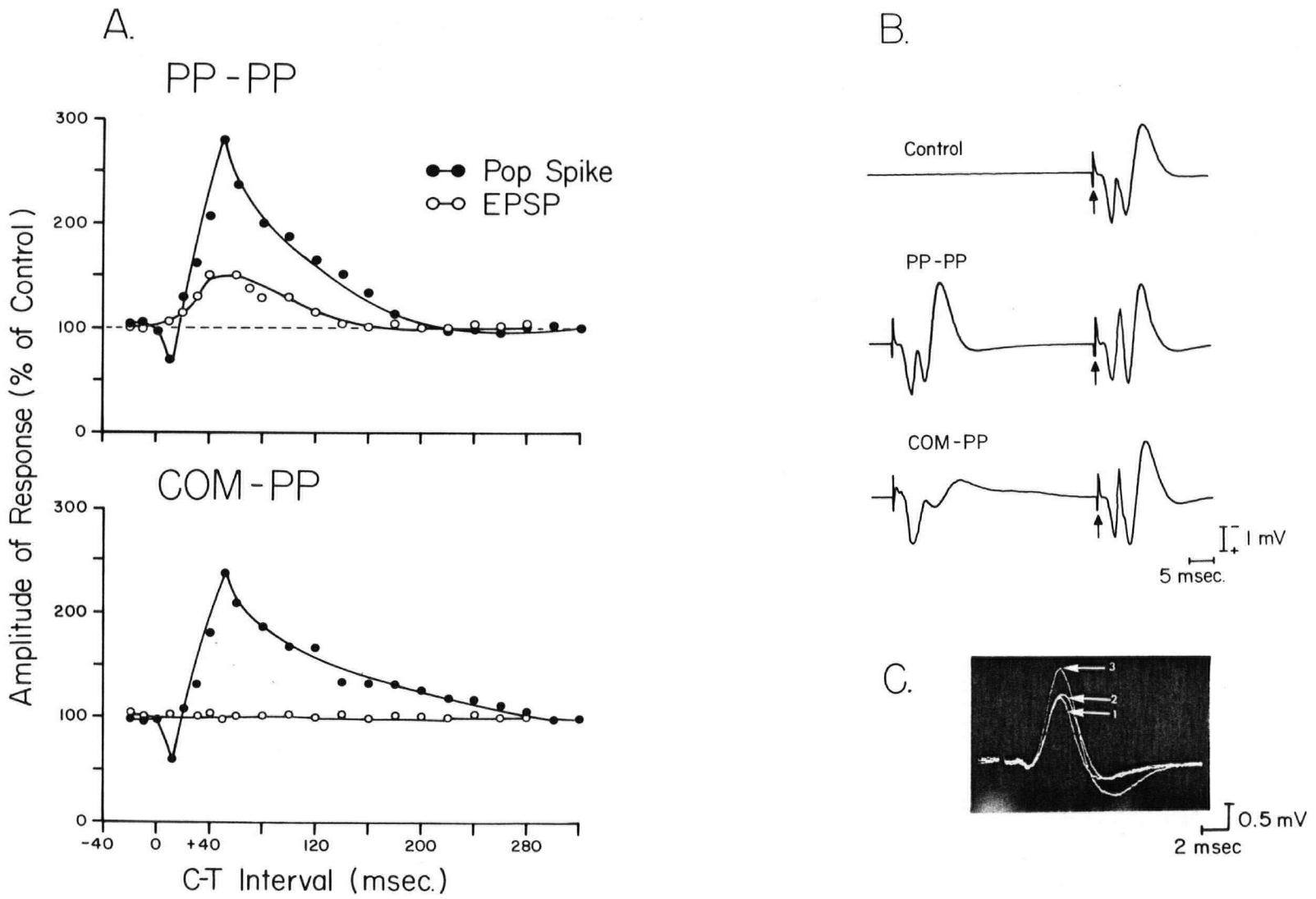
A.
PP-PP
Pop Spike
EPSP
COM-PP
Amplitude of Response (% of Control)
300
200
100
0
-40
0
+40
120
200
280
C-T Interval (msec.)
B.
Control
PP-PP
COM-PP
1 mV
5 msec.
C.
3
2
1
0.5 mV
2 msec

TABLE II

EFFECTS OF CONDITIONING STIMULATION OF THE COMMISSURAL PATHWAY ON PP-EVOKED POPULATION SPIKES

EXPT. NO.	amplitude of population spike* (percent of control)	duration (mSec)
SC-74	275	260
SC-75	135	120
SC-76	240	160
SC-77	260	180
SC-92	500	300
SC-115	350	320
SC-116	250	280
SC-133	280	300
SC-137	325	400
TOTAL = 9	MEAN = 290±33	MEAN = 258±29

*Each value represents the average of 20 trials at a condition-test interval of 35 mSec.

Leaves 291-293 do not exist.

potentiation of PP-evoked population spike was observed following a wide range (3-40 V) of commissural stimuli. However conditioning volleys at intensities higher than 10 V caused a small but significant (110% of control, $p<0.05$) increase in the amplitude of the PP-evoked EPSP recorded in 2 animals and prolonged both the initial period of depression and subsequent potentiation of the PP-evoked population spike in all cases. In the 2 animals in which a small increase in EPSP amplitude was observed the conditioning COMM stimulation evoked prominent late responses. Thus the observed potentiation of the EPSP in those two cases may be due to polysynaptic activation of the test PP pathway.

Evidence That Potentiation Of The Population Spike Was Not Due To Changes In The Test Pathway

An increase in the amplitude of the PP-evoked population spike could be caused by activation of the ipsilateral entorhinal cortex by the conditioning commissural volley. This prior activation of the EC would result in homosynaptic potentiation of the PP-evoked responses. Although the failure to observe potentiation of the PP-evoked EPSP does not support this possibility, further precaution was taken by sectioning the outflow of the EC via the PP in 2 animals. The experimental paradigm consisted of stimulating EC, the PP itself and COMM pathways while recording from the dentate. When the electrodes were

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Leaves 295-297 do not exist.

positioned so that stimulation of EC evoked a fibre volley in the PP and a population spike in the dentate a stereotaxic knife cut was made between the EC and PP. Following the knife cut stimulation of EC no longer evoked a dentate response, but PP stimulation proximal to the cut resulted in a prominent population spike. Commissural conditioning volleys continued to potentiate the PP-evoked population spike suggesting that the EC did not mediate the observed potentiation.

7.4 Discussion

Field Analysis Of The Commissural Input To The Dentate

A) Molecular layer: Stimulation of the COMM pathway results in a short latency extracellular negative field which is maximal in the inner molecular layer. The laminar profile suggests that the zone from which negative fields were recorded extends 50-100 um above the G-cell layer and corresponds to the anatomically verified terminal areas of the COMM pathway (Blackstad, 1956; Gottlieb and Cowan, 1973). This region of the molecular layer, which contains principally the dendritic shafts of G-cells, is below the PP synapses on the distal dendrites of G-cells. In addition to these structures, this layer contains the dendrites and axons of cells in the polymorphic layer.

Unlike the negative dendritic wave which is evoked

by PP stimulation, the COMM negativity has not yet been related to intracellularly recorded EPSP's of individual G-cells (Lomo, 1971a). This makes it difficult to determine precisely which postsynaptic elements are mediating the COMM evoked responses. However, the laminar profiles recorded in the present and previous experiments (Deadwyler et al, 1975) suggest that the short latency (4-7 msec) responses result from a direct action of the COMM pathway on dentate G-cells since 1) the COMM negative field is produced monosynaptically and is preceded by a presynaptic volley, 2) as in the case of the PP input, the negative dipole produced by COMM stimulation is accompanied by a positive wave recorded in G-cell layer which may reflect a passive source for the active sink at the COMM synapses and 3) individual G-cells are sometimes evoked on the positive cell layer field.

B) Responses recorded in G-cell layer: In the region of G-cell body layer COMM stimulation produces a positive waveform upon which individual spikes are superimposed. Increasing the stimulating voltage (from 2-20 V) increased the size of this positive waveform, although it was always smaller than the PP evoked positive field. High stimulating voltages (15-20 V) were required to evoke a field with an amplitude comparable to that elecited by PP stimulation (3-5 V). In 4 of 15 experiments a compound spike similar to the PP-evoked population spike was superimposed on the

positive waveform. Deadwyler et al (1975) were not able to elicit population spikes at all following stimulation of the contralateral CA3. These differences may be related to the fact that Deadwyler et al (1975) used fine monopolar electrodes to stimulate the cells of origin of the COMM pathway which may not be as effective as activating the pathway directly with bipolar electrodes.

The number of single units evoked in the G-cell layer was always lower following COMM stimulation than PP stimulation. In contrast to the low proportion of dentate units which are activated, over 90% of spontaneously firing G-cells are inhibited following COMM stimulation, independent of whether the same units were activated or not. This inhibition may be mediated by collateral activation of basket-type interneurones which have long axons projecting towards G-cell body layer (Cajal, 1911; Andersen et al, 1964).

Since the inhibition produced by COMM stimulation is 2-3 times longer than the inhibition observed following PP stimulation at the same relative intensity (1.5 X T) it becomes necessary to postulate that more inhibitory interneurones are activated following COMM stimulation. These interneurones may be the hilar neurones having dendritic arborization extending to the inner molecular layer in the region of the COMM terminals (Gottlieb and Cowan, 1973; Hjorth-Simonsen

and Laurberg, 1978). The observations that a substantial proportion of COMM terminals are found in the hilar region (Gottlieb and Cowan, 1973; Hjorth-Simonsen and Laurberg, 1978) supports the suggestion that the COMM input may be on inhibitory interneurones in addition to that postulated on G-cells. This postulated feed-forward inhibition of G-cells by COMM fibres would explain the potent inhibition without concomitant activation observed in most cases following COMM stimulation. Since the axons of inhibitory interneurones ramify extensively within the G-cell layer only a few direct connections with those neurones are required to produce the prolonged inhibition observed in this and Deadwyler et al's (1975) study.

Comparison With The Termination Of The Perforant Path

As described in this and the previous chapter, PP and COMM fibres terminate upon the outer and inner molecular layers of the dentate, respectively. The delineation between the terminal areas of PP and COMM pathways is clear with minimal overlap between the two zones (Lynch and Cotman, 1975). This delineation is also apparent from the present field analysis.

The functional significance of these adjacent terminal fields is not yet clear, however, they may underlie the differences in the amplitudes of field

responses and number of G-cells evoked. The COMM extracellular negative field has a slower rise time and smaller amplitude than that evoked by the PP. This may be due to either differences in the effectiveness of PP and COMM terminals to generate synaptic currents or to a greater number of PP synapses in the outer molecular layer. The latter is more likely since the outer molecular layer has a large number of en-passage synapses with dendritic spines which degenerate following PP lesions (Nafstad, 1967). These synapses on dendritic spines would be expected to generate more concentrated synaptic current than COMM synapses on the main dendritic shafts. These differences in synaptic arrangement may also underlie the greater effectiveness of the PP input in discharging G-cells and producing population spikes. Perhaps the physiological role of the COMM system is to alter the effectiveness of the more distal PP input rather than to directly activate G-cells.

Effect Of Commissural Stimulation On PP Evoked Responses

Single pulse stimulation of COMM pathway resulted in a marked increase in the amplitude of the PP-evoked population spike without altering the amplitude or rate of rise of the extracellular EPSP. In the same animals in which conditioning COMM stimulation did not alter the size of the EPSP produced by a test PP volley, paired pulse stimulation of the PP itself resulted in a reliable potentiation of this response. The latter observation is in agreement with the findings of Steward et al (1978) who also failed to find potentiation of the PP-evoked EPSP following conditioning stimulation of the COMM afferent. The additional observation of Steward et al (1978) that paired pulse stimulation of either the PP, COMM or associational pathways result in homosynaptic potentiation of a test EPSP suggests that potentiation of synaptic current can only be produced by prior stimulation of the test pathway itself. A similar conclusion was reached by Andersen et al (1978) and Lynch et al (1978) who observed that tetanic stimulation of an afferent to one dendritic zone of a CA1 pyramidal cell results in long lasting potentiation of the same afferent but not afferents projecting to adjacent dendritic layers.

In contrast to the EPSP, the amplitude of the PP-

evoked population spike was markedly enhanced following conditioning COMM stimulation. Thus unlike potentiation of the EPSP that of the population spike is not restricted to conditioning stimulation of the test pathway. The observed potentiation of the population spike is dependent on the COMM pathway since it was not seen following acute or chronic transection of the ventral psalterium. Furthermore, potentiation of the population spike by COMM stimulation was not eliminated by surgical transection between the entorhinal cortex and the PP thereby eliminating the involvement of the cells of origin of the PP input. The observation that in some animals potentiation of the PP-evoked population spike was observed within 10 msec following a COMM conditioning volley severely restricts the involvement of polysynaptic extrahippocampal systems. In any case the absence of changes in the rate of rise of the EPSP when the population spike is markedly potentiated argues against the possibility that the heterosynaptic potentiation is mediated by presynaptic events leading to increased PP transmitter release.

7.5 Summary

In summary the results of the present chapter demonstrate that stimulation of the COMM pathway results in short latency field responses in the dentate gyrus. The laminar profile and its relationship to the depth profile following stimulation of PP suggests that the COMM input to the dentate depolarizes the dendrites of G-cells at a position more proximal to the cell body than the distally located PP synapses. The response of spontaneously firing G-cells following COMM stimulation causes activation-inhibition sequences in a few cases and pure inhibition in the remainder. Single pulse stimulation of the COMM pathway results in marked potentiation of the PP-evoked population spike without altering the EPSP produced by a test PP volley. On the basis of these data, heterosynaptic potentiation of the population spike may be an additional feature of neuronal transmission through the dentate gyrus.

CHAPTER 8: THE RAPHE-SEROTONIN SYSTEM AND NEURONAL TRANSMISSION IN THE DENTATE GYRUS

8.1 Introduction

The previous two chapters described the laminar profile of the perforant path (PP) input to the dentate and the feasability of using the rate of rise of the extracellular EPSP and amplitude of the population spike as indicators of synaptic current and synchronous neuronal discharges, respectively. The observation made in Chapter 7 that conditioning stimulation of the commissural pathway increases the amplitude of the population spike without altering the rate of rise of the EPSP prompted an examination of the effects of stimulating known monoamine projections to the dentate. Unlike the commissural input the monoamine systems do not substantially innervate the molecular layer of the dentate and are thus unlikely to affect the PP synapses on the distal dendrites of granule cells.

The present chapter examines the effects of stimulating the median raphe nucleus (MR), the site of origin of hippocampal serotonin on field potentials evoked in the dentate by test PP volleys. Whenever possible the response of single dentate units is also examined in order to relate observed changes in field potentials to the activity of individual neurones recorded from the same site.

8.2 Experimental Procedures

The methods used were similar to those described in chapter 7. Bipolar stimulating electrodes were stereotaxically positioned in the angular bundle to activate the perforant path and in the mossy fibres or ipsilateral CA3 (AP = -3.0 mm; L = +3.5 mm; V =-3.5 mm) to activate granule cells antidromically. An additinal stimulating electrode was positioned in the median raphe nucleus (AP = -8.0 mm; L = 0.0 mm; V = -6.0 mm to -6.5 mm). The experiments were performed on 15 normal rats and 5 rats pretreated with para-chlorophenylalanine(p-CPA, 400 mg/kg; i.p.). Immediately following the electrophysiological experiments, some animals (5 normals and 5 p-CPA-treated) were decapitated and the hippocampal formation on both sides removed for subsequent 5-HT assay described in chapter 2.

8.3 Results

Effects Of MR Stimulation On G-cells

The response of 66 spontaneously firing G-cells was studied following stimulation in the region of the median raphe nucleus. Dentate G-cells were identified on the basis of their response patterns following PP stimulation and their relationship to PP-evoked field responses (Fig. 8-1). In some cases (n=15) cells were further identified by antidromically activating them by mossy fibre stimulation.

As shown in Fig. 8-1D, single pulse stimulation (10-20V) of the MR inhibited a total of 56 (85%) G-cells at latencies ranging between 10-25 msec (See also Fig. 8-2). The duration of inhibition lasted for periods of 25-175 msec (mode=43.0 msec). The duration of inhibiton could be lengthened by stimulating the MR with higher intensity or increasing the number of pulses (Fig. 8-2).

Histological verification of effective stimulating electrode placements indicated a direct relationship between the length of inhibition and proximity of the electrode to the MR (Fig. 8-3). In addition stimulating sites 1.0-2.0 mm posterior to the MR did not produce the observed inhibition indicating a specific action of this particular nucleus on G-cells.

FIG. 8- 1: Effects Of MR Stimulation On Dentate Granule Cells.

A: arrangement of recording electrode at the level of the cell body and stimulating electrodes in excitatory (open triangles) and inhibitory (closed triangles) afferents from the entorhinal cortex (EC) and median raphe nucleus (MR), respectively. Collateral of the G-cell axon, the mossy fibre (MF), activates an inhibitory interneuron.

B: oscilloscope traces of the waveforms evoked by PP stimulation evoked at various intensity indicating that recordings were from the G-cell layer.

C,D: post stimulus histograms (bin width = 5 msec) of a G-cell discharge following single pulse stimulation of the PP (C) and MR (D). Inserts show 25 superimposed oscilloscope sweeps. Arrows indicate stimulus artifacts and calibrations are 5 msec/1.0 mV in B and 50 msec/.1 mV in C and D.

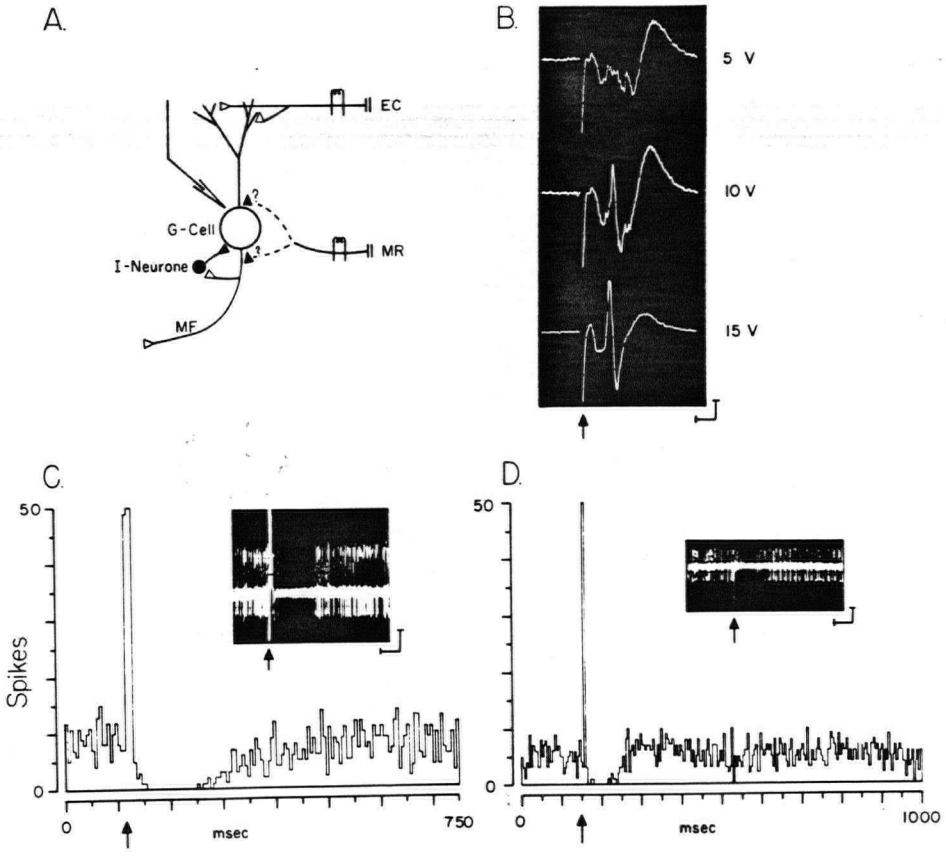
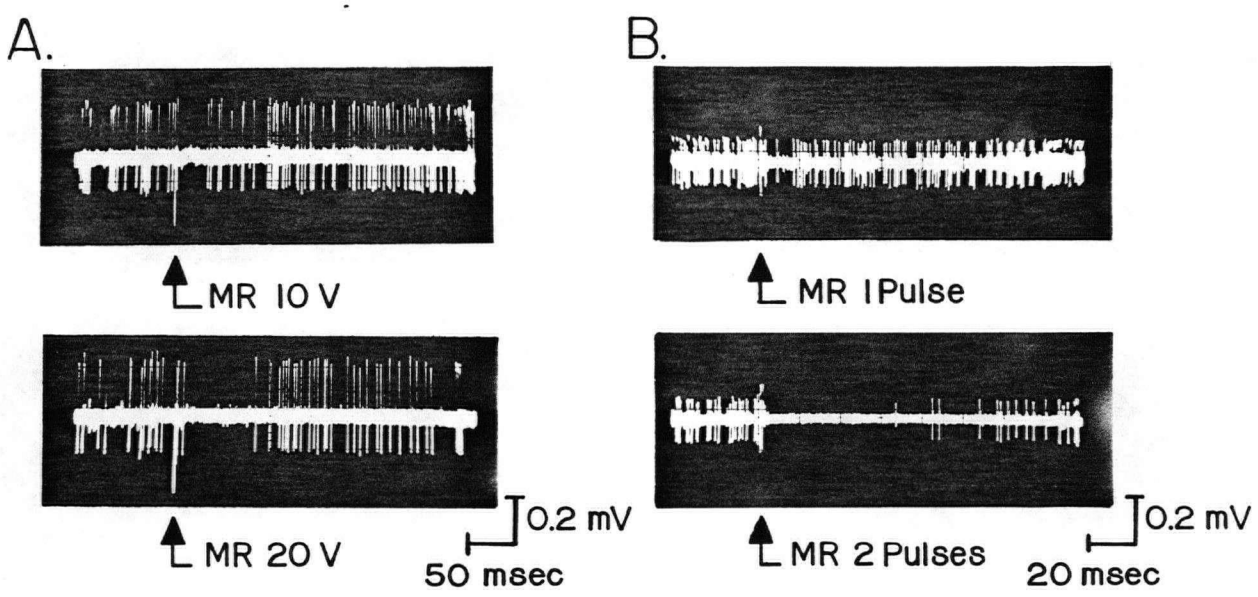

A.
EC
G-Cell
I-Neurone
MR
MF
B.
5 V
10 V
15 V
C.
50
Spikes
0
0
msec
750
D.
50
0
0
msec
1000

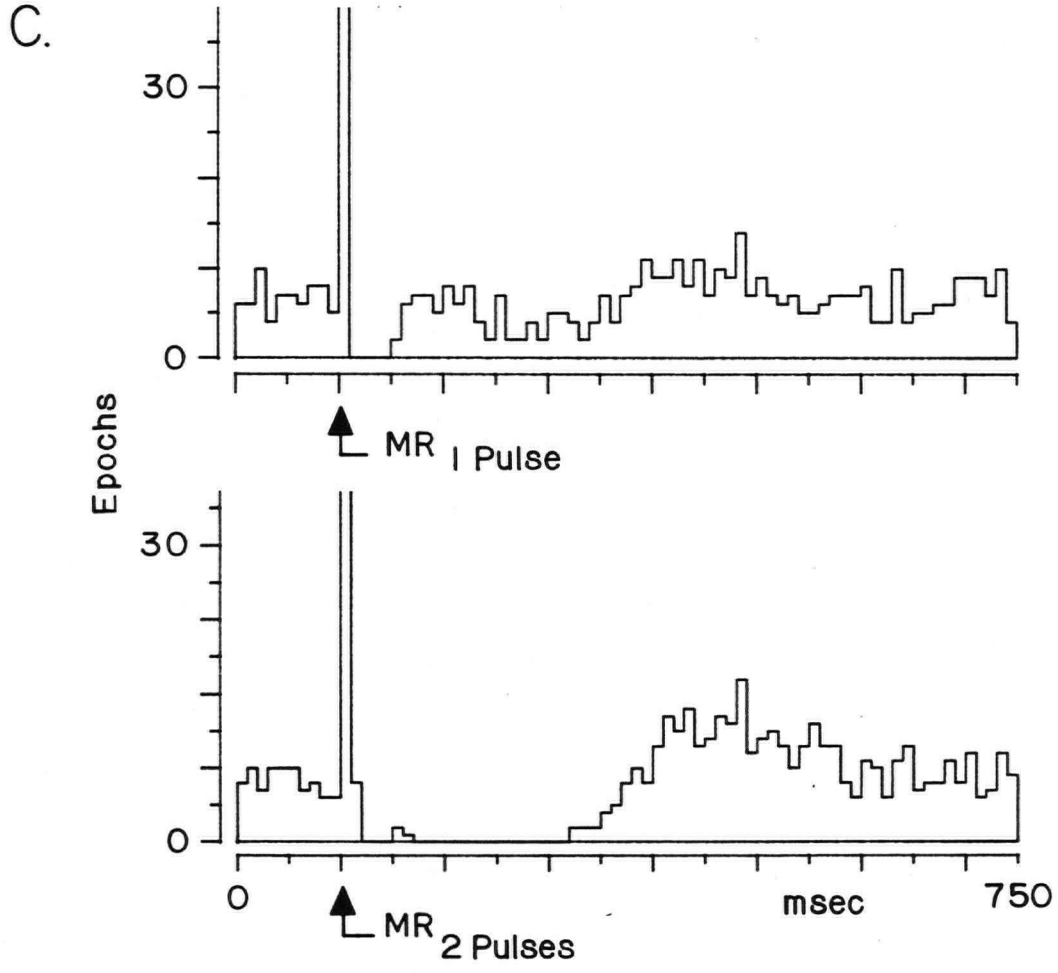
FIG. 8- 2: Inhibition Of Spontaneously Firing Dentate Cells By MR Stimulation.

A: effects of increasing the stimulus intensity on the length of inhibition observed following MR stimulation. (10 superimposed oscilloscope sweeps).

B: effects of increasing the number of pulses delivered to the MR on the length of the observed inhibition. In the lower records the two pulses were repeated by 10 msec. (20 superimposed oscilloscope sweeps).

C: PST histogram of the response of a G-cell to MR stimulation with single or repetitive (300 Hz) pulses. Bin width is equal to 10 msec; 50 trials in each case.


A.
MR 10 V
MR 20 V
0.2 mV
50 msec
B.
MR 1 Pulse
MR 2 Pulses
0.2 mV
20 msec


C.
30
0
Epochs
MR 1 Pulse
30
0
0
MR 2 Pulses
msec
750

Effects Of MR Stimulation On PP-evoked Responses

The response of 12 spontaneously firing G-cells which were excited by the perforant path were examined following stimulation of the MR. As shown in Fig. 8-4, single pulse stimulation of MR, which inhibited the spontaneous discharge of all 12 cells, also blocked the single spike activation of 8 cells (66%) following stimulation of the PP at threshold intensity. However, stimulation of the MR did not reliably block the activation produced by stimulating the perforant path at 2 X T. When the PP was stimulated with 3 x T intensity, thereby evoking a number of G-cells (Fig. 8-4C), MR stimulation resulted in a more synchronous discharge of G-cells which resembled the population spike response. Thus, it became impossible to determine whether individual cells which were evoked in response to control PP stimulation were also evoked by the test PP volley since they may be masked by compound spikes such as that shown in Fig. 8-4C.

In order to overcome this problem, the amplitude of the PP-evoked population spike was measured before and after stimulation of the MR. As shown in Fig. 8-5, a single conditioning volley to MR resulted in an increase in the amplitude of the PP-evoked population spike at critical intervals between 20-70 msec. The rise time of the PP-evoked EPSP was not altered by conditioning pulses that potentiated the amplitude of

FIG. 8- 3: Relationship Between Inhibition Of Dentate Cells And Position Of The Stimulating Electrode In The Region Of MR.

A: schematic of a coronal section of the rat brain at the level of MR (A160) indicating the position of the stimulating electrode along a vertical track which produced the corresponding responses shown in B.

B: the response of a spontaneously firing dentate unit following stimulation in the region of MR at the indicated positions. Each record consists of 10 superimposed oscilloscope sweeps. Abbreviations as on page 130.

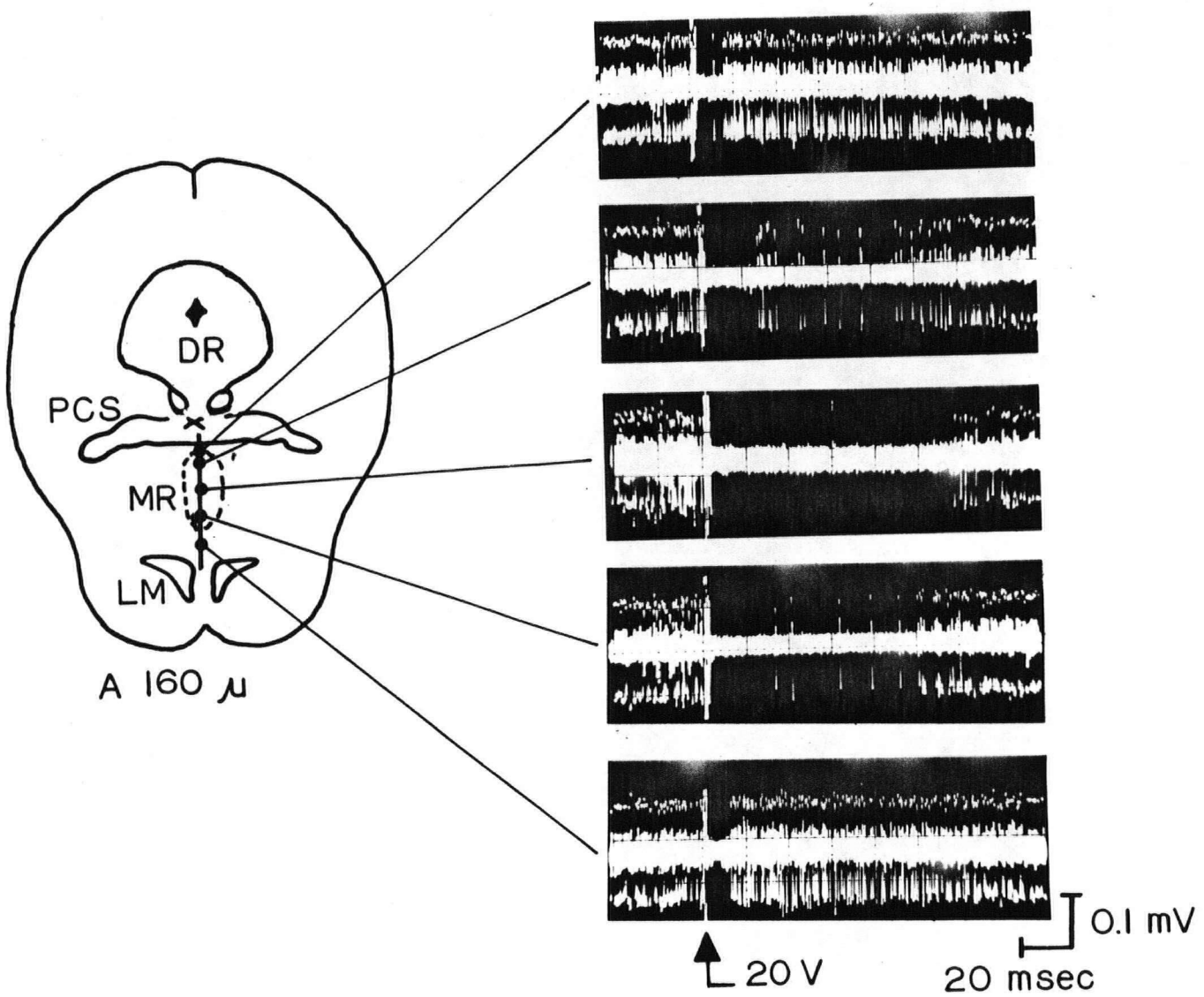

DR
PCS
MR
LM
A 160 μ
0.1 mV
20 V
20 msec

FIG. 8- 4: Effects Of MR Stimulation On PP Evoked Activation Of Dentate Cells.

A: spontaneously firing dentate units which were inhibited by single pulse stimulation of MR (top) and activated by PP (bottom).

B: indicates that the PP-evoked spike (top) is eliminated by a preceding pulse to the MR (bottom). PP stimulus intensity at threshold and C-T interval is 30 mSec. Single spike activation of the above unit. Note the elimination of the PP evoked spike in the lower sweep.

C: same experiment as in B except the PP is stimulated with sufficient intensity to evoke a number of neurones. Note the appearance of a compound spike following MR conditioning stimulation in the lower sweep.

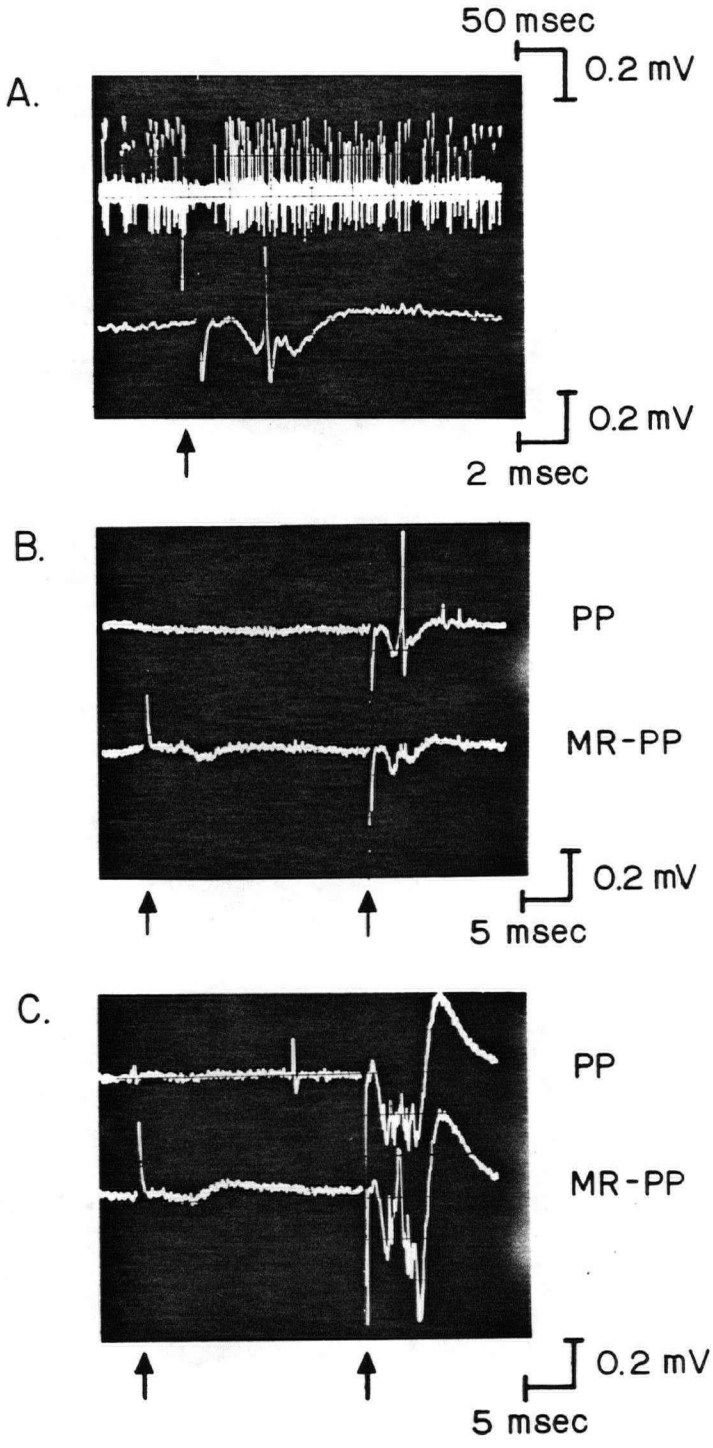
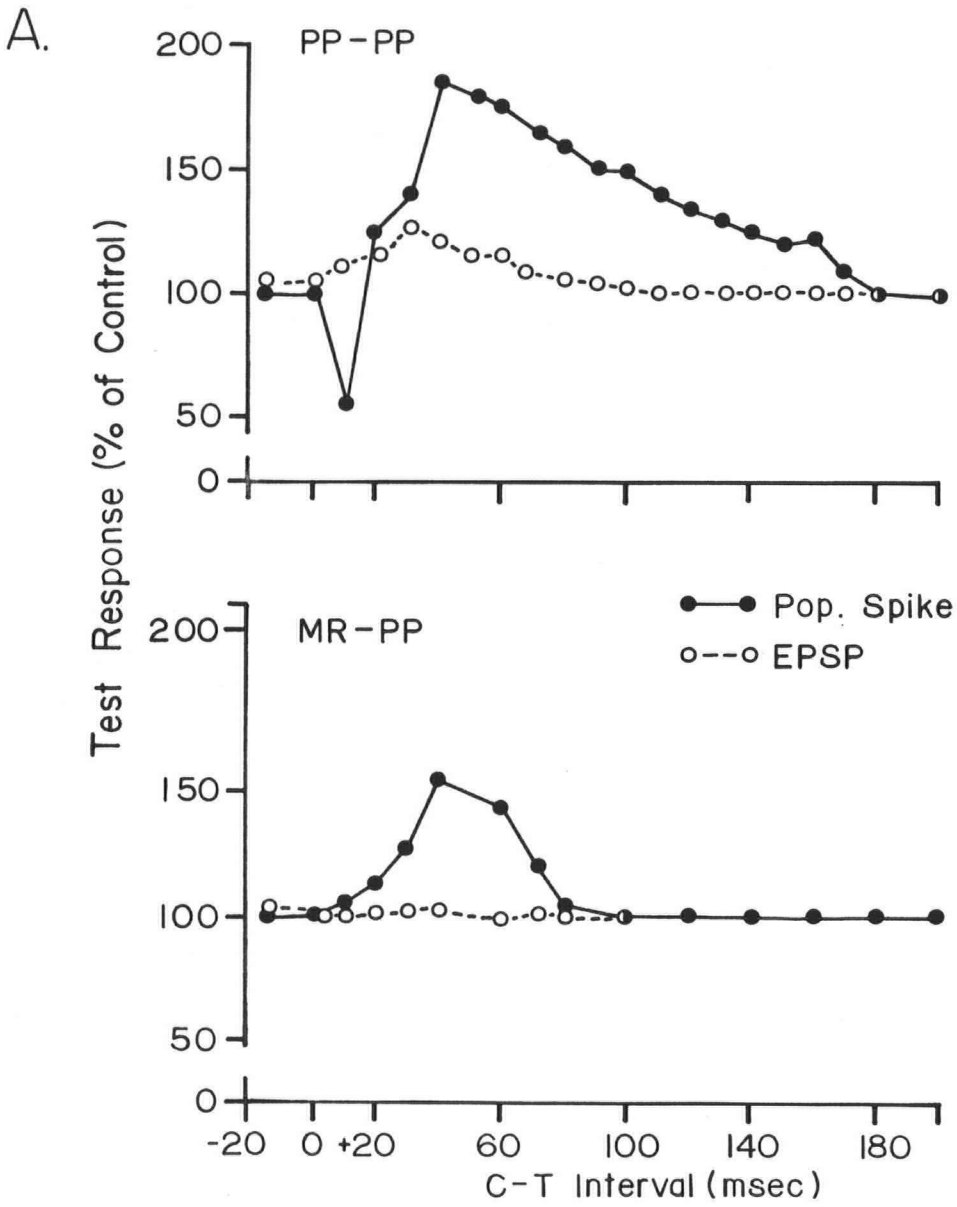
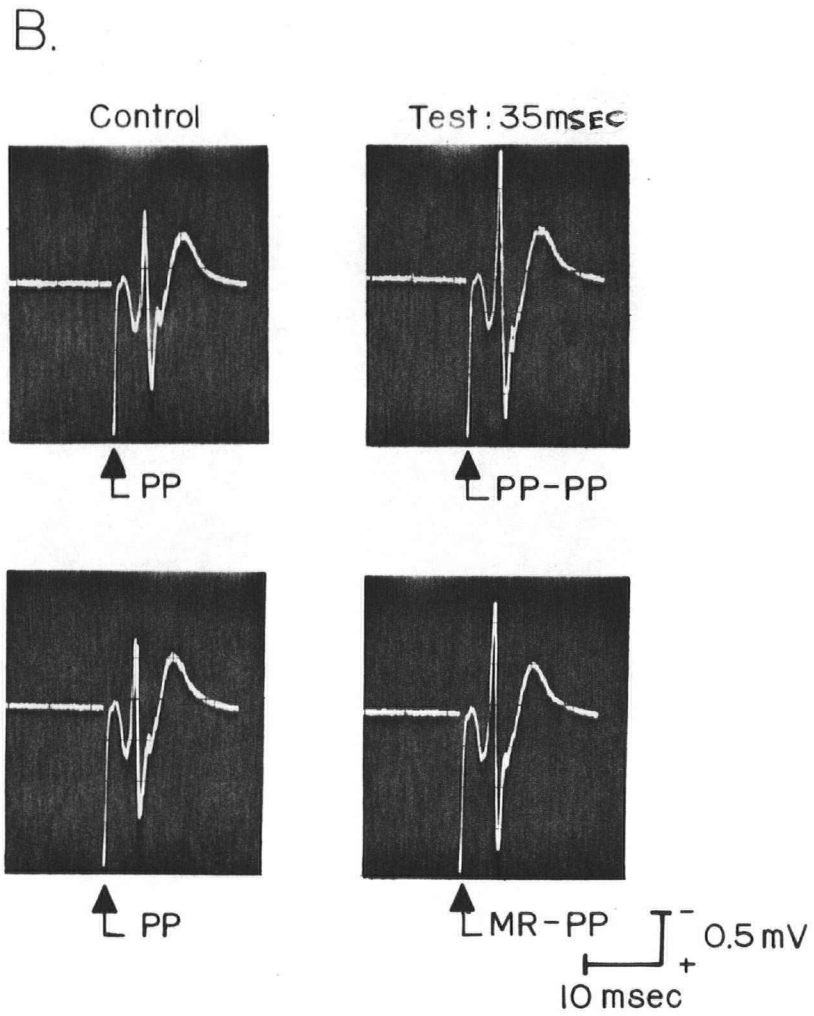

A.
50 msec
0.2 mV
0.2 mV
2 msec
B.
PP
MR-PP
0.2 mV
5 msec
C.
PP
MR-PP
0.2 mV
5 msec

FIG. 8- 5: Effects Of MR Conditioning Pulses On The Field Responses Evoked By The Perforant Path.

A: graphs of the amplitude of population spike and rate of rise of the EPSP evoked by a PP volley (7.5 V) when preceded by a conditioning pulse (15 V) to PP (top graph) or to the MR (lower graph).

C: oscilloscope traces of control PP-evoked population spikes(left) and following conditioning PP (top right) and MR (lower right) pulses.


A.
PP-PP
MR-PP
Pop. Spike
EPSP
Test Response (% of Control)
200
150
100
50
0
-20
0
+20
60
100
140
180
C-T Interval (msec)


B.
Control
Test: 35msec
PP
PP-PP
PP
MR-PP
0.5mV
10 msec

the population spike recorded in the same animals. Potentiation of the population spike was observed in every animal tested with the maximum potentiation ranging between 110% and 170% of control. The degree of potentiation was related to the distance between the stimulating electrode and the center of the MR. Conditioning stimulation in the DR and adjacent areas did not result in reliable potentiation.

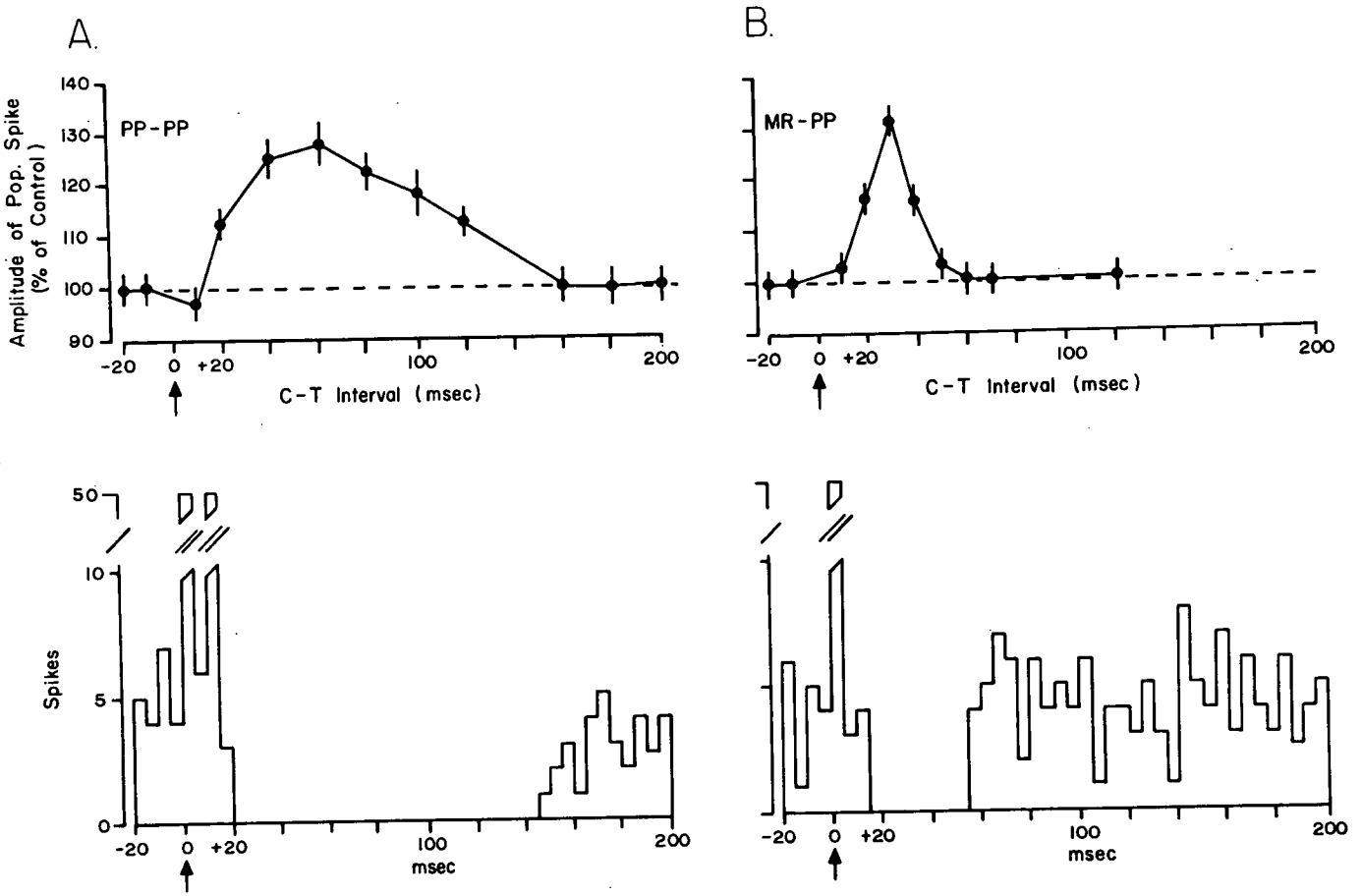
It is noteworthy that at the stimulus intensities used (10-20V) MR stimulation did not result in significant depression of the population spike similar to that which initially follows conditioning stimulation of the PP itself.

Relationship Between Effects Of MR Stimulation On Population Spike And Inhibition Of Single Units.

As illustrated in Fig. 8-6, the time course of the facilitation evoked by the MR conditioning pulses coincided with the period of inhibition of G-cells recorded at the same position or in the immediate vicinity. When the duration of the inhibitory component was altered by changing intensities of stimulation, the period of facilitation of the population spike was altered in the same direction. In Fig. 8-7, this relationship is further illustrated by plotting the amplitude of a representative population spike and the probability that a given G-cell is inhibited at various

FIG. 8- 6: Facilitation Of The Population Spike Response Following PP And MR Conditioning Pulses

The time course of popoulation spike potentiation (upper graphs) following PP (A) and MR (B) conditioning pulses is compared with the response of a spontaneously firing dentate neurone (PST histograms, bin width = 5 mSec)) recorded in the vicinity of the population spike. Note that the duration of potentiation is related to the length of the inhibition elicited by the conditioning pulse in each case.


A.
B.
Amplitude of Pop. Spike (% of Control)
140
130
120
110
100
90
PP-PP
MR-PP
-20
0
+20
100
200
C-T Interval (msec)
50
10
5
0
Spikes
msec

times following MR stimulation. The probability was computed by counting the number of cells (n=66) which were inhibited during 5.0 msec sample bins following MR stimulation. It is noteworthy that the time period when the majority of G-cells were inhibited by MR stimulation is the optimal C-T interval (30-35 msec) for maximal facilitation of the population spike.

Effects Of P-CPA On Respones Evoked By Stimulation Of MR

As shown in table 3, p-CPA pretreatment resulted in a significant depletion (69%) of hippocampal 5-HT measured by the spectrofluorometric assay described in chapter 2. The pretreatment also resulted in a significant attenuation of MR-evoked inhibition of single G-cells and enhancement of the PP population spike response. Moreover, the degree of attenuation of both the inhibition (61%) and population spike enhancement (80%) were similiar to the depletion of hippocampal 5-HT.

FIG. 8- 7: Relationship Between Inhibition Of G-cells And Amplitude Of The Population Spike.

The probability of inhibition (cells inhibited/ cells tested X 100) shown in the histogram and the amplitude of a representative population spike (dashed line) are the left and right ordinates, respectively. The MR was stimulated at time zero and the abscissa represents 5 msec sampling periods of neuronal (n=66) discharges.

Probability of Inhibition [%]
100
80
60
40
20
0
0
20
40
60
80
Time after MR Stimulation (msec)
8.0
6.0
4.0
2.0
0.0
Amplitude of Pop. Spike (mV)

TABLE III

EFFECTS OF p-CPA ON HIPPOCAMPAL 5-HT AND RESPONSES TO STIMULATION OF THE MEDIAN RAPHE

	pp-evoked population spike (% increase)	inhibition of G-cells (no. inhibited / no. tested)	5-HT concentration (ng/g wet tissue)
Control	40±5 (n=15)	55/66 (83%)	545±29 (n=5)
p-CPA	8±3 (n=4)	8/25 (32%)	166±18 (n=4)
percent difference	-80*	-61*	-69**

p-CPA (400 mg/Kg, i.p.) was administered 3 days before recording experiments. 5-HT levels were assayed in both sides of the hippocampal formation using spectrofluoremetric techniques described in chapter 2 (*$p<0.01$; **$p<0.001$).

8.1 Discussion

Effects Of MR Stimulation On Spontaneous Discharge Of G-cells

Single pulse stimulation in the region of the MR results in short latency (10-25 msec) inhibition of the majority of spontaneously firing G-cells. The direct relationship between the length of inhibition and proximity of the stimulating electrode to the MR suggests that the inhibition is mediated by this particular nucleus and not the adjacent areas. It is not possible from the present data to distinguish between mono- and polysynaptic MR evoked inhibition of G-cells. The observation that inhibition occurred at short latencies and in some cases immediately after the stimulus supports a direct input onto G-cells rather than an indirect action involving extrahippocampal systems. Within the dentate the distribution of 5-HT terminals is primarily to the region of the dentate hilus. Histofluorescence and autoradiographic studies (Halaris et al, 1976, see also section 1.1.1) reveal a clear infragranular band of terminals which may be either onto interneurones or the G-cell bodies. Since some of the inhibited neurones were identified as G-cells by antidromically firing them following mossy fibre stimulation these neurones may receive a direct serotonergic input originating in MR.

Effects Of MR Stimulation On PP-evoked Responses

Single pulse stimulation of the MR blocked the activation of G-cells evoked by threshold but not suprathreshold stimulation of the PP. This suggests that at higher PP intensities the activation can overcome the raphe mediated inhibition onto G-cells. Thus when the PP was stimulated at intensities sufficient to evoke a population spike, the amplitude of the population spike was not reduced by perforant path stimulation. In fact an enhancement of the population spike response was observed following MR stimulation. Since potentiation of the population spike was not accompanied by a concomitant increase in the rate of rise or amplitude of the EPSP it was not due to the action of MR on the entorhinal cortex or the region of the PP synapse on distal dendrites. Thus it is not necessary to postulate an increase in transmitter release to account for potentiation of the population spike. Since conditioning stimulation of the commissural pathway (Chapter 2) and the mossy fibres (Lomo, 1971b; Assaf and Miller, unpublished results) potentiate the population spike without altering the EPSP extrinsic afferents to the dentate may alter PP-dentate transmission without changing synaptic current. This point will be developed further in chapter 10.

Relationship Between Inhibition Of G-cells And Potentiation Of The Population Spike

The present data demonstrate a temporal relationship between MR evoked inhibition of G-cells and potentiation of the population spike response such that the onset of inhibition coincides with the critical C-T intervals at which the population spike amplitude is increased. Furthermore, the optimum C-T interval for producing maximum potentiation is when the largest proportion of G-cells is inhibited by MR stimulation. This relationship between inhibition of G-cell discharge and potentiation of the population spike was also demonstrated during paired pulse stimulation of the PP itself. This suggests that inhibitory mechanisms, in part, may mediate the observed potentiation of the population spike without altering synaptic current.

Role For Serotonin:

The inhibition of spontaneously firing G-cells and enhancement of the PP-evoked population spike observed following MR stimulation were substantially reduced by p-CPA pretreatment which decreased 5-HT concentration in the hippocampal formation. Taken together with the relationship between the inhibition of G-cells and position of the stimulating electrode in the MR (Fig. 8-3), these data support the proposal that these

responses were mediated by a raphe-serotonin system.

The observation that single pulse stimulation of MR enhances the amplitude of the PP-evoked population spike was recently confirmed by Winson (1978 and personal communication). He also observed that MR stimulation increases the amplitude of the population spike evoked by PP stimulation at C-T intervals ranging between 20 and 70 msec. However, Winson (1978) examined the effects of MR stimulation in awake as well as in anaesthetized animals. Augmentation of the population spike response was observed in slow wave sleep and in the anaesthetized state but not in the alert state (Winson, 1978). On the basis of these data, Winson (1978) suggested that the effects of a raphe-serotonin system on neuronal transmission in the dentate gyrus may be behaviour-specific.

8.5 Summary

In summary, single pulse stimulation of the MR results in short latency inhibition of spontaneously firing G-cells and potentiation of the PP evoked population spike without altering the rise time of the EPSP. There is a temporal relationship between the period of inhibition of unit activity and critical C-T intervals for potentiation. Depletion of 5-HT in the hippocampal formation attenuates the MR-elicited respones suggesting that they are mediated by a serotonin-containing system.

CHAPTER 9: THE LOCUS COERULEUS AND NEURONAL TRANSMISSION IN THE DENTATE GYRUS

9.1 Introduction

Conditioning stimulation of the commissural pathway (Chapter 7) and the median raphe nucleus (Chapter 8) increase the amplitude of the perforant path (PP)-evoked population spike. Like the serotonin innervation of the dentate, noradrenaline (NA)-containing terminals, which originate in the locus coeruleus (LC), are sparse in the zone of PP synapses but relatively dense in the dentate hilus. This suggests that LC is unlikely to affect PP synapses or the EPSP directly but may alter the discharge of G-cells at some other site. The present chapter examines the effects of stimulating LC on field potentials evoked in the dentate by a test PP volley. The response of single dentate cells is also examined in an attempt to relate changes in field potentials to activity of individual neurones.

9.2 Experimental Procedures

The methods used were similar to those described in chapters 7 and 8. In addition to all the electrode placements provided in chapter 8 (PP, DG,CA3, MR), a bipolar stimulating electrode was positioned in the locus coeruleus (AP = 1.5 mm posterior to stereotaxic ear bars; L = 1.1 mm from the midline; V = 5.5 to 6.5 mm below the surface of cerebellar cortex) of 15 normal rats and 10 rats which had previously (15-20 days) recieved a bilateral injection of 6-OHDA (4 uq in 2 ul of 0.15M saline with 0.2 mg/ml ascorbic acid) into the dorsal NA bundle. Following the electrophysiological experiments the hippocampal formation was removed and tissue samples assayed for NA as described in Chapter 2.

9.3 Results

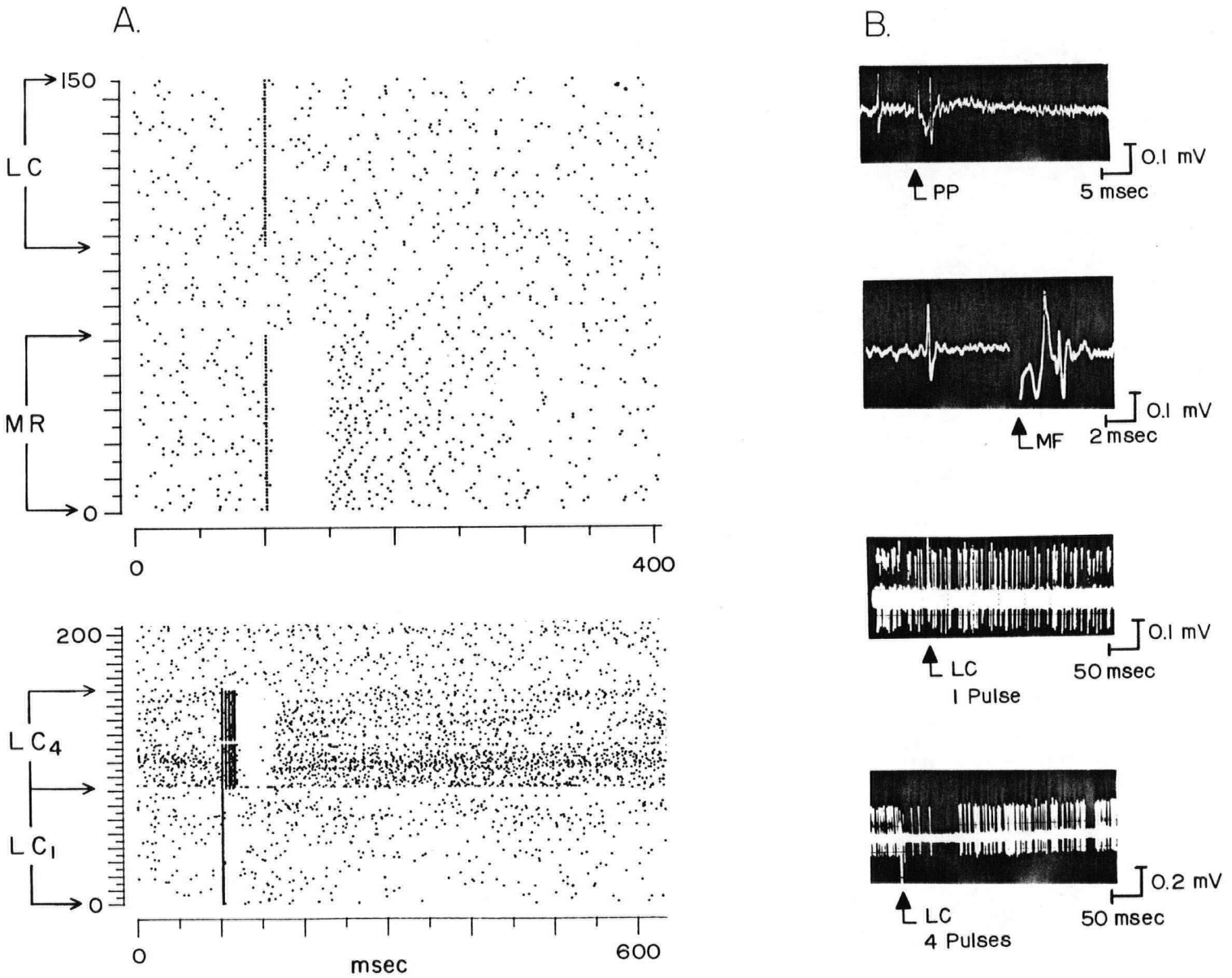
Effects Of LC Stimulation On Dentate Cells

The responses of 63 spontaneously firing dentate neurones were examined following single or multiple pulse stimulation of the locus coeruleus. Of the 63 cells, 56 were identified as G-cells on the basis of thier response to stimulation of PP as well as by histological localization of the recording electrode in the dentate cell body layer. In addition 12 of the 56 G-cells were activated antidromically by mossy fibre

FIG. 9- 1: Effects Of LC Stimulation On Dentate Neurones.

A: raster display showing the response of a spontaneously firing neurone recorded in the G-cell layer following single pulse stimulation of LC (top) and MR (bottom). Response of the same neurone to single and multiple pulse (4 pulses, 300 Hz) stimulation of LC is shown by the lower raster.

B: oscilloscope records showing a G-cell which was monosynaptically evoked by PP stimulation, antidromically evoked by mossy fibre (MF) stimulation. Single pulse stimulation of LC was without effect while multiple pulses resulted in inhibition.


A.
150
LC
MR
0
0
400
200
LC4
LC1
0
0
600
msec
B.
0.1 mV
PP
5 msec
0.1 mV
MF
2 msec
0.1 mV
LC
1 Pulse
50 msec
0.2 mV
LC
4 Pulses
50 msec

stimulation. 7 neurones could not be identified as G-cells since they were synaptically activated by mossy fibre stimulation and were recorded below the G-cell layer. The latter neurones were tentatively identified as hilar neurones, possibly intrinsic to the dentate gyrus.

The discharge pattern of the majority of G-cells (88%) was not altered by single pulse stimulation of the LC at intensities ranging from 0-25V (Fig. 9-1). Inhibition at short latencies (less than 25 msec) was observed in 4 (7%) units and single spike activation was observed in 3 (5%) G-cells. Only 1 of 7 non G-cells was inhibited.

During stable recordings from G-cells the stimulating electrode was moved in the dorsal-ventral plane to insure that the response of the cell was observed at different stimulating depths. At depths 1.0-1.5 mm below the level of the LC facial movements were evoked by the stimulation, these movements caused a large field immediately following the stimulus artifact and may be related to activation of the nucleus of the fifth cranial nerve.

Electrodes were placed in both LC and MR in 4 animals allowing a comparison between the responses of 21 neurones following MR and LC stimulation (e.g. Fig. 9-1, top) single pulse stimulation of MR inhibited the discharge of 16 neurones whereas that of LC

inhibited only 2 of the neurones. The inhibition following MR stimulation was similar in latency (less than 25.0 msec) and duration (mode=45 msec) to that described in the previous chapter.

The effects of repetitive stimulation of the LC (2-5 pulses, 300 Hz) were examined on 15 G-cells recorded in 5 animals. As shown by the lower raster in Fig. 9-1, stimulation of the LC with 4 pulses resulted in reliable inhibition in 8 (55%) neurones while single pulses had no effect. The onset of inhibition was less than 30 msec following the last pulse of the train and continued for 50-100 mSec. The background discharge rate of the neurone was often (4 of the 6 neurones) higher during stimulus presentations than control periods. Slight facial movements were observed during stimulation, therefore the increased discharge rate of the neurone may be related to the activation of sensory-motor nuclei adjacent to LC.

Effect Of LC Stimulation On PP-evoked Field Responses.

The effects of stimulating LC with single and repetitive (2-5 pulses, 15V, 300 hZ.) pulses on amplitude of the population spike and rise time of the EPSP was examined in 6 control rats. As shown in Fig. 9-2, repetitive but not single pulse stimulation of LC reliably potentiated the population spike response in all 6 rats but did not alter the rise time of the EPSP in the same animals.

The critical C-T intervals for potentiation of the population spike ranged between 20-80 msec following the last pulse of the brief train. Maximum potentiation (150-175% of control) was observed at optimal C-T intervals between 35 and 50 msec.

Fig. 9-3 indicates that the degree of potentiation is related to the number of stimulating pulses since single pulse did not reliably result in potentiation whereas 2-4 pulses produced reliable potentiation. The response following a stimulus train of 5 pulses was not substantially different than that observed following 6 or more pulses, thus a 4-5 pulse stimulus train was adopted as the test stimulus for inter-animal comparisons.

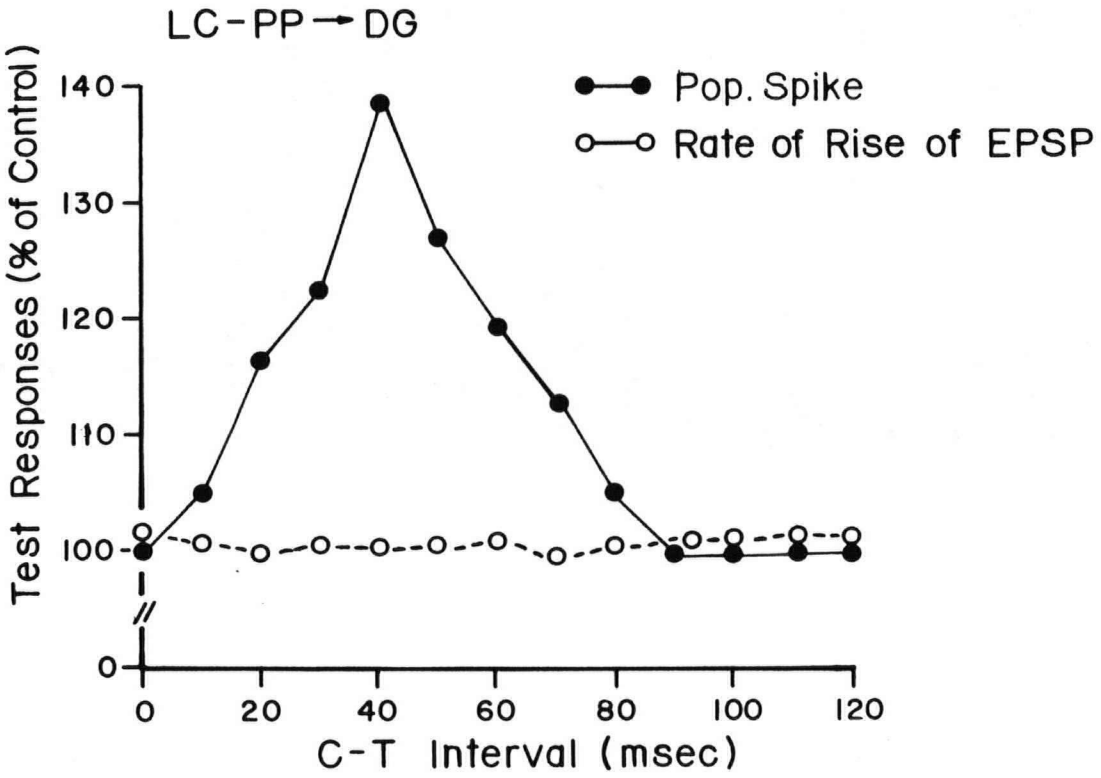
As shown in Fig. 9-4, there was a direct relationship between depth of the stimulating electrode tip and the degree of potentiation. Placements

FIG. 9- 2: Effects Of LC Stimulation On PP-evoked Dentate Population Responses.

A: the effects of conditioning LC stimulation (15V,5 pulses at 300 Hz.) on PP-evoked population spike and EPSP.

B: oscilloscope sweeps showing the control (lower arrow) PP-evoked population spike and potentiation of this response (top arrow) by preceding stimulation of LC (C-T = 35 mSec).

A.


LC-PP→DG
Pop. Spike
Rate of Rise of EPSP
Test Responses (% of Control)
140
130
120
110
100
0
0
20
40
60
80
100
120
C-T Interval (msec)

B.

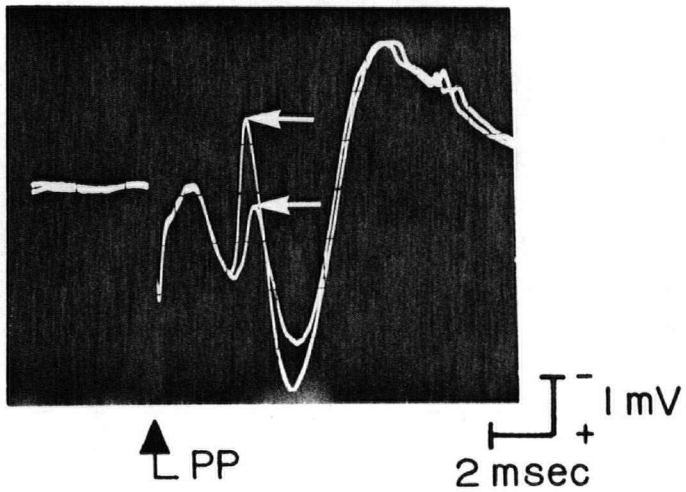

PP
1 mV
2 msec

FIG. 9- 3: Relationship Between The Number Of LC Pulses And Amplitude Of The Population Spike.

Each point is the average of 20 trials at a constant condition-test interval (35 msec). Frequency of the pulse train was 300 Hz.

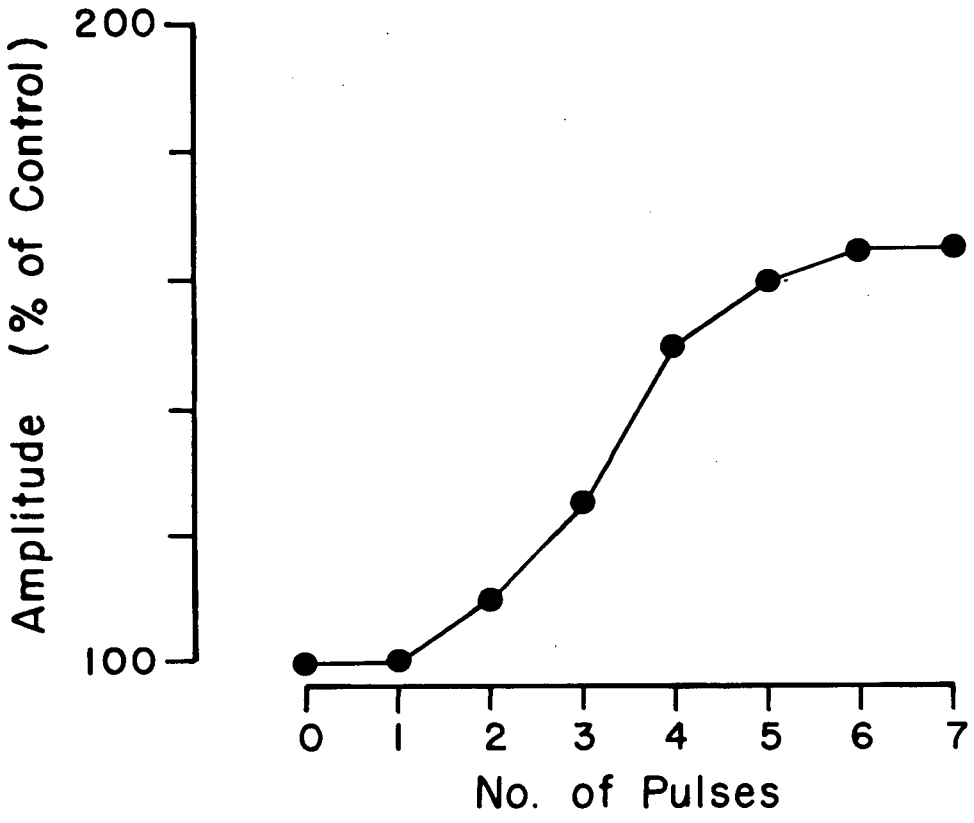
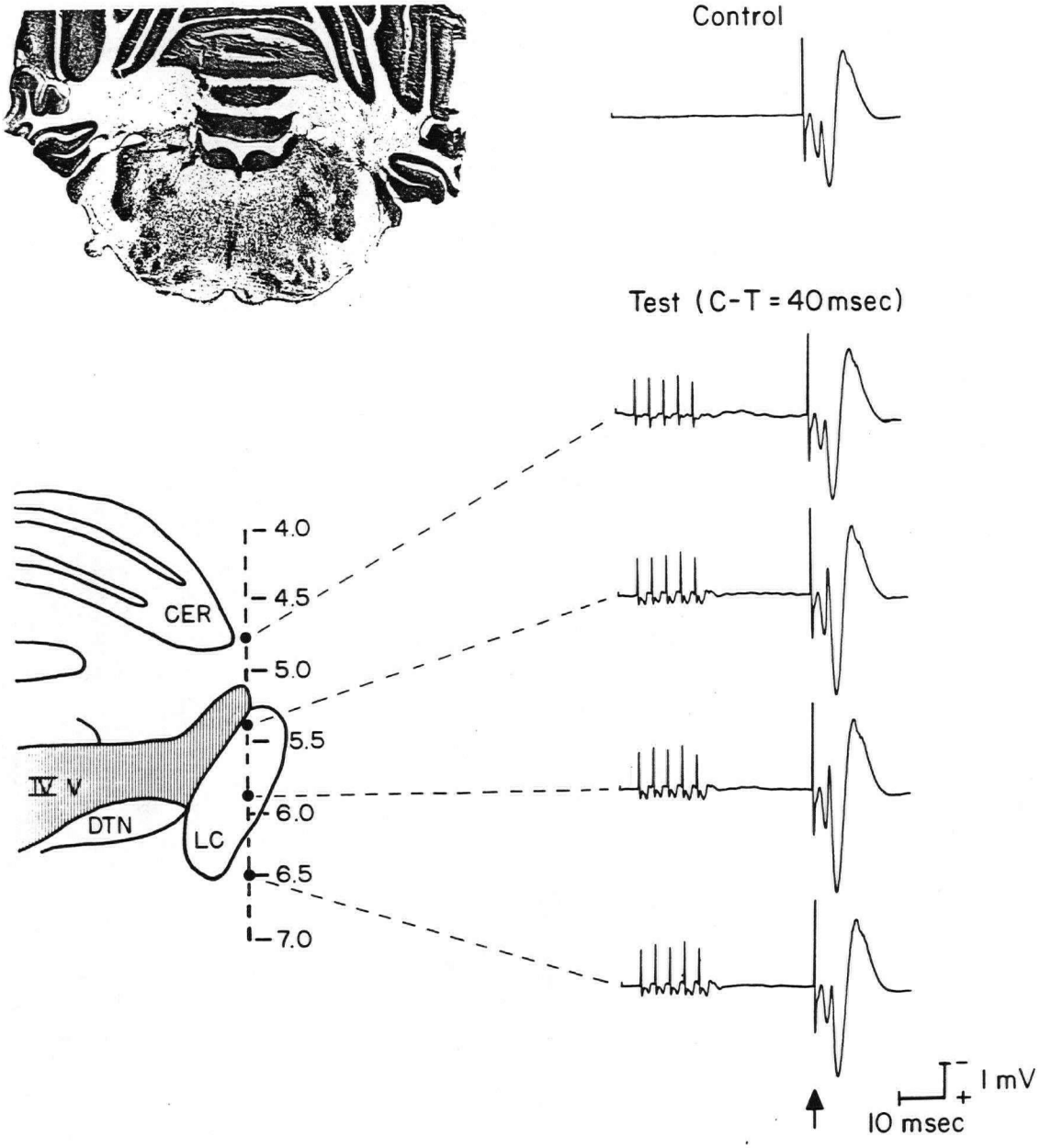

200
100
Amplitude (% of Control)
0
1
2
3
4
5
6
7
No. of Pulses

FIG. 9- 4: Histological Localization Of Stimulation Electrode In LC.

The photomicrograph taken at the rostral level of LC shows the stimulating electrode placement (arrow). The lower schematic and corresponding profiles indicate the relationship between proximity of the stimulating electrode to LC and enhancement of the PP-evoked population spike. Each record is the average of 30 trials.

Abbreviations.

CER	cerebellum
DTN	dorsal tegmental nucleus
LC	locus coeruleus
IVV	fourth cerebral ventricle


Control
Test (C-T = 40msec)
CER
4.0
4.5
5.0
5.5
6.0
6.5
7.0
IV V
DTN
LC
1 mV
10 msec

localized to the locus coeruleus, especially the rostral pole, produced significantly more potentiation than extra LC placements.

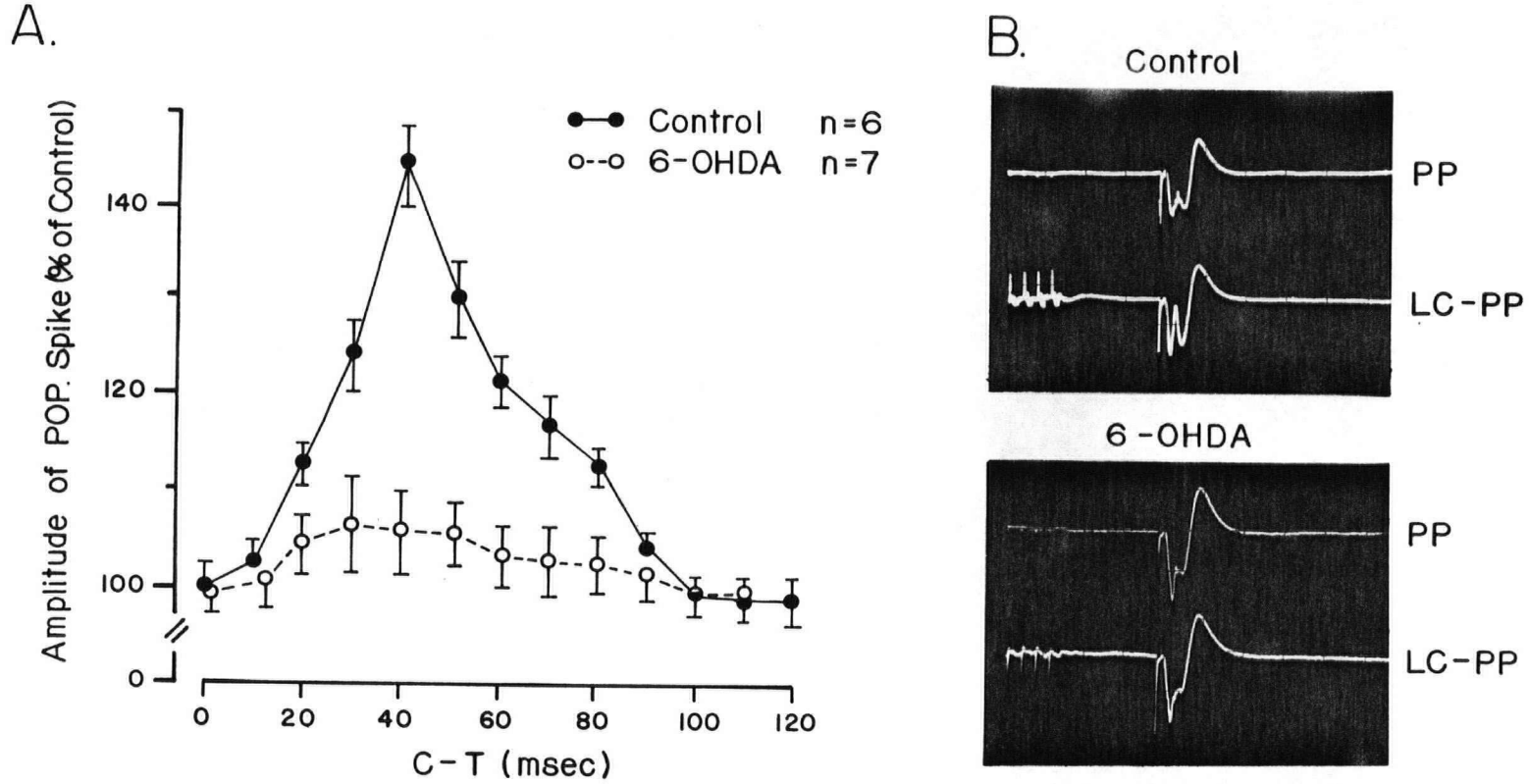
Effects Of 6-OHDA Lesions Of The Dorsal Noradrenergic Pathway

Bilateral injection of 6-OHDA (4 uq in 2 ul saline) into the dorsal NA bundle resulted in substantial depletions (mean=91%) of hippocampal NA. As shown in Fig. 9-5 electrical stimulation of LC in 6-OHDA treated rats did not significantly potentiate the population spike response. In the same animals paired pulse stimulation of PP continued to produce a significant potentiation indicating that the capacity for increase in amplitude was not eliminated by 6-OHDA lesions. It is noteworthy that the only lesioned animal in which a low degree (120-125%) of potentiation was observed had the least effective depletion of NA (30%).

FIG. 9-5: Effects Of 6-OHDA On The Response To LC Stimulation.

A: amplitude of the test population spike plotted as a function of condition-test (C-T) interval. The vertical bars indicate standard errors of the mean.

B: oscilloscope traces of control (PP) and test (LC-PP) population spikes indicating that spike amplitude is increased following electrical stimulation of LC in control (top photo) but not in 6-OHDA lesioned rat (lower photo).


A.
Control n=6
6-OHDA n=7
Amplitude of POP. Spike (% of Control)
140
120
100
0
0
20
40
60
80
100
120
C-T (msec)
B.
Control
PP
LC-PP
6-OHDA
PP
LC-PP

9.4 Discussion

Effects Of LC Stimulation On G-cell Discharge

In contrast to the inhibition observed following single pulse stimulation of the MR, single volleys delivered to the LC did not reliably influence the discharge of the majority of dentate cells. Trains of stimuli were much more effective resulting in inhibition of 55% of the dentate cells at latencies consistently less than 30 msec following the onset of train. However, stimulation of LC with repetitive trains usually resulted in facial movements and an increase in the background firing of the recorded neurone. Previous studies have also indicated that repetitive stimulation of LC is required to inhibit hippocampal (Segal and Bloom, 1974) and cortical neurones (Phillis and Kostopoulos, 1976). However these studies do not report whether they observed facial twitches. Nevertheless, their records (Segal and Bloom, 1974b Fig. 2; Phillis and Kostopoulos, 1976 Fig. 1) reveal a higher background discharge of the neurone being recorded. An increase in discharge rate may be related to activation of adjacent sensory-motor regions due to current spread resulting from high frequency stimulation (Bagshaw and Evans, 1976). This increased rate of discharge could obscure LC-evoked inhibition.

The reasons that stimulation of LC with repetitive

stimuli at low intensities is more effective than a single strong volley may be due to the organization of the LC and its mode of termination upon the recorded cells. Anatomical investigations have revealed that a relatively small population of LC cell bodies (estimate range between 1400-2000) give rise to thin unmyelinated axons which bifurcate considerably to terminate in wide regions of the forebrain (Fuxe, 1965; Ungerstedt, 1971; Swanson and Hartman, 1975 ; Jones et al, 1977). Perhaps repetitive stimulation overcomes the high probability of conduction failure inherent in a large number of axon bifurcations (Rall, 1974). In the absence of acceptable reports on the discharge patterns of identified LC neurones no comparisons can be made at this time between optimal stimulation parameters and the spontaneous or physiological discharge pattern of LC neurones. In the present study the number of pulses was kept minimal (3-5 pulses) whereas in other reports (Segal and Bloom, 1974b) very long trains (up to 10 seconds) were used. Such differences make it difficult to compare the latencies, potencies and duration of evoked responses between studies.

In light of the diffuse distribution of NA terminals in the dentate, special care was taken to identify the cells that were recorded. This resulted in the inclusion of only 15 cells recorded in 8 rats for the purposes of data analysis. All 15 cells were monosynaptically activated by PP stimulation, inhibited

by MR and were localized to the vicinity of the G-cell layer. These criteria suggest that these cells were in fact G-cells, however, it is not possible to determine whether the inhibition observed following multiple pulse LC stimulation was mediated by a monosynaptic input directly onto G-cells.

Effect Of LC Stimulation On PP-evoked Responses

Conditioning stimulation of LC resulted in an increase in the amplitude of the PP-evoked population spike without altering the amplitude or rise time of the PP-evoked EPSP. The effective C-T intervals ranged between 20-80 msec and maximum potentiation (150-175% of control) was observed following 3-5 conditioning pulses whereas no potentiation was observed following single pulse stimulation of LC. The observation that the degree of potentiation was related to the proximity of the stimulating electrode to LC proper further supports a specific action of this particular nucleus on PP-evoked population spike. The additional observation that 6-OHDA injections which deplete hippocampal NA block the potentiation, strongly suggests involvement of a noradranergic system.

Since potentiation of the population spike amplitude was not accompanied by a concomittant increase in the rate of rise of the EPSP it is not likely to be due to the action of LC on the entorhinal

cortex or the region of the PP synapse on the distal dendrites of G-cells. In this respect the actions of LC stimulation on PP-evoked responses may be similar to those observed following stimulation of other extrinsic afferents including the commissural input and the MR.

9.5 Summary

Electrical stimulation of LC caused an increase in the amplitude of the dentate poulation spike which was evoked by a test PP volley. The same conditioning stimulation did not result in an increase in the rate of rise of the EPSP suggesting that presynaptic mechanisms could not account for enhancement of the population spike response. Injections of 6-OHDA into the dorsal NA bundle resulted in depletion of hippocampal NA and abolished the effects of LC stimulation on the population spike response. On the basis of these data, it was proposed that a noradrenergic system modifies neuronal transmission in the dentate gyrus.

CHAPTER 10: GENERAL DISCUSSION

The results of the experiments described in this thesis have demonstrated that serotonergic and noradrenergic systems originating in MR and LC, respectively, modulate RSA and/or the population response of G-cells to stimulation of the perforant path (PP) input. Detailed discussions of the present and previous data which support this conclusion were presented at the end of each chapter. However, the physiological implications of the present data and how they relate to existing concepts of the functional role of the hippocampal formation have not been discussed.

Previous electrophysiological and behavioural studies have emphasized that RSA may reflect or predict 1) activity of other brain areas such as the neocortex and the septal area (Green and Arduini, 1954; Petsche et al, 1962) 2) overt behaviour (Vanderwolf, 1969; Ranck, 1975) and 3) inferred mental processes (Gray, 1970; Nadel and O'Keefe, 1974). However, the mechanisms underlying RSA and the involvement of specific neuronal systems in this response have recieved little attention. In contrast, hippocampal population responses have been well characterized (Gloor et al, 1964; Andersen and Lomo, 1966; Lomo, 1971a) and mechanisms which alter their magnitude were, and continue to be, extensively investigated (Lomo, 1971b Bliss and Lomo, 1973; Andersen et al, 1977; Lynch et al, 1977; Dunwiddie and

Lynch, 1978). More recently, studies which examine the relationship between hippocampal population responses , behaviour (Winson and Abzug, 1978) and RSA (Assaf and Miller in preparation) have been initiated.

The present thesis has 1) delineated mechanisms which could underlie the effects of pharmacologically distinct neuronal systems on hippocampal electrical activity and 2) indicated that extrinsic afferents to the DG such as the commissural input (Chapter 7) and monoaminergic projection (Chapters 8 and 9) alter the amplitude of the PP-evoked population spike. Furthermore, the observation that a raphe serotonin system, wich increased the amplitude of the population spike, also produced hippocampal desynchronization suggests that the magnitude of the population spike response may be related to or dependent on ongoing patterns of hippocampal electrical activity.

10.1 Patterns Of Hippocampal Electrical Activity

Previous studies had postulated that distinct neuronal systems, probably originating in the brainstem, mediate the characteristic patterns of hippocampal electrical activity (Green and Arduini, 1954; Torii, 1961; Macadar et al, 1974). However, these studies did not elucidate anatomically or pharmacologically distinct neuronal systems that could serve as a model for studying the mechanisms mediating

the different patterns. The present observation that a serotonin-containing system produced desynchronization has facilitated the delineation of neuronal mechanisms within the septal area that could underlie this response. The postulated mechanisms, which included inhibition of irregularly firing I-neurones and subsequent disruption of the bursting of B-neurones, were consistent with previous anatomical (Tombol and Petsche, 1969) and electrophysiological (Petsche et al, 1962) studies indicating a heterogenous population of neurones in the medial septal nucleus. Although the present data did not eliminate the possibility that other intra-and extraseptal mechanisms can underlie the desynchronization, they provide a model which could be readily tested. The additional observations that 1) MS neurones may be differentiated on the basis of their discharge patterns or their hippocampal projections and 2) kainate lesions, which destroy cell bodies without influencing fibers of passage (Assaf and Miller, 1978d) eliminate RSA, facilitate future studies on the role of intra- and extraseptal mechanisms in the generation of hippocampal electrical acitivty.

On the basis of previous reports (Macadar et al, 1974), LC stmulation was expected to produce RSA specifically. The neuronal mechanisms mediating this response could then be compared with the desynchronization reulting from MR stimulation. However, the results of chapter 5 indicated that 6-OHDA

injections which depleted forebrain NA did not block RSA elicited by stimulation in the region of LC. The additional observation that electrolytic lesions of LC did not abolish RSA suggested that examination of this system may not be suitable for delineating mechanisms which mediate RSA. These findings, which were recently confirmed by Robinson (1978) in the freely moving rat, do not entirely eliminate noradrenergic involvement in the generation of RSA since: 1) stimulation in a diffuse region in the vicinity of LC also initiates RSA, thus the effects of 6-OHDA injections on a noradrenergic component may be masked or even compensated for by these other systems 2) NA-containing nuclei located caudal to LC project to the septal area (Moore, 1978) and may be involved in the generation of RSA 3) multiple pathways and postulated transmitters (reviewed in section 1.3) are implicated in the generation of RSA and none of them, with exception of the septal area, may be critical for the generation of this response. Perhaps, as Gray and his colleagues (1975) have proposed, noradrenergic systems lower the threshold for a particular frequency (7.7 Hz) which is initiated by the septal area. Activity of the septal area, perhaps reflected by the output of B-neurones, may result from the interaction between various neuronal systems which modulate hippocampal electrical activity in a manner appropriate to ongoing physiological states.

10.2 The Significance Of Hippocampal Population Responses.

Previous studies (Gloor et al, 1964; Lomo, 1971a), taken together with the results of chapter 6, support the interpretaion that the field potentials recorded extracellularly in DG following PP stimulation reflect the direct action of the entorhinal cortex on dentate granule cells. Interest in these responses stemmed from the observations that paired-pulse stimulation of PP resulted in an increase in the rate of rise of the EPSP and amplitude of the population spike (Lomo, 1971b; Steward et al, 1976). This potentiation has been studied either following a brief period of repetitive conditioning stimulation (usually 1-3 seconds at 50-100 Hz) which resulted in long-term potentiation of the extracellular EPSP and population spike (Bliss and Gardner-Medwin, 1973) or following a single conditioning volley which produced potentiation of the same parameters for a short (less than 1 second) period of time (Lomo, 1971b; Steward et al, 1976). Since both short and long-term potentiation have been demonstrated in the PP-dentate projection and there is no evidence to indicate that different fundamental mechanisms are involved, we will assume, for the purpose of the present discussion, that they share common mechanisms.

Initially it was proposed that augmented transmitter release underlies the greater synaptic

current and subsequent increase in the number of G-cells evoked by the test PP pulse (Lomo, 1971b). Previous speculations that an increased quantal release of transmitter accounts for post-tetanic potentiation of neuromuscular transmission (Eccles and Rall, 1951; Liley, 1956) provided impetus for this proposal. However, in the initial studies (Lomo, 1971b; Bliss and Lomo, 1973) increases in the amplitude of the PP-evoked population spike were sometimes observed in the absence of changes in the EPSP. For this reason, Bliss and Lomo(1973) and Bliss (personal communication) proposed that mechanisms other than augmentation of synaptic current could enhance the responsiveness of the postsynaptic cells.

The present thesis provides support for the latter suggestion since conditioning stimulation of Comm, MR and LC increases the amplitude of the PP-evoked population spike without altering the PP synaptic potential. In the same animals, conditioning pulses delivered to the PP itself potentiated both the EPSP and the population spike. These observation imply that neuronal transmission from the entorhinal cortex to DG could be modulated by either 1) presynaptic mechanisms which increase the efficiency of the afferent input or 2) postsynaptic mechanisms which enhance the responsiveness of the receiving cell or its effector mechanisms. Both mechanisms could account for the potentiation observed during paired-pulse stimulation

of the PP but only postsynaptic mechanisms could account for the increase in population spike amplitude following conditioning pulses to extrinsic afferents which did not alter the EPSP.

The effects of stimulating extrinsic afferents on the PP evoked responses have not been studied in detail previous to the present work. However, Lomo (1971b) has shown that conditioning stimulation of the mossy fibre system, which antidromically activates G-cells, results in an increase in the amplitude of the PP evoked population spike without changing the rate of rise of EPSPs. More recently, Alvarez and Gardner-Medwin (1976) reported a similar effect following stimulation of the medial septal area. These data, taken together with the results of chapters 7-9, suggest that conditioning stimulation of sites other than the PP can somehow increase the number of G-cells which are evoked by a constant PP synaptic current. Therefore presynaptic mechanisms can not account for the observed potentiation.

Postsynaptic mechanisms that could account for the increase in the amplitude of the population spike include 1) activation of excitatory interneurones which partially depolarize the G-cell soma (Andersen et al, 1967) and 2) removal of factors normally preventing the PP from evoking a given G-cell. The contribution of excitatory interneurones is unlikely since in the

present study potentiation is observed in the absence of any excitation. Moreover, there is no evidence for the existence of excitatory interneurones in the immediate vicinity of G-cells. In fact, the common feature of all the inputs presently shown to produce potentation of the population spike response, is inhibition of G-cells either directly, as in the case of MR, or subsequent to an activation as in the case of the PP and commissural inputs.

The relationship between inhibition of G-cells and potentation of the population spike was examined in most detail following stimulation of MR since this particular nucleus produced a potent inhibition which was not preceded by activation. The duration of inhibition of G-cells was related to the length and degree of potentiation. Several mechanisms could account for this apparent paradox between depression of spontaneous G-cell discharge and facilitation of the PP evoked population spike. At the simplest level inhibition of a large number of G-cells may reset their random activity thereby allowing a more synchronous discharge in response to a prepotent PP volley. Since the amplitude of the population spike reflects, in part, the synchronous discharge of a large number of G-cells, it will be enhanced. This synchronizing mechanism may also be operative during rhythmical septal-hippocampal activity. Perhaps, as Vinogrova (1975) had suggested earlier, information arriving via

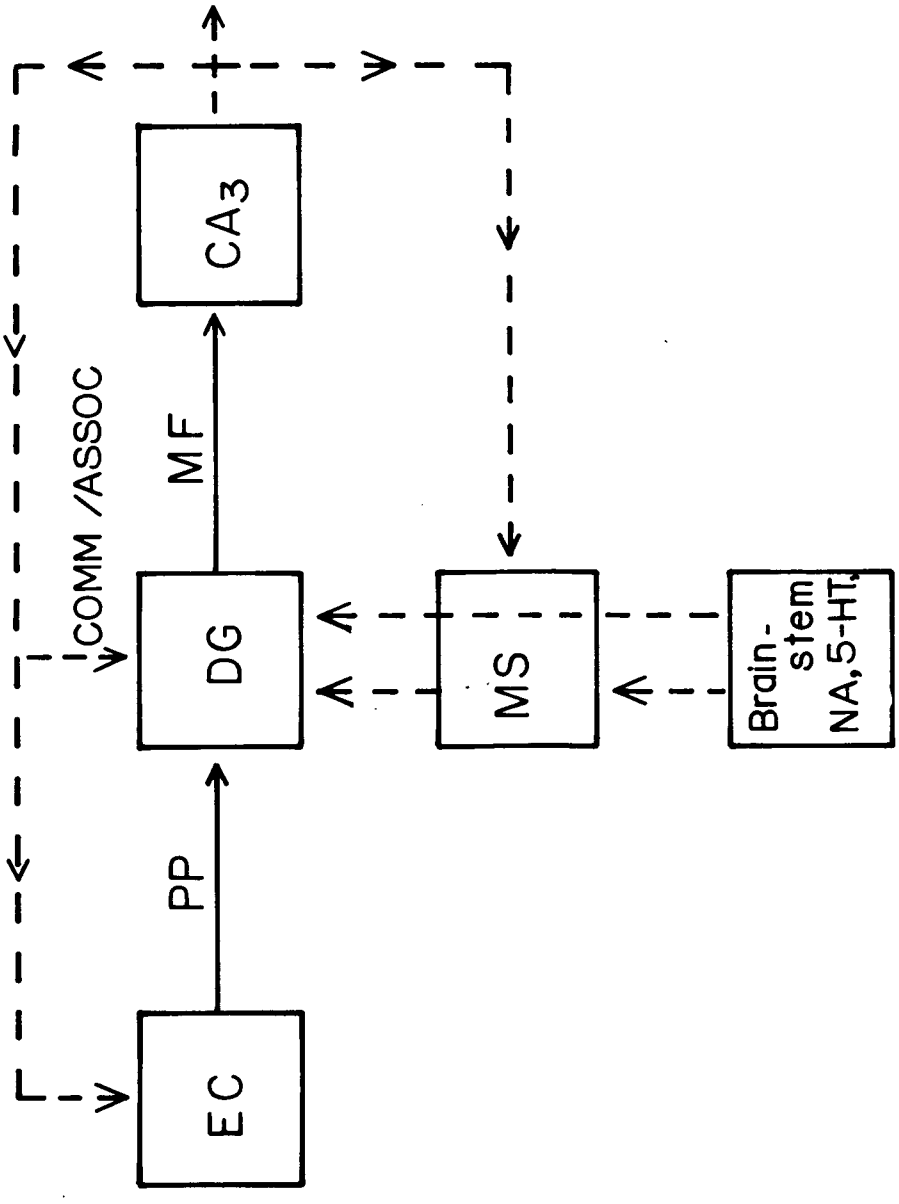
the PP is modulated by the bursting discharge pattern of septal or hippocampal neurones.

10.3 Rhythmical Activity And Neuronal Transmission In the Dentate Gyrus

The discussion to this point has been limited to mechanisms within the dentate gyrus itself which could increase the amplitude of the population spike response. An additional approach is to regard the population spike response as the output of the dentate via the mossy fibre system onto CA3. Perhaps this output is regulated in a manner appropriate to ongoing physiological states by adjusting the responsiveness of dentate G-cells. As indicated in Fig. 10-1, the CA3 region would play a pivotal role since it receives almost the entire dentate output and has both direct connections with the dentate via the associational/commissural systems and indirect connections through the septal area. A decrease in mossy fibre output, as in the case of G-cell inhibition, would initiate mechanisms that enhance the population spike response to a given PP input. The direct feedback may be mediated by changes in the depolarization of proximal dendrites of G-cells thereby enhancing or shunting PP synaptic current generated on the distal dendrites. The indirect septal loop may attenuate the input by initiating the bursting discharge pattern of septal neurones (McLennan and

FIG. 10- 1: Proposed Extrinsic Influences On Neuronal Transmission In The Dentate Gyrus.

The solid lines indicate that information may be transmitted from the entorhinal cortex (EC) to the dentate gyrus (DG) via the perforant path (PP). The output from DG is via the mossy fibre system (MF) onto CA3 region of the hippocampus. The dotted lines indicate possible feedback control of EC-DG transmission by CA3 via the associational/commissural system, the medial septal area (MS) and brainstem NA and 5-HT-containing systems.


EC
PP
DG
MF
CA3
COMM /ASSOC
MS
Brain-stem NA, 5-HT.

Miller,1976) which may then 'gate' the incoming PP input (Vinogradova, 1975). The additional feature offered by the indirect septal loop would be to match CA3 output to ongoing physiological states which produce the characteristic discharge patterns of septal neurones (chapter 3).

The recent findings of behaviourally specific modification of the PP evoked population spike support the above suggestions (Winson and Abzug, 1978). Moreover, preliminary experiments (Assaf and Miller, 1978e) show that the amplitude of the PP evoked population spike is dependent on the ongoing pattern of hippocampal electrical activity and septal unit discharge. If the PP is stimulated during RSA or when B-neurones are bursting the amplitude of the population spike is reliably smaller than that evoked by the same PP volley delivered during hippocampal desynchronization. It is interesting to note that Winson and Abzug (1978) report that the PP evoked population spike is smallest when the freely moving rat is in an alert or aroused state and largest during slow wave sleep. Previous studies have shown that RSA occurs during alert states and desynchronized or irregular hippocampal activity is recorded during slow wave sleep (Vanderwolf, 1972; Robinson, 1978).

Taken together these studies suggest that PP-dentate transmission may be enhanced during hippocampal

desynchronization and attenuated during RSA. It is noteworthy that when electrical activity of the neocortex consists of desynchronized fast activity, as in arousal states, rhythmical slow activity is prominent in the hippocampal formation (Green and Arduini, 1954). Perhaps this apparent reciprocity is a reflection of compensatory mechanisms within the septal-hippocampal axis which maintain constancy of cortico-hippocampal-thalamic transmission.

The implication of this constancy and whether long-term potentiation is an integral component of it must await further experiments. Likewise, understanding the relevance of PP-dentate transmission to overall brain function depends on knowing 1) what is the nature of the information relayed by the PP, 2) where does the hippocampus relay this information and 3) how do extrinsic inputs to the hippocampal formation such as those originating in the septal area and monoamine-containing nuclei modify this information?

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