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An Anatomical and Physiological Investigation of Hippocampal Connections Involved in Cardiovascular Control

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AN ANATOMICAL AND PHYSIOLOGICAL INVESTIGATION OF
HIPPOCAMPAL CONNECTIONS INVOLVED IN
CARDIOVASCULAR CONTROL

by

Kenneth Gary Ruit

A Dissertation Submitted to the Faculty of the Graduate
School of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

September

1988

DEDICATION

To Mom, Dad, Linda, Ron, and Kim

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Edward J. Neafsey, for his dedication and commitment to his graduate students. This work could not have been completed without his guidance and undying enthusiasm for scientific discovery. I would also like to thank the additional members of my dissertation committee, Dr. Anthony J. Castro, Dr. John F. Disterhoft, Dr. Thackery S. Gray, and Dr. Robert D. Wurster for their willingness to critically evaluate this work. Their input was invaluable. I would also like to acknowledge the technical assistance of Dr. John McNulty, Dr. Ross Kosinski, Linda Fox, and especially Vanessa Alones who assisted with the electron microscopic study. I would like to thank all the members of the Anatomy Department for their support, encouragement, and senses of humor. Special thanks to Mike, Bob, and Terry--we were a foursome that no serious golfers wanted to be associated with. Finally, a very special "thank you" to my wonderful wife, Kim, whose constant love, support, and prayers made it all worthwhile.

VITA

The author, Kenneth G. Ruit, is the oldest son of Garret and Joyce Ruit. He was born on May 1, 1961 in Paterson, New Jersey.

His secondary education was obtained at Eastern Christian High School in North Haledon, New Jersey, from which he graduated in June, 1979. In September of 1979 he entered Wheaton College in Wheaton, Illinois and graduated with a Bachelor of Science degree in Biology in May of 1983. In August of 1983, he entered the Department of Anatomy of the Graduate School at Loyola University of Chicago. While at Loyola, he received a Basic Science Fellowship and taught in the gross anatomy, histology, and neuroscience courses. In 1987 he received a Biomedical Research Support Grant from the Neuroscience and Aging Institute of Loyola University Medical Center. He is a member of the Society for Neuroscience, the Society of Sigma XI, and the American Scientific Affiliation. He has also been active as the Graduate Student Representative on the Graduate Committee of the Department of Anatomy, and as Treasurer of the Graduate Student Council.

He is married to Kimberly Ann Ruit.

After completion of his doctorate, he will begin a post-doctoral fellowship in the Department of Neurology and Neurological Surgery at Washington University Medical Center in St. Louis, Missouri under the supervision of Dr. William D. Snider.

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CHAPTER I

INTRODUCTION

The literature on the hippocampus has grown extensively since the turn of the century, yet "despite extensive investigations of the hippocampus, we have only a vague understanding of its global functional role. . ." (Eichenbaum et al., 1987). Functional evaluations of the hippocampal formation have evolved from associating it with olfactory functions (see Brodal, 1947) to the view of the hippocampus as a cognitive map (O'Keefe and Nadel, 1978). The early studies of Papez (1937) implicated the hippocampus along with the hypothalamus, the anterior thalamus, and the cingulate gyrus as an anatomical basis for emotional expression. MacLean (1949) broadened this concept, calling these limbic system structures the "visceral brain" based on his observations of the interrelationships between differing emotional states and interoceptive "gut" feelings or reactions. However, as the role of the hippocampus in learning and memory became increasingly popular (Siefert, 1983), its possible role in the modulation of autonomic functions was forgotten, chiefly because of ambiguities in the results of electrical stimulation and lesion experiments (Kaada, 1960).

Recently, the medial frontal cortex of the rat has been shown to receive major efferent projections from the CA1 and subicular regions of the ventral hippocampus (Swanson, 1981; Ferino et al., 1987), prompting the suggestion that this prefrontal region is involved primarily in limbic system functions. The medial frontal cortex also has a direct projection to the nucleus of the solitary tract, the

primary visceral afferent nucleus in the dorsal medulla (van der Kooy et al., 1982, 1984; Terreberry and Neafsey, 1983, 1987). It is likely that this pathway is at least partially responsible for the cardiovascular and gastrointestinal changes observed in response to electrical stimulation of the medial frontal cortex (Kaada, 1951; Lofving, 1961; Terreberry and Neafsey, 1984, 1988; Burns and Wyss, 1985; Hurley-Gius and Neafsey, 1986). The projection zone from the hippocampus closely overlaps the cortical cells which send their axons to the brainstem; this connectivity suggests that the hippocampus has direct access to a visceral control system of the brain and that via the medial frontal cortex the hippocampus may indeed play an important role in autonomic regulation allowing an organism to respond appropriately to different environmental cues.

CHAPTER II

REVIEW OF RELATED LITERATURE

The hippocampus

At the base of these [cerebral] ventricles, which faces in toward the median line, an elevation of white substance rises up and, as it were, grows there. This is raised up from the inferior surface like an appendage . . . In its length it extends toward the anterior parts and the front of the brain, and is provided with a flexuous figure of varying thickness. This recalls the image of a Hippocampus, that is, of a little sea-horse.

This is the first description of the gross appearance of the hippocampal formation recorded by Julius Caesar Arantius in 1587. Since that time many scientists have dedicated themselves to the study of the hippocampal formation in an effort to understand its anatomical connections, biochemical processes, electrophysiological characteristics, pharmacological constitution, and overall role in higher brain functions.

The pioneering works of Santiago Ramon y Cajal and his student Lorente de No in the late nineteenth and early twentieth centuries described in depth the cytoarchitecture of the different subdivisions (the CA1, CA2, CA3, CA4 cell regions of Ammon's horn, the subiculum, and the dentate gyrus) of the hippocampal formation as well as its afferent, efferent, commissural, and associational connections (Cajal, 1893; Lorente de No, 1934). Subsequent studies up to the present have utilized a variety of techniques in order to elaborate on the findings of Cajal and Lorente de No. Axonal degeneration studies (Powell and Cowan, 1955; Nauta, 1956, 1958; Valenstein and Nauta, 1959; Blackstad, 1956, 1958; Blackstad et al., 1970; Raisman et al., 1965, 1966; Raisman, 1966; Cragg, 1965; Siegel and Tassoni, 1971; Hjorth-Simonsen,

1971, 1972, 1973; Hjorth-Simonsen and Jeune, 1972; Siegel et al., 1974), autoradiography (Gottlieb and Cowan, 1973; Steward, 1976; Rosene and Van Hoesen, 1977; Meibach and Siegel, 1977; Swanson and Cowan, 1977, 1979; Swanson et al., 1978), the use of physiologically transported tracers (Wyss et al., 1979; Swanson et al., 1980, 1981; Irle and Markowitsch, 1982; Ino et al., 1987), and electrophysiological techniques (Green and Adey, 1956; Poletti et al., 1973; DeFrance et al., 1973a&b; McLennan and Miller, 1974; Deadwyler et al., 1975; Poletti and Sujatanond, 1980; Finch and Babb, 1980; Finch et al., 1984, 1986; Yang and Mogenson, 1984) have made the anatomical and neurophysiological relationships of the hippocampal formation some of the most well known in the brain.

The major afferent projection of the hippocampus is the "perforant path" first described by Cajal (1911) and substantiated by Blackstad (1958), Raisman et al. (1965), and Steward (1976). The "perforant path" arises in the entorhinal cortex and terminates primarily on the granule cells of the dentate gyrus, the first synapse in a tri-synaptic flow of information through the hippocampus (Figure 1). The axons ("mossy fibers") of the granule cells project onto the prominent dendritic spines on the pyramidal cells of the regio inferior or CA3 region of Ammon's horn (Cajal, 1893). These cells' axons or "Schaffer collaterals" (Schaffer, 1892) project onto the dendrites of the pyramidal cells in the regio superior or the CA1 cell region of Ammon's horn and also to the subiculum. The cells of the CA1 region

project to the subiculum, and together with axons from the subiculum and CA3 form the principle efferent pathway of the hippocampal formation, the fimbria-fornix.

Other major afferents to the hippocampus include those from the medial septum (Raisman, 1966; Swanson and Cowan, 1979), the opposite hippocampus (Blackstad, 1956; Swanson et al., 1978), the cingulate gyrus (White, 1959; Raisman et al., 1965; Domesick, 1969), and neocortical associational areas (Van Hoesen and Pandya, 1975a&b). Other subcortical afferents of the hippocampus arise in the thalamus, the hypothalamus and mammillary complex, and the brainstem including noradrenergic input from the the locus coeruleus, serotonergic input from the median raphe nuclei, dopaminergic input from the ventral tegmental area, and inputs from the substantia nigra, dorsal and laterodorsal tegmental nuclei, and central gray (see Wyss et al., 1979).

Major terminations of hippocampal efferent projections via the fornix include the lateral septal complex, the mammillary complex, the bed nucleus of the stria terminalis (Swanson and Cowan, 1977), the nucleus accumbens (Fox, 1943; De France et al., 1980; Lopes da Silva et al., 1984), and the infralimbic area (Swanson and Cowan, 1977; Swanson, 1981). In addition, there also exist caudally directed, non-fornix projections to the entorhinal and retrosplenial areas of the cerebral cortex (see Swanson and Cowan, 1977).

Concomitant with anatomical studies of Ammon's horn appeared

studies which speculated on the function of the hippocampus. The earliest studies associated the hippocampus with olfactory functions (see Brodal, 1947). Brodal points out in his review that conclusions about the hippocampus' role in olfaction were based on rather flimsy anatomical and physiological evidence associating the hippocampus with other olfactory structures. He argued that, although some functional aspects of a structure can be inferred from anatomical observations, the validity of that inference is acceptable "only provided that the anatomical methods employed allow a detailed mapping out of the relevant fiber connections." His review of anatomical studies showed that the hippocampus is only very indirectly associated with olfactory structures, refuting previous suggestions that the role of the hippocampus is primarily olfactory.

Further studies of hippocampal connections and physiological studies implicated the hippocampus in a variety of other functions. Papez (1937) discussed the anatomical interconnections between the hippocampus, the hypothalamus, the anterior thalamus, and the cingulate gyrus, components of the "limbic lobe" of Broca forming the so-called Papez circuit, as the "anatomic basis of the emotions" and not as olfactory. The conclusions of Papez were strengthened by the landmark work of Kluver and Bucy (1939) in which temporal lobe lesions including the hippocampus in the rhesus monkey induced "psychic blindness", strong oral tendencies, and marked emotional behavioral changes.

Scoville and Milner (1957) worked with a young male human patient

(H.M.) who had the medial portion of both temporal lobes (including the hippocampus) removed to treat temporal lobe epilepsy. They found that following these lesions, H.M. did not have the capacity to form any new memories. This study popularized the association of the hippocampal formation with learning and normal memory function (see Green, 1964; Adey, 1967; Segal and Olds, 1972; Segal et al., 1972; Mishkin, 1978; Olton et al., 1979; Squire, 1982; Swanson et al., 1982; Siefert, 1983; Halgren, 1984; Heit et al., 1988). The neural activity of the hippocampus is also associated with epileptogenic discharges (see Adey, 1959; Green, 1964; Prince, 1978), voluntary motor systems and motor activity, (Vanderwolf, 1969; Heimer and Wilson, 1975), correlates of spatially-oriented behaviors (O'Keefe, 1976; O'Keefe and Conway, 1978; Olton et al., 1978), and neuroendocrine control (Mangili et al., 1966; McEwen et al., 1979; Ganten and Pfaff, 1982; McEwen et al., 1986). Although these and many other studies have enumerated various hippocampal formation functions, the studies in this dissertation will deal specifically with the role of the hippocampus in autonomic regulation, which may be linked to the proposed role for the hippocampus in "emotion."

"Emotion", as described by Papez (1937), has two components initiated by limbic system structures: a way of acting or outward behavior and subjective feeling or experience. MacLean elaborated this concept when he called the limbic system the "visceral brain" which "interprets and gives expression to its incoming information in terms

of feeling. . .[or] the 'gut' component of memory" (MacLean, 1952). This "incoming information" includes olfactory input to the amygdala and the hippocampus (Powell et al., 1965; White, 1965; Steward, 1976), gustatory afferents relayed to the lateral hypothalamus, the amygdala, and the bed nucleus of the stria terminalis (Norgren, 1974; Norgren, 1976), and general visceral afferent information relayed to the hypothalamus, the amygdala, and the thalamus (Ricardo and Koh, 1978; Sawchenko, 1983).

Anatomically, the dense projection from the hippocampus to the hypothalamus, a region of the brain that is, among other functions, associated with cardiovascular control (Loewy and McKellar, 1980), suggests that the hippocampus may be involved in the control of autonomic activity (Brodal, 1947). Another major subcortical projection of the hippocampus is to the lateral septal region of the brain (Swanson and Cowan, 1979), and it has been shown that electrical and chemical stimulation of the septum elicits modifications in heart rate and blood pressure (Calaresu and Mogenson, 1972; Gelsema and Calaresu, 1987). These changes in cardiovascular activity are usually associated with the projection from the septum to the hypothalamus (Calaresu et al., 1976).

Electrical stimulation of the hippocampus in primates, cats, dogs, and guinea pigs induces "somatic" behavioral modifications such as attention (Kaada et al., 1953) and attack responses (Siegel and Flynn, 1968), "agitation", defensive reactions, growling, escape

reactions (MacLean and Delgado, 1953), grooming, and "bewilderment and confusion" (MacLean, 1957). Additional responses included visceral or autonomic modifications such as decreases in heart rate and increases in pulse pressure (Smith, 1944), decreases in blood pressure with either increases or decreases in heart rate (Anand and Dua, 1956), respiratory inhibition (Kaada and Jasper, 1952; Andy and Akert, 1955; Liberson and Akert, 1955; Anand and Dua, 1956), inhibition of pyloric antral peristalsis (Kaada, 1951), and sympathetic discharges indicated by pupillary dilatation and increased blood pressure (Carlson et al., 1941; Andy and Akert, 1953). Stimulation of the fornix and hippocampus also evokes activity in neurons in the dorsal vagal complex of the cat (Akert and Gernandt, 1962).

More recently, studies of the hippocampus in primates and rats have focused on the role of the hippocampus in the pituitary-adrenal axis and its involvement in stress-related mechanisms. Extensive stimulation or lesion studies have shown that the hippocampus, which contains the highest concentration of receptors for glucocorticoids in the mammalian brain (McEwen, 1982), exerts an inhibitory influence over adrenocortical activity and participates in glucocorticoid feedback on plasma corticosterone levels (Wilson and Critchlow, 1973-74; Casady and Taylor, 1976; Frankel et al., 1978; Dunn and Orr, 1984). Chronic stress or exogenous corticosterone administration down-regulates the corticosterone receptors in the hippocampus, yet this down-regulation is reversible and receptor concentrations soon return to normal levels

(Sapolsky et al., 1984). Also the number of corticosterone receptors in the hippocampus declines in the aged rat (Sapolsky et al., 1983). This decline has been correlated with the observation that aged rats, in response to stress, are not able to exert negative feedback on increased plasma corticosterone levels and, hence, exhibit a corticosterone hypersecretion following the end of stress (Sapolsky et al., 1984).

In contrast to these reports, many studies have not been successful in eliciting somatomotor, autonomic, or endocrine responses by stimulating the hippocampus proper. Instead, electrical stimulation of the hippocampus and fornix in cats and dogs did not produce any significant blood pressure effects except at extremely high currents (Kaada, 1951). Similarly, neither electrical nor chemical stimulation produced any significant autonomic manifestations in awake cats (MacLean, 1957). This led Kaada to conclude that "There is at present no evidence either from stimulation or from ablation experiments to justify the application of this term [visceral brain]. . .to the hippocampus-fornix system" (Kaada, 1960).

The medial frontal cortex

The cortex located on the medial surface of the cerebral hemispheres extending from the genu of the corpus callosum to the frontal pole is designated as the medial frontal cortex. In the rat, the medial aspect of the frontal cortex has been subdivided

cytoarchitecturally by Krettek and Price (1977) and Vogt and Peters (1981) into the anterior cingulate area, the prelimbic area, and the infralimbic area (Figure 2).

Numerous studies have demonstrated the afferent connections of the medial frontal cortex in the rat, cat and monkey (see Kolb, 1984; Finch et al., 1984; Cavada and Reinoso-Suarez, 1985; Musil and Olson, 1988a&b). Afferents to the medial frontal cortex arise in the insular cortex (Saper, 1982; Markowitsch and Guldin, 1983), the thalamus (Domesick, 1969, 1972; Leonard, 1969; Beckstead, 1976; Krettek and Price, 1977a; Herkenham, 1979), the amygdala (Krettek and Price, 1974, 1977b; Price, 1981; Amaral and Price, 1984), the hippocampus (Rosene et al., 1976; Swanson and Cowan, 1977; Swanson, 1981; Ferino et al., 1987), the substantia nigra and ventral tegmental area (Lindvall et al., 1974; Fallon and Moore, 1978), and the parabrachial nucleus (Fulwiler and Saper, 1984).

Efferents of the medial frontal cortex terminate homotypically in the contralateral cortex (Domesick, 1969; Beckstead, 1979; Ferino et al., 1987) and reciprocally in structures which project to the medial frontal cortex such as the insula, the amygdala, the thalamus, and the ventral tegmental area (see Domesick, 1969; Leonard, 1969; Beckstead, 1979; Kolb, 1984; Room et al., 1985). Other specific efferents of the medial frontal cortex include projections to the periaqueductal gray, the superior colliculus, the pontine gray, (Hardy and Leichnetz, 1981; Wyss and Sripanidkulchai, 1984; Neafsey et al., 1986), and the solitary

nucleus (van der Kooy et al., 1982, 1984; Terreberry and Neafsey, 1983, 1987; Neafsey et al., 1986).

The medial frontal cortex has been implicated in spatially-oriented learning behaviors (Silva et al., 1986; Wolf et al., 1987), self-stimulation and spontaneous motor activity (Mora and Myers, 1977; Mora, 1978; Vives et al., 1986), the regulation of emotional states in response to stress as an integral part of the mesocortical dopaminergic system (Thierry et al., 1976), modulation of plasma corticosterone levels (Dunn and Miller, 1987), and visceral control (Kaada, 1951; Lofving, 1961; Burns and Wyss, 1985; Terreberry and Neafsey, 1984; Hurley-Gius and Neafsey, 1986; Fryzstak and Neafsey, 1987; Verberne et al., 1987).

The relationship of the hippocampus to the medial frontal cortex

A heavy ipsilateral projection to the medial frontal cortex arises in the ventral CA1 region of Ammon's horn and the subiculum, terminating specifically in the prelimbic and infralimbic regions (Swanson and Cowan, 1977; Swanson, 1981; Ferino et al., 1987) (Figure 3). The physiological function of this projection remains unclear. Some have speculated that this projection may be a link between caudal parts of the hippocampal formation and primary sensory regions of the neocortex via this frontal "associational" cortex (Swanson, 1981) or that this projection represents a portion of the neural circuitry associated with ascending dopaminergic input from the ventral

mesencephalic tegmentum to both the medial frontal cortex and the hippocampal formation (Ferino et al., 1987).

Review of recent anatomical studies shows that the projection from the hippocampus to the medial frontal cortex appears to overlap the cortical origin of the recently characterized direct descending projection to the solitary nucleus in the dorsomedial medulla (van der Kooy et al., 1982, 1984; Terreberry and Neafsey, 1983, 1987) (Figure 3). The solitary nucleus plays a major role in the integration of visceral afferent information carried over the trigeminal, facial, glossopharyngeal, and vagus nerves (Torvik, 1956; Contreras et al., 1982). It receives afferent inputs including taste, gastrointestinal afferents, pulmonary stretch and irritant receptor inputs, and cardiovascular afferents including baroreceptor and chemoreceptor inputs over the carotid sinus nerve, aortic nerve, and vagus nerve from the heart (Norgren, 1978; Kalia and Mesulam, 1980a&b; Kalia and Welles, 1980; Davies and Kalia, 1981; Kalia and Kropilak, 1982; Onai et al., 1987). The solitary nucleus is also involved in the generation of respiratory rhythm (Baumgarten and Nakayama, 1964; von Euler et al., 1973; Cohen, 1979). The solitary nucleus is in a position to modulate both parasympathetic and sympathetic neural activity via its connections with the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus, the visceral efferent nuclei of the brainstem (Norgren, 1978; Geis and Wurster, 1980; Kalia and Mesulam, 1980a&b; Rogers et al., 1980; Kalia and Sullivan, 1982), and a connection it has

with neurons in the intermediolateral cell column in the thoracic spinal cord (Loewy, 1981).

Electrical stimulation of the ventral portion of the medial frontal cortex elicits changes in heart rate, blood pressure, respiration (Kaada, 1951; Lofving, 1961; Burns and Wyss, 1985; Terreberry and Neafsey, 1984, 1988), and gastric motility (Hurley-Gius and Neafsey, 1986). These responses plus the direct connection the ventral medial frontal cortex has with the solitary nucleus have led to this region of the cerebral cortex being termed the "visceral motor cortex." In addition, electrical stimulation of the ventral hippocampus has been shown to alter single unit activity in the medial frontal cortex (Ruit and Neafsey, 1986). These observations have led to the suggestion that the hippocampus has direct access to a central visceral control system (Ruit and Neafsey, 1986, 1987, 1988a&b), and that the concept of the limbic system as the "visceral brain" (MacLean, 1949) may still have merit.

Figure 1.

A line drawing of a horizontal section through the rat hippocampus illustrating the tri-synaptic flow of information through the hippocampus originating in the entorhinal cortex (Ent), innervating the dentate gyrus (DG), passing through the CA3 and CA1 cell layers of Ammon's horn, the subiculum (Sub), and ultimately traveling through the main efferent pathway of the hippocampus, the fimbria-fornix (fi).

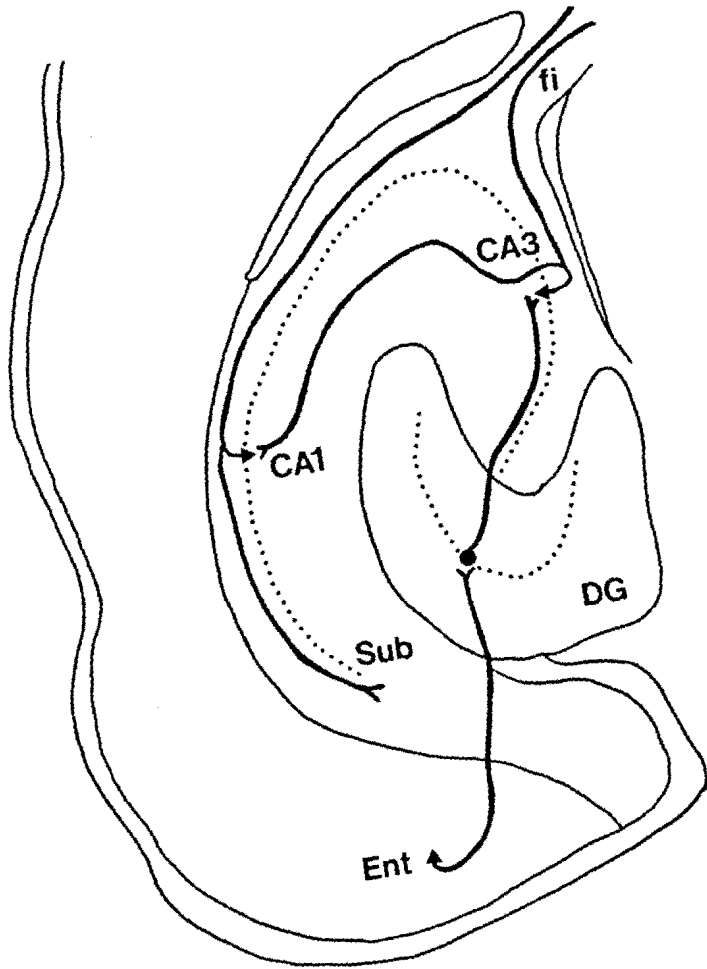


Figure 2.

A-D. Rostral to caudal series of nissl-stained coronal sections of the medial frontal cortex at 500 um intervals. The arrows indicate the cytoarchitectural boundaries between regions of the cortex. AgM = medial agranular cortex; ACd = dorsal anterior cingulate cortex; ACv = ventral anterior cingulate cortex; PL = prelimbic cortex; IL = infralimbic cortex; dpc = dorsal peduncular cortex; tt = tenia tecta; cc = corpus callosum.

E. Surface diagrams of the medial aspect of the cerebral hemisphere of the rat which demonstrate the topography of the cortical areas discussed in this study. I. The organization of the medial frontal cortex used in this study. II. The organization of the medial frontal cortex from Krettek and Price (1977). MO = medial orbital cortex; PrCm = medial precentral area; RsAg = agranular retrosplenial area; RsG = granular retrosplenial area; Th = thalamus.

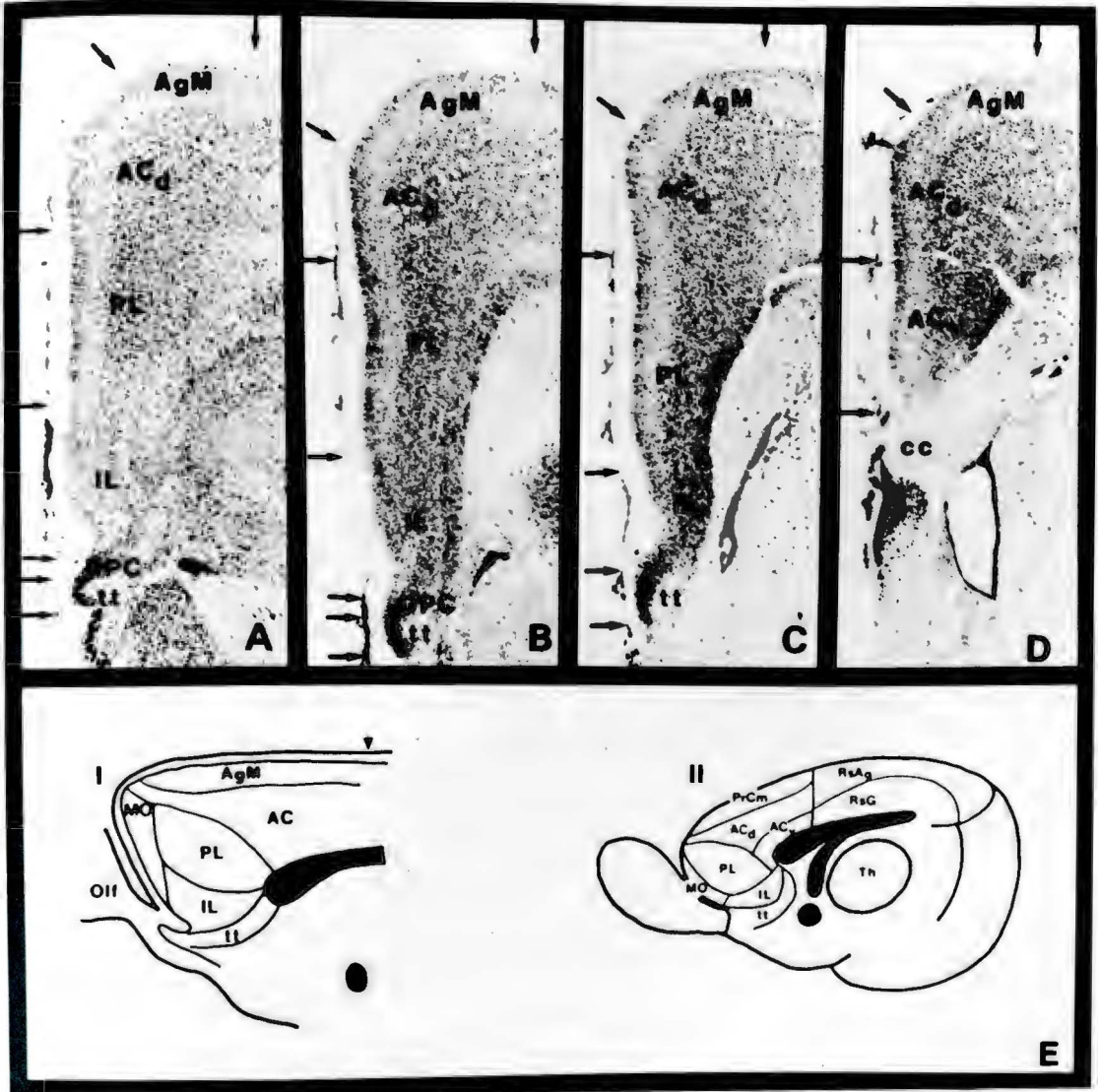
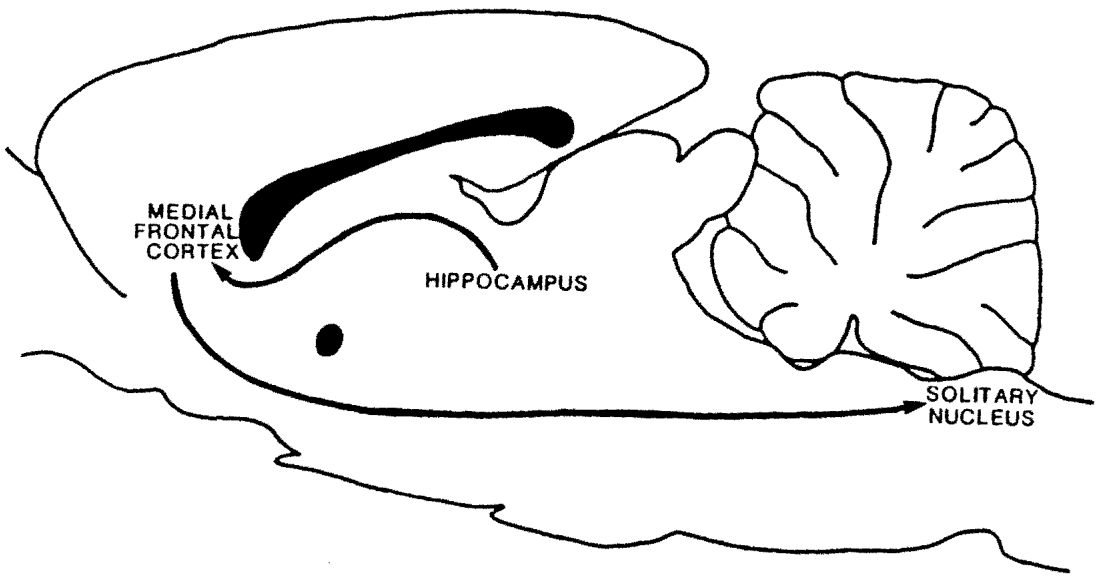


Figure 3.

Line drawing of mid-sagittal section of the rat brain illustrating the pathways which will be studied in this dissertation.



CHAPTER III

SPECIFIC AIMS

The experiments of this dissertation are designed to examine physiologically and anatomically the organization and function of hippocampal connections involved in cardiovascular control. The specific objectives of the research are:

- 1) to determine the effects of electrical and chemical stimulation of the hippocampus on heart rate, blood pressure, and respiration,
- 2) to determine anatomically hippocampal projections to the medial frontal cortex from points in the hippocampus found to alter heart rate, blood pressure, and respiration using light and electron microscopic techniques,
- 3) to determine electrophysiologically the degree of convergence of hippocampal input on neurons in the medial frontal cortex which have previously been identified as projecting to the solitary nucleus.

CHAPTER IV

CARDIOVASCULAR AND RESPIRATORY RESPONSES
TO ELECTRICAL AND CHEMICAL STIMULATION OF
THE HIPPOCAMPUS IN ANESTHETIZED AND
AWAKE RATS

ABSTRACT

The possible role of the hippocampal formation in the modulation of autonomic functions has been overlooked due to the focus of recent research on its role in learning and memory. This study was undertaken to systematically investigate the function of the hippocampus in visceral control. Electrical (30-60 second trains of 0.25 msec pulses at 25 Hz, currents 10-150 uamps) and chemical (microinjections of 0.1-0.5 ul of a 1.0M glutamate solution) stimulation of the hippocampal formation in the anesthetized and the awake rat evoked marked decreases in heart rate, blood pressure, and slower, more regular respirations. Artificial ventilation (2 cc/breath; 100 breaths per minute) had no effect on the cardiovascular responses, indicating that these effects were not secondary to respiratory changes. Administration of methyl atropine (0.4 mg/kg) eliminated the bradycardia response and attenuated or obliterated the blood pressure response but did not alter the respiratory response. This suggests that the cardiovascular responses were mediated primarily by the vagus nerve. Ablation of the medial frontal cortex, a visceral motor region which projects directly to the nucleus of the solitary tract and which receives a heavy direct projection from the CA1 and subicular cell fields of the ventral hippocampus, markedly attenuated or eliminated the cardiovascular and respiratory responses to stimulation of the ventral but not the dorsal hippocampus. The possibility that the medial frontal cortex may be a

relay by which the hippocampus influences cardiovascular responses, including those observed during stress, is discussed.

INTRODUCTION

The hippocampus of the mammalian brain has been the focus of many anatomical, physiological, pharmacological, and behavioral studies, yet we still have only a dim understanding of its role in brain function. As a component of the limbic system, the hippocampus has been associated with mechanisms of emotion (Papez, 1937) and learning and memory (Siefert, 1983). However, MacLean (1952) defined the limbic system as the "visceral brain" based on its visceral afferent inputs; these include gustatory afferents relayed to the lateral hypothalamus, the amygdala, and the bed nucleus of the stria terminalis (Norgren, 1974; Norgren, 1976), general visceral afferent information relayed to the hypothalamus, the amygdala, and the thalamus (Ricardo and Koh, 1978; Sawchenko, 1983), and olfactory input to the hippocampus and amygdala (Powell, 1965; White, 1965; Steward, 1976).

Electrical stimulation of the hippocampus in primates, cats, dogs, and guinea pigs induces "somatic" behavioral modifications such as attention (Kaada, 1953), and attack (Siegel and Flynn, 1968) responses, "agitation", defensive reactions, growling, escape reactions (MacLean and Delgado, 1953), grooming, and "bewilderment and confusion" (MacLean, 1957). Other findings included visceral or autonomic

modifications such as decreases in heart rate and increases in pulse pressure (Smith, 1944), decreases in blood pressure with either increases or decreases in heart rate (Anand and Dua, 1956), respiratory inhibition (Kaada and Jasper, 1952; Andy and Akert, 1955; Liberson and Akert, 1955; Anand and Dua, 1956), inhibition of pyloric antral peristalsis (Kaada, 1951), and sympathetic discharges indicated by pupillary dilatation and increased blood pressure (Carlson et al., 1941; Andy and Akert, 1953). In contrast to these reports, many studies have not been successful in eliciting autonomic responses by stimulating the hippocampus proper. For example, electrical stimulation of the hippocampus and fornix in cats and dogs did not produce any significant blood pressure effects except at extremely high currents (Kaada, 1951). Similarly, neither electrical nor chemical stimulation produced any significant autonomic manifestations in awake cats (MacLean, 1957). Autonomic activity has been more commonly associated with electrical stimulation of the amygdala than with electrical stimulation of the hippocampus (MacLean and Delgado, 1953; Mogenson and Calaresu, 1973). The ambiguous results of the hippocampal studies led Kaada to conclude that "There is at present no evidence either from stimulation or from ablation experiments, to justify the application of this term [visceral brain] . . . to the hippocampus-fornix system" (Kaada, 1960).

Recently, the infralimbic and prelimbic regions of the medial frontal cortex (areas 25 and 32, respectively) of the rat (Krettek and

Price, 1977) have been shown to receive major afferent projections from the CA1 and subicular regions of the ventral hippocampal formation (Swanson, 1981; Ferino et al., 1987). The infralimbic and prelimbic regions also have been described recently as having a direct projection to the nucleus of the solitary tract, the primary visceral afferent nucleus in the dorsal medulla (van der Kooy et al., 1982, 1984; Terreberry and Neafsey, 1983, 1987). Electrical stimulation of this region of the cortex elicits changes in heart rate, blood pressure, respiration (Kaada, 1951; Lofving, 1961; Terreberry and Neafsey, 1984, 1988; Burns and Wyss, 1985), and gastric motility (Hurley-Gius and Neafsey, 1986). Since the hippocampal projections overlap the cortical origin of the direct projection from the medial frontal cortex to the solitary nucleus, the hippocampus has ready access to central visceral control systems. The objective of this study was to determine the effects of electrical and chemical stimulation of the hippocampal formation on heart rate, blood pressure, and respiration in both anesthetized and awake rats.

MATERIALS AND METHODS

Anesthetized preparations

Long-Evans hooded rats (n=19) were anesthetized with ketamine HCl (100 mg/kg, intraperitoneally). A heparinized (50 units/ml) catheter was inserted into the femoral artery and connected to a Statham ID23

pressure transducer attached to a Grass Model 5 polygraph for blood pressure recording. Needle electrodes were placed in the back and chest musculature in order to monitor the electrocardiogram (ECG). R waves were detected in the ECG by a window discriminator, and the window pulses were fed into a rate meter whose output was recorded on the polygraph. A Y-shaped cannula was inserted into the trachea in order to prevent fluid accumulation in the upper respiratory passages and facilitate artificial respiration if necessary; a Grass thermistor was inserted into one arm of the cannula in order to monitor respiratory activity on the polygraph.

The animals were placed into a stereotaxic apparatus, the cisterna magna opened to prevent cortical swelling, and a unilateral craniotomy performed over the hippocampus. Stimulation points were located in the hippocampus according to the stereotaxic coordinates of Paxinos and Watson (1986). A glass insulated tungsten microelectrode (100 μ m tip exposure) (Neafsey, 1981) was lowered into the hippocampus with the aid of a micromanipulator. Unilateral electrical stimulation was delivered using a 60 second train of 0.25 msec negative pulses at 25 Hz with a maximum current of 50 uamps. A Grass S88 stimulator with a PSIU6C photoelectric constant current stimulus isolation unit was used to deliver the stimulus; current intensity was read off the S88 VOLTS dial (accurate to \pm 20%). Stimulation began at 50 uamps and, if a response was observed, the threshold current was determined by gradually reducing the current until the response was no longer

elicited. Points were considered non-responsive if a 50 uamp stimulus did not elicit a response. There was at least a one minute interval between all stimulations.

Several electrode tracks were made in each animal; the medial/rostral tracks passed through the dorsal hippocampus while the lateral/caudal tracks traversed the ventral hippocampus. In this study, "dorsal" hippocampus refers to the dorsal portions of the hippocampal formation between 1.8 mm and 4.3 mm caudal to bregma as illustrated in the Paxinos and Watson atlas (1986); "ventral" hippocampus refers to the more ventral (>3.0 mm from the cortical surface) and lateral (>4.0 mm off the midline) portions of the hippocampal formation between 4.8 mm and 6.8 mm caudal to bregma according to Paxinos and Watson. Since stimulation was delivered every 250-500 um through the hippocampus, possible differential effects (Dunn and Orr, 1984) of subicular, CA1, CA3, dentate gyrus, or entorhinal cortex stimulation were examined. Small marking lesions (10 uamps direct current for 10 seconds) were made along each electrode track to facilitate histological reconstruction. In some cases, where stimulation evoked clear and consistent heart rate, blood pressure, and respiratory responses, a suction lesion was placed ipsilaterally in the medial frontal cortex in an attempt to determine the central pathway underlying the response. The lesion was made in two steps. First, the most dorsal 2.0 mm of the medial frontal cortex were removed between bregma and 5.0 mm rostral to bregma and between the midline and 2.0 mm

lateral to it, and the responses to hippocampal stimulation observed. Then, the more ventral medial frontal cortex between 2.0 mm and 5.0 mm deep was removed and the responses to hippocampal stimulation observed.

Four animals received intraperitoneal injections of methyl atropine (0.4 mg/kg) to determine whether the response was vagally mediated. The hippocampal stimulation was repeated 3-5 minutes following drug injection and the effect on the responses noted.

Hippocampal stimulation was also performed on three artificially ventilated (2.0 cc/breath; 100 breaths/minute) rats in order to monitor heart rate and blood pressure changes in direct response to hippocampal stimulation independent of the effects of changes in respiratory depth or frequency.

Finally, in four animals, microinjections of sodium glutamate, an excitatory amino acid, were made unilaterally in the dorsal and ventral hippocampus. These experiments were performed because electrical stimulation excites both cell bodies and fibers of passage. The injection of sodium glutamate has been shown to selectively activate only cell bodies (Goodchild et al., 1982) making it possible to determine whether physiological responses are elicited by activation of cell bodies rather than fibers of passage. A solution of sodium glutamate was made by adding 10M sodium hydroxide to a 1.0M solution of L-glutamic acid dissolved in deionized water until the pH was 8.0. Pressure injections of glutamate (0.1-0.5 ul) were made in the hippocampus via a glass micropipette (tip diameter = 50 um) attached to

a 1.0 ul Hamilton syringe. The syringe was attached to a micromanipulator and was moved stereotaxically as described above. Control injections of an equal volume of isotonic saline were made at responsive points as a control for volume effects.

Following all experiments, the animals were sacrificed by an overdose of sodium pentobarbital and perfused transcardially with 0.9% saline followed by 10% buffered formalin. The brains were removed and 60 um frozen coronal sections were cut, mounted on glass slides, stained with 0.1% cresyl violet, and coverslipped. The sections were drawn on paper using a projection microscope. The electrode tracks from the electrical stimulation experiments were reconstructed, and the location of stimulation points which elicited heart rate, blood pressure, and/or respiratory responses were determined and plotted on representative sections through the entire hippocampal formation. Only stimulation points located within 250 um of hippocampal cell laminae were used for analysis; points in the white matter were discarded. The injection sites in the chemically stimulated brains were plotted by locating the pipette tracks and estimating the location of the pipette tip from depth readings. Measurements of blood pressure, heart rate, and respiration were expressed as mean values \pm standard error of the mean, and the statistical significance of physiological changes in response to stimulation of the hippocampus was determined using a paired Student's t-test at a significance level of $p < 0.05$. Mean arterial pressure was calculated by adding one third of the pulse

pressure to the diastolic pressure.

Awake preparations

In an initial surgery, the animals were anesthetized with ketamine HCl (100 mg/kg, intraperitoneally), and two flexible electrocardiogram leads were placed in the back and chest musculature. A heparinized catheter was placed in the femoral artery for blood pressure recordings. The electrocardiogram wires and the arterial catheter were then led subcutaneously to the back of the neck where they were exteriorized. The arterial catheter was heat sealed. The wounds were sutured, the animal placed in a stereotaxic frame, and small holes drilled in the calvarium over the hippocampus. Glass insulated tungsten stimulating microelectrodes (tip exposure 100 μm) were stereotaxically lowered into the dorsal and ventral hippocampus of each animal. Final electrode placement was determined by stimulating and eliciting a heart rate and/or blood pressure response; once a responsive point was found, each electrode was cemented in place using dental acrylic. Flexible wires with amphenol connectors were attached to each electrode, and each wire was led to the opposite side of the head, along with a reference wire for each electrode attached to a screw in the calvarium, where a plug was fashioned and cemented to the surface of the skull. The wound was sutured and the animal allowed to recover. Following a 1 to 2 day recovery period, the animal was allowed to move freely in its cage while the heart rate was monitored

on a rate meter, the blood pressure monitored via a pressure transducer connected to a polygraph, and electrical stimulation delivered via a flexible cable attached to the plug cemented to the animal's head. Stimulation parameters were 30 second trains of 0.25 msec pulses at 25 Hz with current intensities between 50-150 uamps; the responses were observed over 2-3 recording sessions. Hippocampal electrical activity was monitored by recording through the stimulating electrode implanted in the hippocampus. In these cases, the stimulator was shut off at peak response and the electrical activity in the hippocampus was monitored on the polygraph up to five minutes following the response. After the final session, small marking lesions were made at the stimulation points in the hippocampus to facilitate histological reconstruction of the electrode track. Here, too, only stimulation points histologically located within 250 um of hippocampal cell laminae were used for analysis.

RESULTS

Anesthetized preparations

Electrical stimulation. Low intensity (<50 uamps) electrical stimulation of all cell fields of the hippocampal formation produced significant decreases in heart rate and blood pressure (Figure 1) and several types of respiratory responses which varied from a slower, more shallow and regular breathing pattern (Figure 1A) to no response

(Figure 1B). The most common respiratory response, however, is illustrated in Figure 2; it was typically a slower, more regular respiratory pattern. The heart rate, blood pressure, and respiratory responses, as shown in Figures 1 and 2, occurred within 5-10 seconds of stimulus onset; the heart rate, blood pressure, and respiratory activity usually returned to baseline levels before the 60 second stimulus train was terminated; some responses lasted the duration of the stimulus and returned to baseline only when the stimulator was turned off. The shortest latency seen for blood pressure, heart rate, and respiratory responses was within 1 second of stimulus onset.

Table 1 illustrates mean arterial pressure and heart rate at baseline levels, at peak response levels during a 50 uamp stimulus, and the net change. Stimulation in both the dorsal and ventral regions of the hippocampal formation produced large, statistically significant decreases in both heart rate and blood pressure ($p < 0.0001$). The average change in respiration during 50 uamp stimulations at nine points in the ventral or dorsal hippocampus was a decrease from 178 ± 9 breaths per minute to 68 ± 7 breaths per minute ($p < 0.001$), and the change in respiratory frequency also seemed to coincide with a deeper, more regular respiratory pattern (Figure 2). Responsive points were found in all regions of the hippocampus; stimulus intensities as low as 25 uamps elicited responses from CA1, CA3, both the inner and outer blades of the dentate gyrus, and the subiculum. The cardiovascular and respiratory responses with the lowest thresholds (10 uamps) were

observed upon stimulation of the CA1 cell field (Figure 3); no cell fields other than CA1 exhibited consistent responses with currents as low as 10 uamps. In addition, no cardiovascular or respiratory responses were observed upon electrical stimulation of the entorhinal cortex (28 points in 3 animals).

Chemical stimulation. The physiological effects of microinjections of sodium glutamate in the ventral and dorsal hippocampus were recorded in four animals. Unilateral injections of 0.1 to 0.5 ul of 1M sodium glutamate elicited marked decreases in blood pressure, heart rate, and respiratory rate (Figure 4). The physiological effects of injection of glutamate into the ventral hippocampus occurred within 1 minute of injection. Following the peak response, the blood pressure and heart rate remained depressed for an additional 2 to 3 minutes. Responses to injection of glutamate into the dorsal hippocampus did not occur until at least 1.5 to 2 minutes following the injection, and the response was a more gradual depression in blood pressure and heart rate than the immediate drop usually seen following ventral hippocampal injections. Respiratory responses to glutamate injection into the ventral or dorsal hippocampus were usually a decrease in both rate and depth. Injections of up to 0.5 ul of saline into the ventral or dorsal hippocampus as a control elicited no physiological responses.

Artificial ventilation. Statistically significant decreases in heart rate (-60 ± 6 beats per minute; paired t-test, $p < 0.05$) and blood

pressure (-24 ± 1 mmHg; paired t-test, $p < 0.05$) were also observed during a 25 uamp stimulus in either the dorsal or ventral hippocampus in all three animals that had been artificially ventilated (2.0 cc/breath; 100 breaths/minute) (Figure 5), indicating that cardiovascular responses can be elicited in the absence of any changes in respiratory activity. Although the case illustrated in Figure 5 shows a somewhat larger response during artificial ventilation, in most cases the magnitude of these responses was similar to those seen in spontaneously breathing animals.

Administration of methyl atropine. Intraperitoneal injections of methyl atropine (0.4 mg/kg) increased the baseline heart rate within five minutes of administration (Figure 5). In all four animals tested, subsequent 25 uamp stimulation at a point in the hippocampus which had previously been shown to significantly decrease heart rate (-42 ± 6 beats per minute; paired t-test, $p < 0.007$) and blood pressure (-23 ± 2 mmHg; paired t-test, $p < 0.001$) failed to elicit a bradycardia response (typical example in Figure 5), indicating that the bradycardia associated with electrical stimulation of the hippocampus is mediated by the vagus nerve. Blood pressure responses following atropine administration were either absent, as in Figure 5, or still present but diminished (-5 ± 3 mmHg; paired t-test, $p > 0.2$). This indicates that the blood pressure responses were, for the most part, secondary to control by parasympathetic influences on the heart via the vagus nerve. Respiratory responses (not shown) were unchanged following

administration of methyl atropine, indicating that these responses are independent of the changes in heart rate and blood pressure.

Medial frontal cortex lesions. In an attempt to characterize a possible pathway mediating the cardiovascular responses to hippocampal stimulation, ablation of the ipsilateral medial frontal cortex by aspiration was performed. In cases of stimulation sites in the dorsal hippocampus ($n = 3$ animals), neither the dorsal nor the subsequent lesion in the ventral medial frontal cortex produced responses significantly different than those evoked before the lesion (Dunn-Bonferroni multiple comparisons t-test (Wilcox, 1987), $p > 0.05$; Figure 6, Figure 7). On the other hand, although the responses from stimulation in the ventral hippocampus ($n = 5$ animals) were not significantly changed by removal of the dorsal medial frontal cortex ($p > 0.05$), the heart rate and blood pressure responses were significantly attenuated by subsequent removal of the ventral medial frontal cortex ($p < 0.05$; Figure 6, Figure 7). In the case shown in Figure 5, although the baseline mean arterial pressure dropped from that observed before the lesion, typical blood pressure and heart rate decreases were seen following stimulation in the ventral hippocampus after removal of the dorsal medial frontal cortex. The same responses were eliminated, however, by removal of the ventral medial frontal cortex while the baseline mean arterial pressure remained the same. This rules out the possibility that elimination of the response following removal of the ventral medial frontal cortex could be due to a lowering of the mean

arterial pressure or heart rate to near or below the "lower limit" of the system. Respiratory responses typically exhibited no change from the response before the lesion except in response to removal of the ventral medial frontal cortex and subsequent stimulation in the ventral hippocampus; in this case, the respiratory response was always obliterated. In all cases, the lesion involved the entire ipsilateral medial frontal cortex but spared the septum (Figure 8).

Awake preparations

Results of electrical stimulation in the awake animal were obtained in four preparations. Stimulation parameters were 30 second trains of 0.25 msec pulses at 25 Hz at current intensities between 50 and 150 uamps. Each of the animals exhibited autonomic responses indicated by pupillary constriction, decreases in overall heart rate as well as decreases in the variability of the heart rate, and decreases in blood pressure during stimulation of both the dorsal and ventral hippocampus (Figure 9). Respirations in awake animals were not monitored. The cardiovascular responses occurred within 10 seconds of stimulus onset, lasted approximately 10 seconds, and tended to return to baseline near the end of the stimulus trial. In many cases, the decreases in heart rate and blood pressure were followed by an increase in heart rate and blood pressure during the last part of the stimulus, as in Figure 9, or following the offset of the stimulus; these increases were associated with large seizure electrical discharges in the hippocampus which began late in the stimulus (25-30 seconds after

stimulus onset) or after the offset of the stimulus, and lasted for approximately 60 to 80 seconds. During and following the stimulus, the animal also exhibited various behavioral alterations including "wet dog" shakes, grooming, foraging, and feeding behavior characterized by vigorous eating of rat chow and inappropriate eating of bedding and droppings. The increases in heart rate and blood pressure during the last part of the stimulus may also be secondary to the increase in muscle activity associated with these behavioral alterations. After a stimulation session lasting approximately one half hour to 45 minutes (10-15 stimulation trials), the animal became lethargic, laid down, and slept, making somatomotor or autonomic modifications of behavior increasingly more difficult to elicit.

DISCUSSION

The observed cardiovascular and respiratory effects of electrical and chemical stimulation of the hippocampus confirms the linkage of the hippocampus, especially its CA1 cell field, with visceral control. At all responsive points in all animals used in this study, anesthetized or awake, the response consisted of decreases in heart rate, blood pressure, and respiratory rate. The ability to reproduce these effects of electrical stimulation with chemical stimulation demonstrates that it is indeed the cells of the hippocampus which are producing these responses and not simply just the activation of fibers of passage. The

fact that blood pressure and heart rate decreases were also seen in artificially ventilated animals suggests that these effects are not secondary to changes in respiratory patterns. However, since the animals were artificially ventilated but not paralyzed, some phrenic nerve respiratory pattern changes may still have persisted. The elimination of responses to ventral but not dorsal hippocampal stimulation by lesions of the ventral medial frontal cortex suggests that the projection from the ventral hippocampus to the ventral medial frontal cortex is the pathway activated by stimulation that produces the responses.

One of the principle subcortical projections of the hippocampus is to the lateral septal region of the brain (Swanson and Cowan, 1979), and it has also been shown that electrical and chemical stimulation of the septum elicits modifications in heart rate and blood pressure (Calaresu and Mogenson, 1972; Gelsema and Calaresu, 1987). These changes in cardiovascular activity are usually associated with the projections from the septum to the hypothalamus (Calaresu et al., 1976), a region of the brain that is, among other functions, associated with cardiovascular control (Loewy and McKellar, 1980). However, the ventral CA1 and subicular regions of the hippocampus have also been shown to send a large projection to the ipsilateral prelimbic and infralimbic regions of the medial frontal cortex (Swanson, 1981; Ferino et al., 1987) which project directly to the solitary nucleus in the dorsal medulla (van der Kooy et al., 1982, 1984; Terreberry and

Neafsey, 1983, 1987); there are no projections from the dorsal hippocampus to the medial frontal cortex. Low intensity electrical stimulation of the medial frontal cortex elicits prominent changes in heart rate, blood pressure, and respiration (Burns and Wyss, 1985; Kaada, 1951; Lofving, 1961; Terreberry and Neafsey, 1984, 1988). Stimulation of the ventral hippocampus has been shown to alter single unit activity of cells in the medial frontal cortex, thereby establishing an electrophysiologically functional link between the hippocampus and a central visceral control system (Ruit and Neafsey, 1986). The elimination of the cardiovascular response to stimulation of the ventral hippocampus following ablation of the medial frontal cortex indicates that this cortico-cortical relay is important in producing the cardiovascular response.

The projection of the medial frontal cortex to the brainstem terminates in the solitary nucleus which is a visceral afferent or sensory nucleus. In addition to receiving visceral afferent information, the solitary nucleus is also in a position to modulate both parasympathetic and sympathetic visceral motor activity via its connections with the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus, the sites of vagal preganglionic neurons which project to the heart (Norgren, 1978; Geis and Wurster, 1980; Kalia and Mesulam, 1980a&b; Rogers et al., 1980; Kalia and Sullivan, 1982), and a connection the solitary nucleus has with sympathetic preganglionic neurons in the intermediolateral cell column in the thoracic spinal

cord (Loewy, 1981) which innervate the vasculature. The solitary nucleus is also involved in the generation of respiratory rhythm via direct influences on phrenic and spinal motor neurons which control respiratory musculature (Baumgarten and Nakayama, 1964; von Euler et al., 1973; Cohen, 1979) (Figure 10). Therefore, input to this visceral sensory nucleus can ultimately affect visceral motor output.

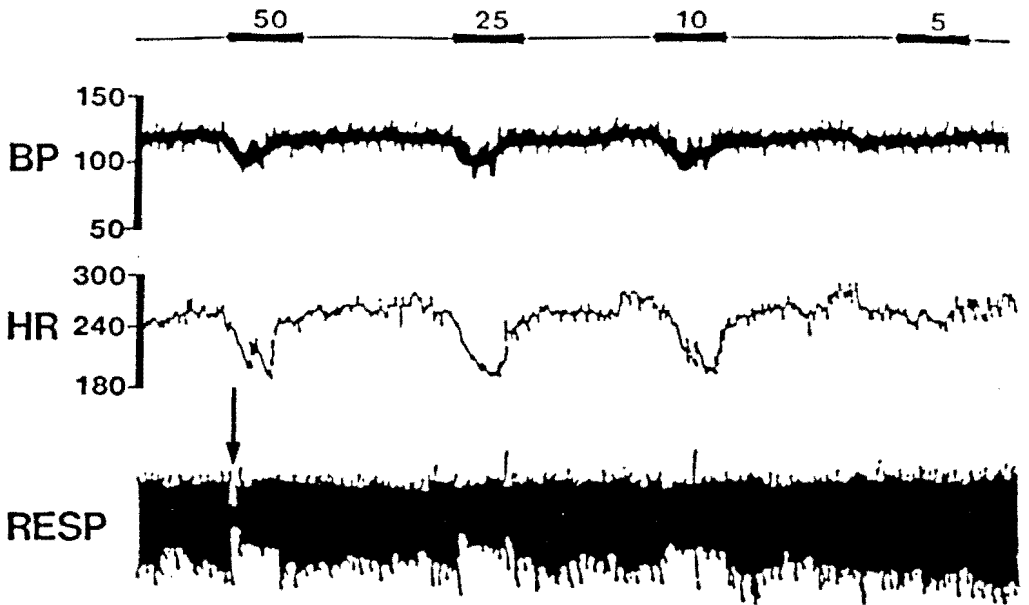
The role of the hippocampus in behavior remains unclear. The hippocampus has, however, been clearly linked to regulation of the corticosterone response to stress. The hippocampus contains the highest concentration of receptors for glucocorticoids in the mammalian brain (McEwen, 1982), exerts an inhibitory influence over adrenocortical activity, and participates in glucocorticoid feedback on plasma corticosterone levels (Wilson and Critchlow, 1973-74; Casady and Taylor, 1976; Frankel et al., 1978; Dunn and Orr, 1984; Sapolsky et al., 1984a). The number of corticosterone receptors in the hippocampus has been shown to decline in the aged rat (Sapolsky et al., 1983). This decline has been correlated with the observation that aged rats, in response to stress, are unable to exert negative feedback on increased plasma corticosterone levels and, hence, exhibit a prolonged corticosterone hypersecretion following the end of stress (Saplosky et al., 1984b). The present results suggest that the hippocampus may also be related to cardiovascular and respiratory responses to stress. The interrelationship of the hippocampus and the medial frontal cortex may be important in this regard because the medial frontal cortex has been

implicated in responses to stress as an integral part of the mesocortical dopamine system (Thierry et al., 1976) and because of its relationship to cardiovascular and respiratory control (Terreberry and Neafsey, 1984, 1988; Fryzstak and Neafsey, 1987; Verberne et al., 1987). Interestingly, stimulation of the medial frontal cortex has recently been reported to increase plasma corticosterone levels in urethane anesthetized rats (Dunn and Miller, 1987).

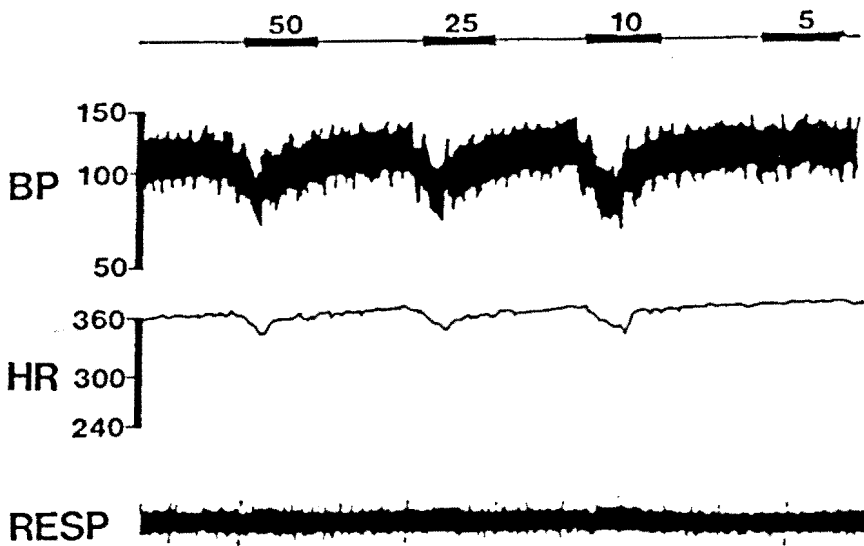
The results of the present study suggest that this hippocampal/frontal cortical pathway may play a specific and important role in the regulation of visceral motor function in allowing an organism to respond appropriately to environmental stimuli.

Figure 1.

Blood pressure (BP), heart rate (HR), and respiratory (RESP) responses evoked by electrical stimulation of the ventral (A) and dorsal (B) hippocampus in anesthetized rats. The top tracing in each panel indicates stimulus on/off; stimulus train duration is 60 seconds. The stimulus intensities in microamps are indicated above the stimulus bars. The blood pressure is calibrated in mmHg; the heart rate is calibrated in beats per minute. The respiratory response to stimulation in the ventral hippocampus shows a brief period of apnea (arrow) followed by respirations of smaller amplitude. Dorsal hippocampal stimulation, in this case, shows no outstanding effects on respiration. At each site in the ventral and dorsal hippocampus, responses were evoked with currents as low as 10 uamps with no responses at 5 uamps.



A. Ventral hippocampus.



B. Dorsal hippocampus.

Figure 2.

Typical respiratory response evoked by electrical stimulation of the hippocampus. The chart recorder paper speed is increased compared to that in Figure 1 in order to observe more easily the characteristics of the response. Points indicating "stimulus on" and "stimulus off" are marked by the arrows. Time scale = 5 seconds. The response is characterized by a large expiration (upward deflection) followed by a short period of apnea. Slower, deeper respirations follow, but the pattern returns to baseline levels before the offset of the stimulus. This type of respiratory response was the most commonly seen accompanying cardiovascular responses similar to those illustrated in Figure 1.

49

Stimulus ON

Stimulus OFF

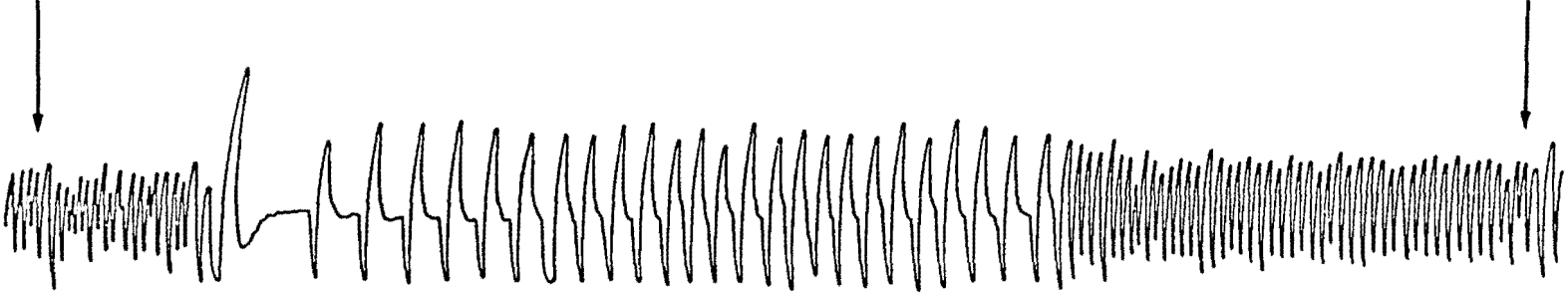


Figure 3.

A. Nissl stained coronal section through the ventral hippocampus showing the extent of one electrode track. The arrow marks a lesion indicating the location of a stimulation point in the CA1 cell field which elicited the cardiovascular responses shown in Figure 1A. The asterisk marks the end of the electrode track. S = subiculum; DG = dentate gyrus; bar denotes 1.0 mm.

B. Nissl stained coronal section through the dorsal hippocampus showing the extent of two electrode tracks. The midline is to the right of the photomicrograph. The arrow marks a lesion indicating the location of a stimulation point in the CA1 cell field which elicited the cardiovascular responses shown in Figure 1B. The asterisks mark the ends of the electrode tracks. DG = dentate gyrus; LP = lateral posterior nucleus of the thalamus; bar denotes 1.0 mm.

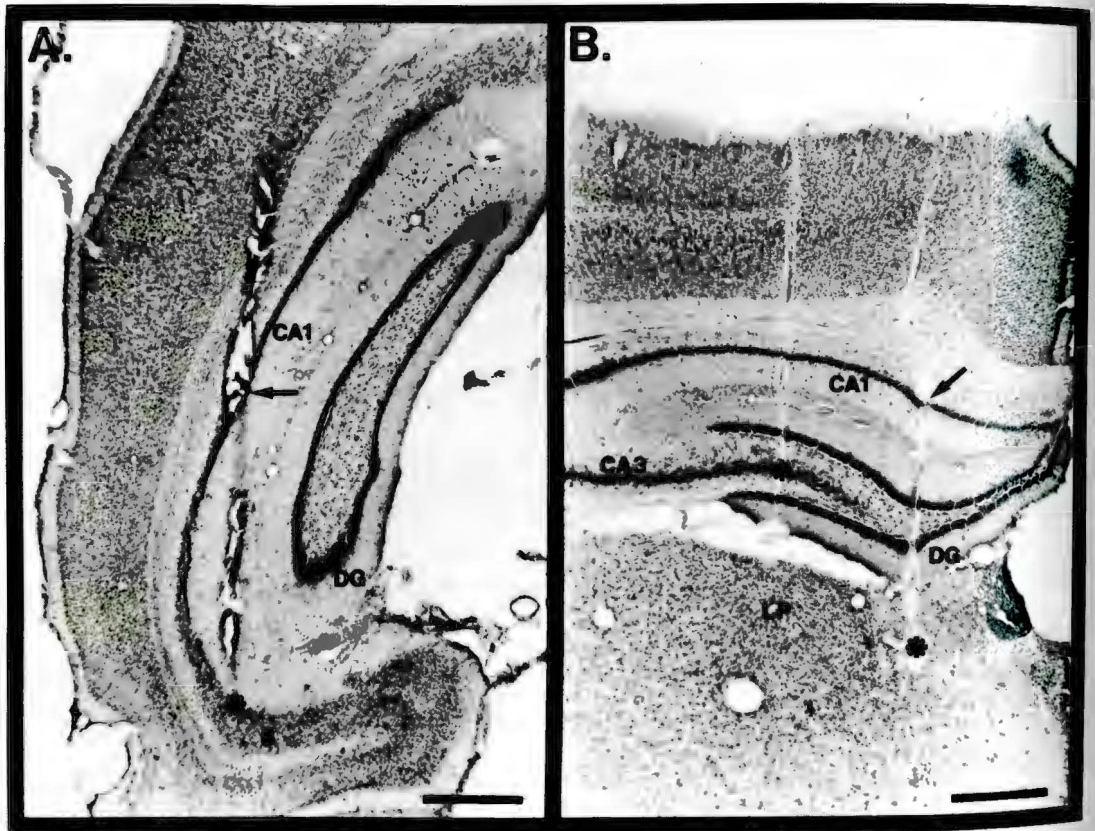


Figure 4.

Blood pressure (BP), heart rate (HR), and respiratory (RESP) responses to microinjections of sodium glutamate (indicated by arrowheads) into the ventral hippocampus (A) and the dorsal hippocampus (B). Panel C illustrates BP, HR, and RESP tracings following control microinjections of saline into the hippocampus. The corresponding line drawings illustrate the location of injection sites (asterisks) within the ventral or dorsal hippocampus. BP is calibrated in mmHg; HR in beats per minute. Scale bar in physiograph tracings = 1 minute; scale bar in line drawings = 1 mm. Injection of glutamate (in these cases, 0.2 ul) into the ventral or dorsal hippocampus elicited decreases in BP and HR and slower respirations within 1 to 2 minutes of administration. Injection of equal volumes of saline produced no physiological effects.

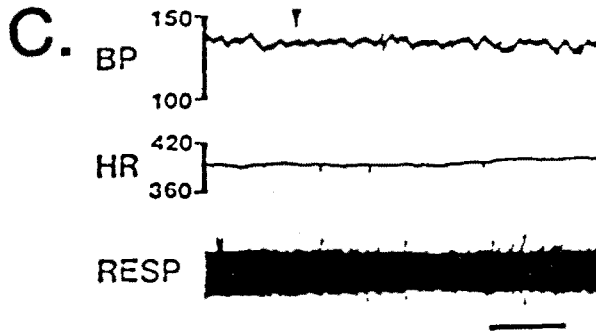
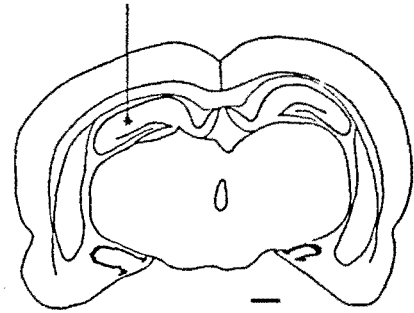
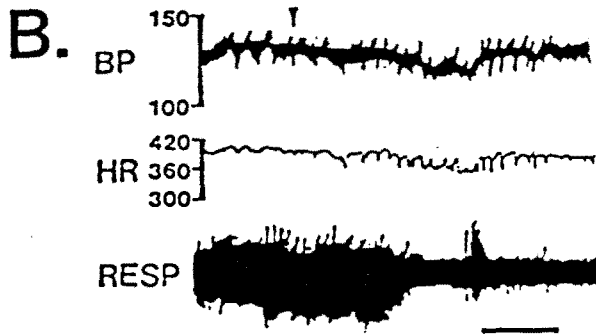
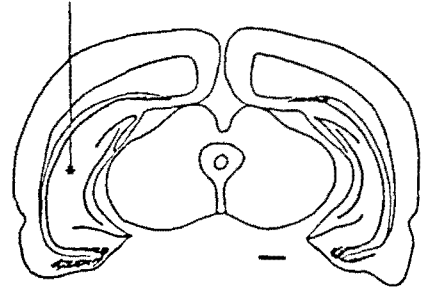
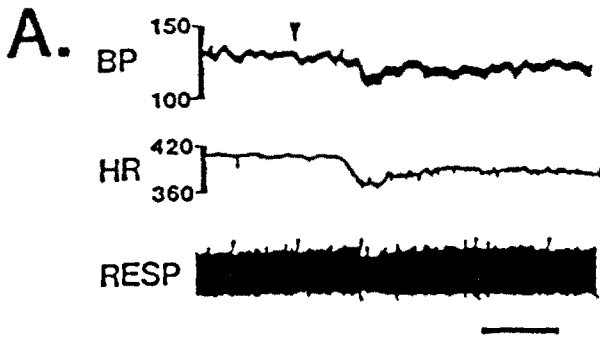
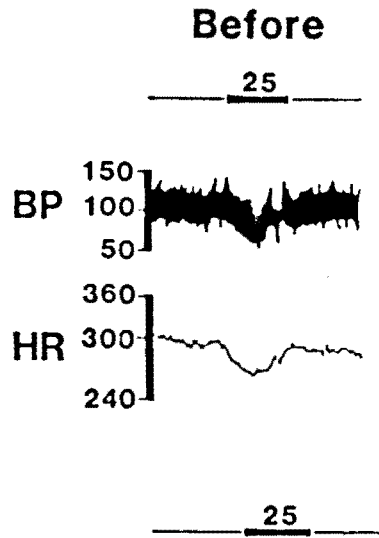
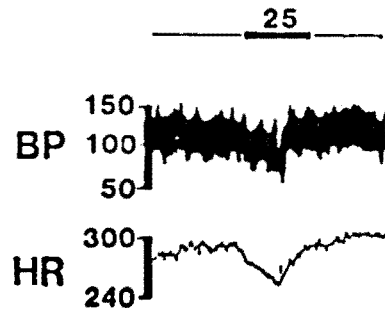
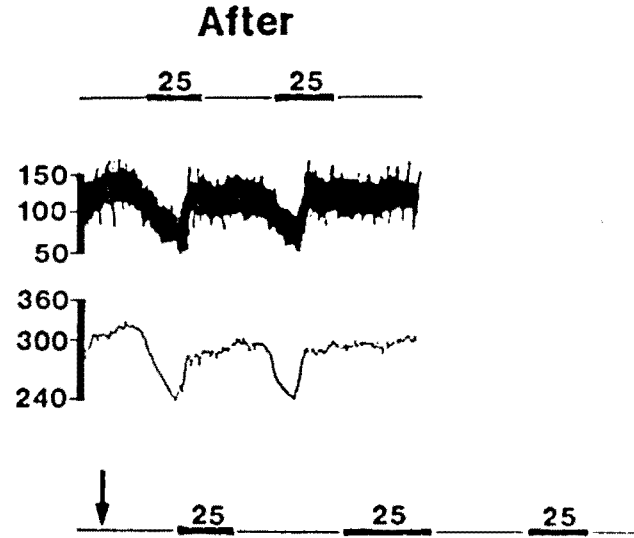


Figure 5.

Blood pressure (BP) and heart rate (HR) responses to electrical stimulation of the hippocampus before and after artificial ventilation (top) or administration of methyl atropine (bottom). The top tracing in each panel indicates the 60 second stimulus train. The stimulus intensities in microamps are indicated above the stimulus bars. The blood pressure is calibrated in mmHg; the heart rate is calibrated in beats per minute. The physiological responses are still present and, in this case, somewhat larger following artificial ventilation. Administration of methyl atropine (arrow indicates time of injection) increases the baseline heart rate, eliminates the heart rate response to hippocampal stimulation, and, in this instance, abolishes the blood pressure response.



**Artificial
ventilation**



**Methyl
atropine**

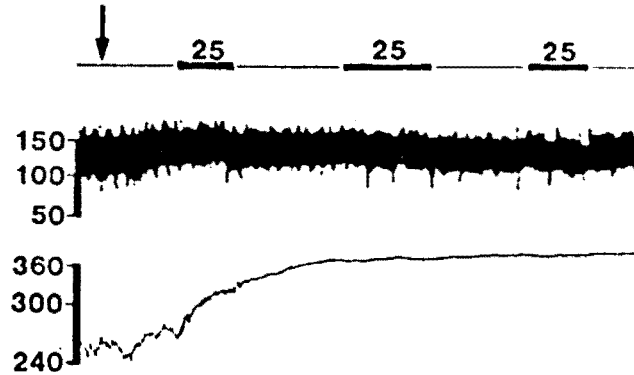


Figure 6.

Blood pressure (BP, mmHg) and heart rate (HR, beats per minute) responses to dorsal and ventral hippocampal electrical stimulation before a medial frontal cortex lesion (left), following removal of only the dorsal medial frontal cortex (middle), and following subsequent removal of the ventral medial frontal cortex (right). The top tracing in each panel indicates the delivery of 60 second stimulus trains. The stimulus intensities in microamps are indicated above the stimulus bars. Removal of the medial frontal cortex has no effect on the response elicited from stimulation in the dorsal hippocampus. Although in the middle lower panel the baseline mean arterial pressure is decreased following the lesion of the dorsal medial frontal cortex, the response is still present following stimulation in the ventral hippocampus. However, upon removal of the ventral medial frontal cortex, the response elicited from stimulation in the ventral hippocampus is obliterated.

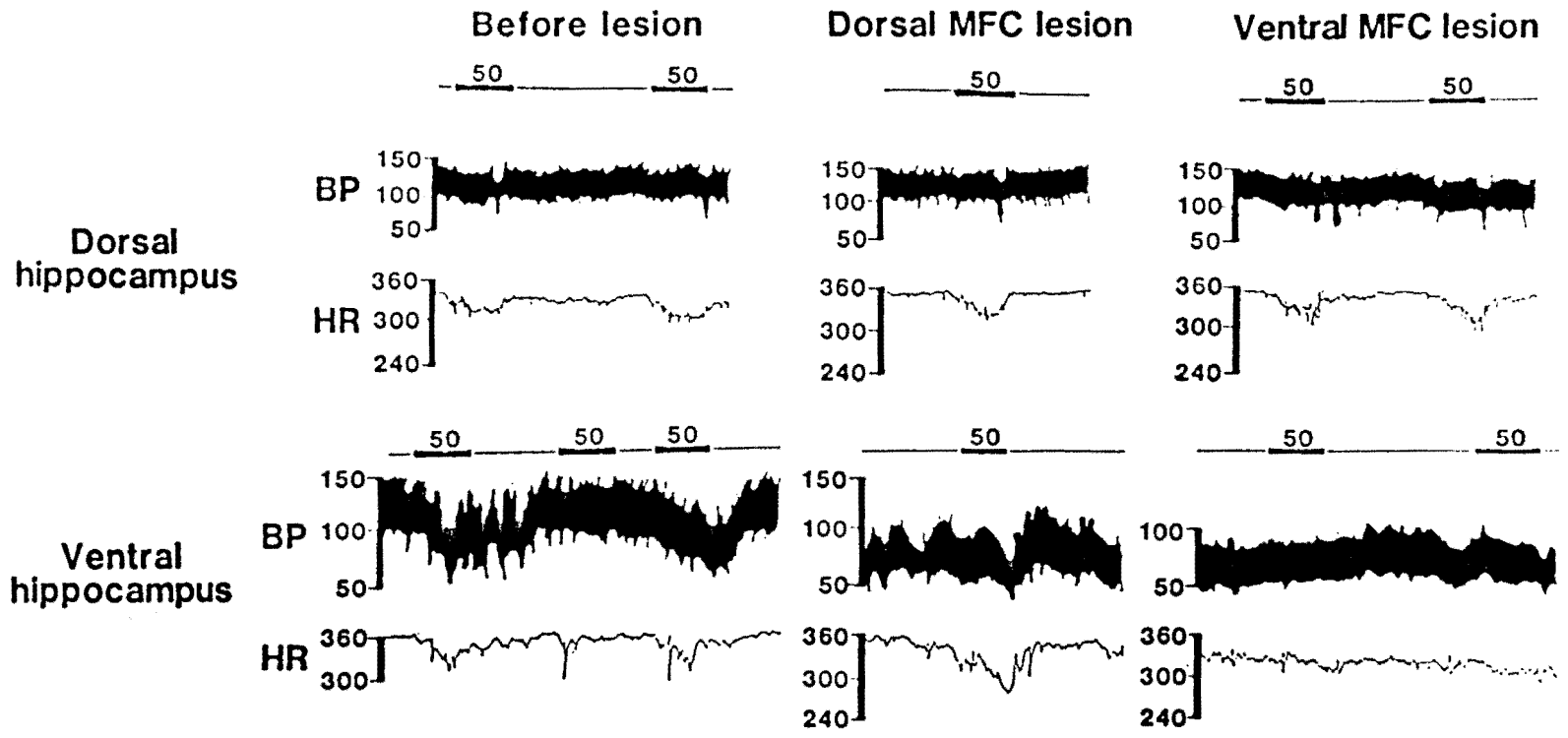
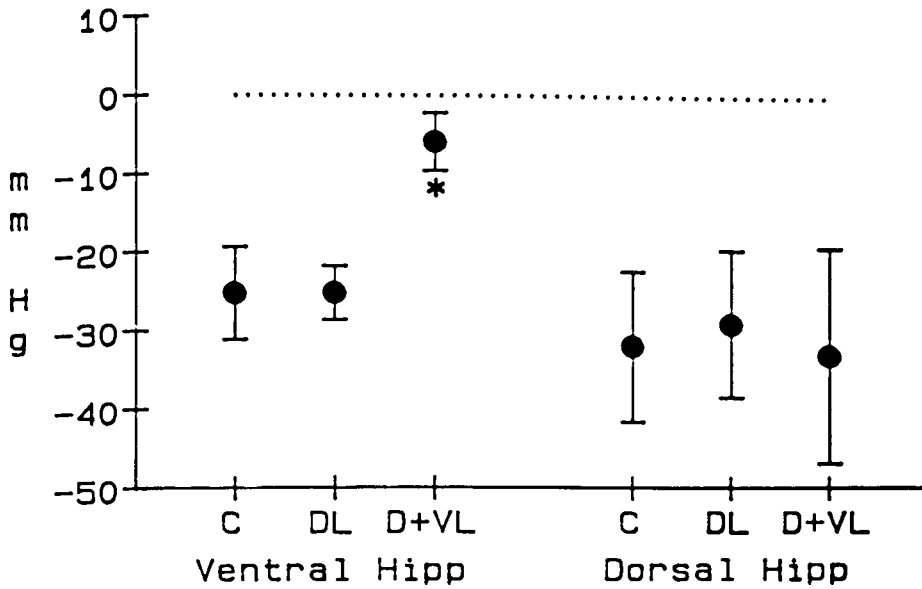


Figure 7.

Graphs representing mean absolute values of changes in blood pressure and heart rate in response to stimulation in the ventral hippocampus (Ventral Hipp; n = 5) and dorsal hippocampus (Dorsal Hipp; n = 3) at control values (C), following removal of the dorsal part of the medial frontal cortex (DL), and following subsequent removal of the ventral medial frontal cortex (D+VL). Blood pressure is represented in mmHg; heart rate in beats per minute. Mean blood pressure and heart rate responses to stimulation in the ventral hippocampus following dorsal lesions in the medial frontal cortex are not significantly different than control. However, stimulation in the ventral hippocampus following removal of the ventral part of the medial frontal cortex elicits physiological responses which are significantly different (* ; $p < 0.05$) from control or dorsal medial frontal cortex lesion values i.e. the blood pressure and heart rate responses are essentially abolished by the ventral lesion. Stimulation in the dorsal hippocampus following dorsal and ventral lesions of the medial frontal cortex does not elicit responses significantly different than control.

BLOOD PRESSURE CHANGE FROM BASELINE



HEART RATE CHANGE FROM BASELINE

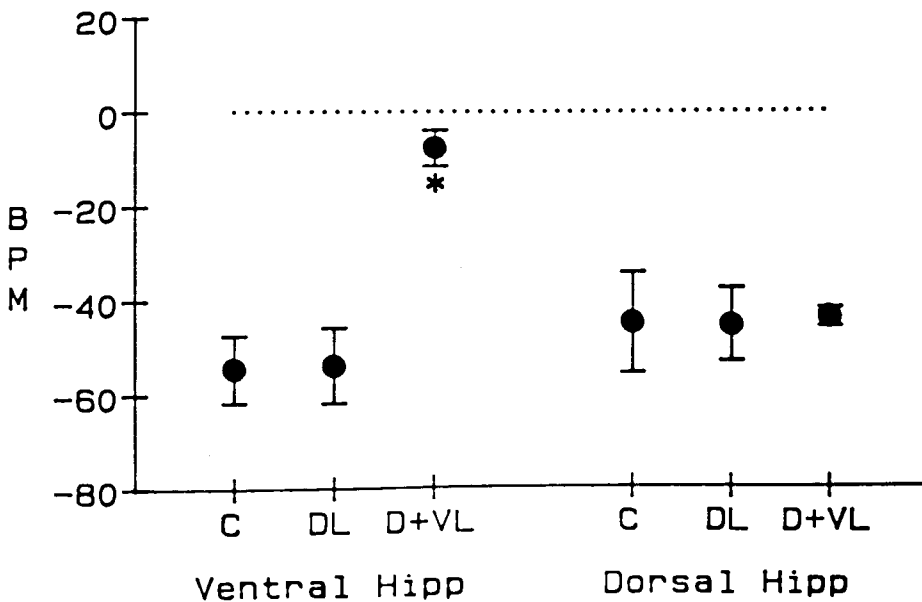


Figure 8.

Series of line drawings (rostral => caudal, A-E) illustrating the extent of medial frontal cortex suction lesions. In sections A-D, the cross-hatched areas indicate the extent of the dorsal medial frontal cortex lesion whereas the stippled areas delineate the subsequent ventral medial frontal cortex lesion. All lesions involved the prelimbic and infralimbic regions of the medial frontal cortex, but spared the septum (Sep, section E). Bar denotes 1.0 mm.

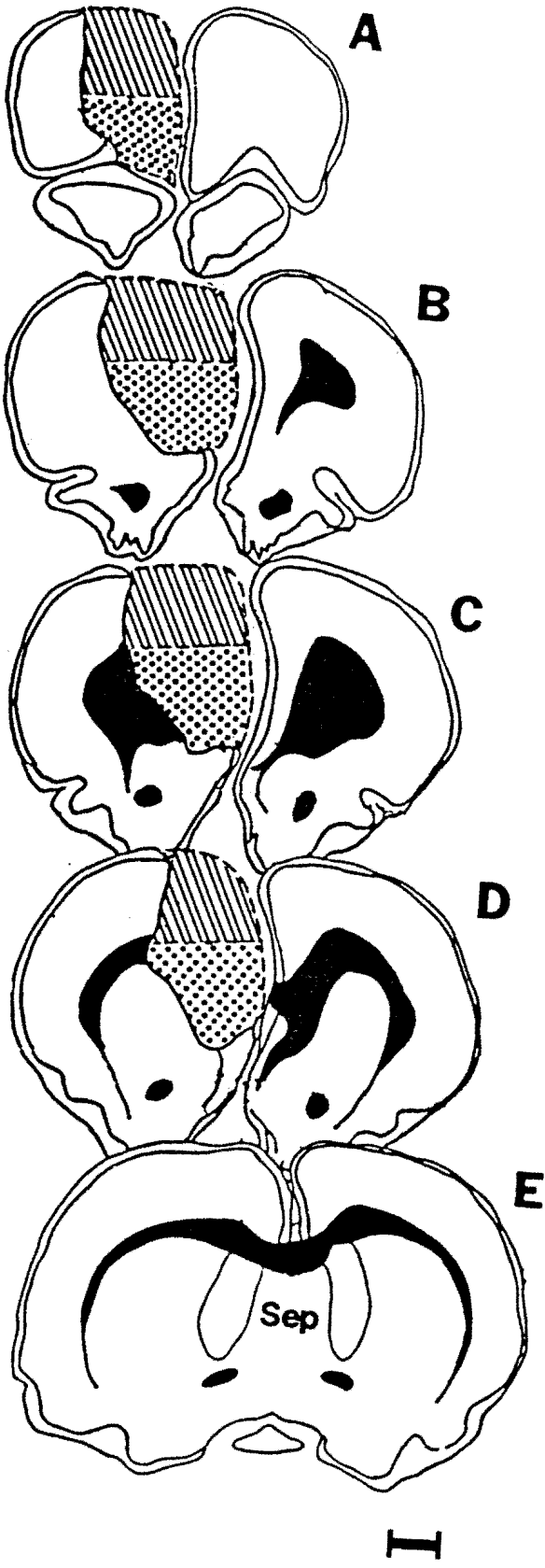


Figure 9.

A. Blood pressure (BP) response evoked by electrical stimulation of the hippocampus in the awake rat. The arrow marks the point at which the 100 uamp stimulus began and the stimulus duration = 30 seconds. The blood pressure is calibrated in mmHg. The tracing shows an initial decrease in blood pressure followed by a sharp rise which coincided with seizure electrical activity in the hippocampus and increased muscle activity.

B. Heart rate (HR, bpm = beats per minute) response evoked by electrical stimulation of the hippocampus in the awake rat. Each dot on the figure indicates the heart rate derived from the R-R interval calculated at each heart beat. The stimulus was turned on at time = 0 and continued for 30 seconds. At approximately a 10 second latency the heart rate decreased and returned to baseline by the end of the stimulus.

A



B

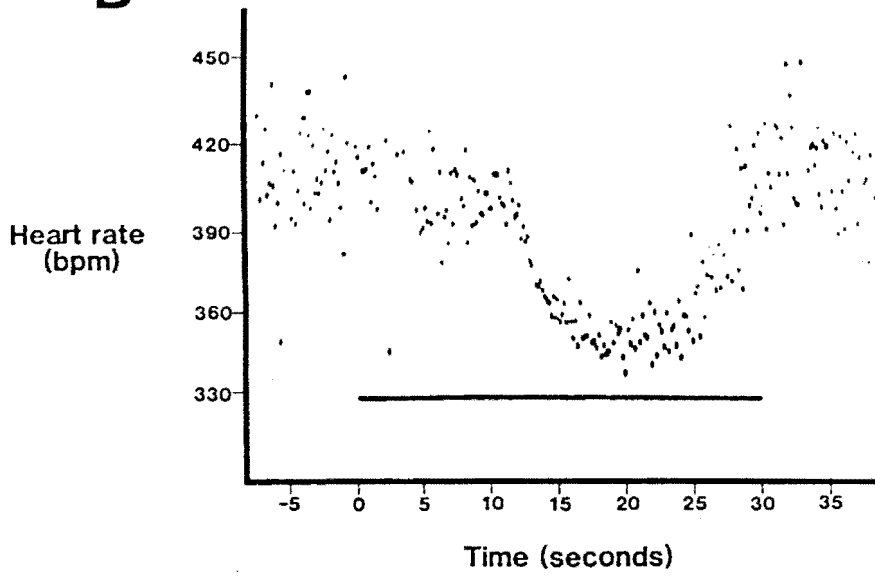


Figure 10.

Schematic diagram illustrating how input from the hippocampus and medial frontal cortex to the visceral afferent solitary nucleus ultimately results in changes in respiration, blood pressure, and heart rate.

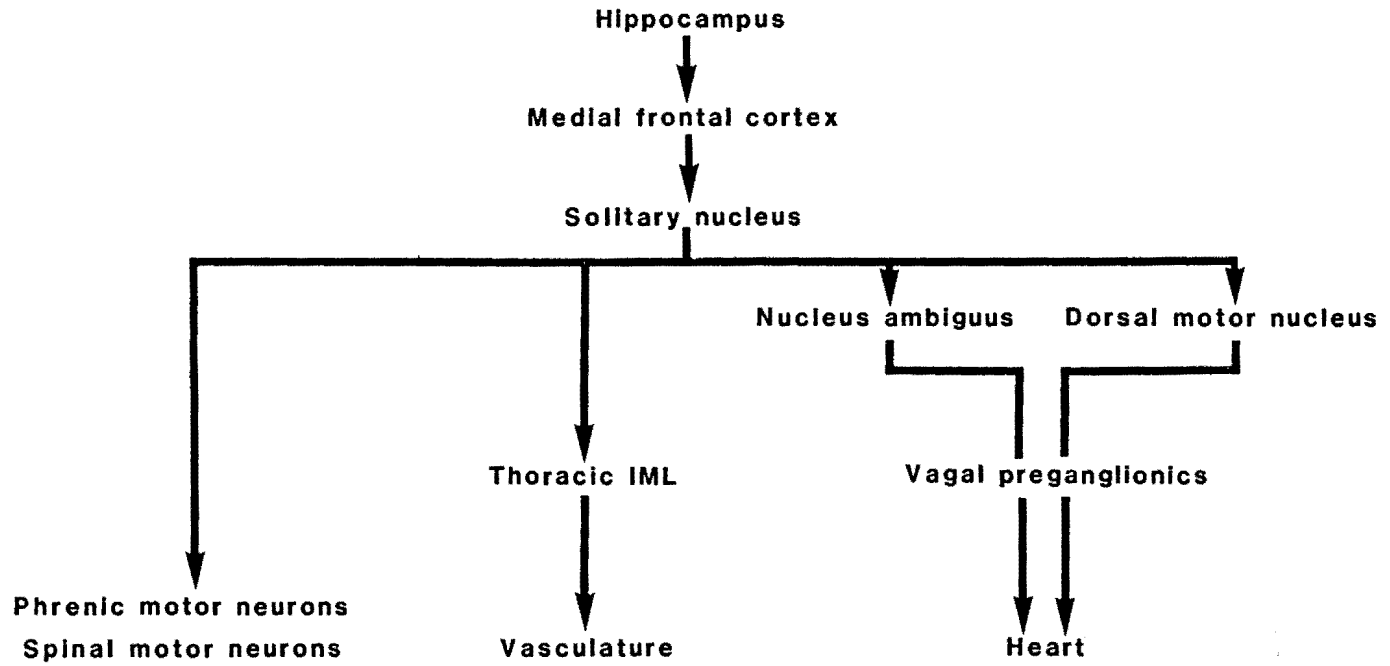


Table 1.

Statistical analysis of blood pressure and heart rate expressed as mean arterial pressure (mmHg) and mean heart rate (beats per minute) recorded at baseline levels, peak response levels, and the resulting differences. Asterisks represent statistically significant ($p < 0.05$; actual values were $p < 0.0001$) changes in mean arterial pressure and heart rate determined by a paired Student's t-test. "n" represents the number of different stimulation points in the dorsal and ventral hippocampus from which blood pressure and heart rate data was accumulated.

Ventral
hippocampus
n = 73

Dorsal
hippocampus
n = 32

Mean Arterial Pressure			Heart Rate		
Baseline	Peak response	Change	Baseline	Peak response	Change
102 ± 3	64 ± 2	-38 ± 2*	353 ± 6	304 ± 7	-48 ± 3*
112 ± 4	76 ± 4	-36 ± 4*	329 ± 9	273 ± 10	-56 ± 4*

CHAPTER V

CONVERGENCE OF HIPPOCAMPAL INPUT TO
THE MEDIAL FRONTAL CORTEX ON
NEURONS WHICH PROJECT TO THE SOLITARY NUCLEUS:
AN ANATOMICAL STUDY

ABSTRACT

The medial frontal cortex has been implicated as a relay for ventral hippocampal influences on cardiovascular function. This study was undertaken to determine anatomically the relationship of hippocampal input to the medial frontal cortex with neurons in the medial frontal cortex which project to the solitary nucleus, a cardiovascular control center in the brainstem. Injections of the anterograde and retrograde neuroanatomical tracer wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) into the solitary nucleus retrogradely labelled cells in the infralimbic and prelimbic areas of the medial frontal cortex. Injections of WGA-HRP into the ventral hippocampus anterogradely labelled terminals in the medial frontal cortex which, at the light microscopic level, closely overlapped the origin of the descending projection from the medial frontal cortex to the brainstem. Electron microscopic analysis revealed that anterogradely labelled terminals make synaptic contact primarily on dendritic processes in the neuropil adjacent to retrogradely labelled cells. In addition, anterogradely labelled terminals did, in some instances, make synaptic contact on the somas of retrogradely labelled cells. The results of this study suggest that the hippocampus is strongly linked anatomically with a region of the brain involved in central cardiovascular control.

INTRODUCTION

Numerous anatomical studies have focused on the connections of the hippocampal formation. Among the structures the hippocampus projects to are the septum, the hypothalamus, and the infralimbic region of the medial frontal cortex (Swanson and Cowan, 1977, Swanson, 1981; Ferino et al., 1987), all of which are regions of the brain associated with central cardiovascular control (Calaresu and Mogenson, 1972; Loewy and McKellar, 1980; Terreberry and Neafsey, 1984, 1988; Gelsema and Calaresu, 1987). The infralimbic region of the medial frontal cortex projects directly to the nucleus of the solitary tract in the dorsal medulla (van der Kooy et al., 1982, 1984; Terreberry and Neafsey, 1983, 1987). Since low threshold electrical stimulation of the hippocampal formation elicits changes in cardiovascular activity, and ventral medial frontal cortex lesions attenuate or obliterate cardiovascular responses to hippocampal stimulation, we have suggested that the medial frontal cortex is a relay between the hippocampus and the brainstem by which the hippocampus influences cardiovascular function (Ruit and Neafsey, 1988a).

In the present study, light microscopy was utilized to characterize the projection from the hippocampus to the medial frontal cortex following injections of the anterograde and retrograde neuroanatomical tracer wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) into points in the hippocampus where electrical stimulation

influenced cardiovascular function. Ultrastructural methods were also employed to determine if terminals in the medial frontal cortex anterogradely labelled by hippocampal WGA-HRP injections synapse on medial frontal cortical neurons retrogradely labelled by injections of WGA-HRP into the solitary nucleus, indicating a direct, monosynaptic connection between the hippocampus and a "visceral motor" control system.

MATERIALS AND METHODS

Light microscopic studies

Long-Evans hooded rats (n=6) were anesthetized with ketamine HCl (100 mg/kg, intraperitoneally), and needle electrodes were placed in the back and chest musculature in order to monitor the electrocardiogram (ECG). R waves in the ECG were detected by a Haer window discriminator, and the window pulses were fed into a rate meter whose output was recorded on a Grass Model 5 polygraph. The animals were placed in the stereotaxic apparatus, the cisterna magna opened to prevent cortical swelling, and a unilateral craniotomy performed over the dorsal or ventral hippocampus. In this study, "dorsal" hippocampus refers to the dorsal portions of the hippocampal formation between 1.8 mm and 4.3 mm caudal to bregma as illustrated in the Paxinos and Watson atlas (1986); "ventral" hippocampus refers to the more ventral (>3.0 mm from the cortical surface) and lateral (>4.0 mm off the midline)

portions of the hippocampal formation between 4.8 mm and 6.8 mm caudal to bregma according to Paxinos and Watson. Stimulation points were located in the hippocampus according to the stereotaxic coordinates of the Paxinos and Watson atlas. A glass insulated tungsten microelectrode (100 μ m tip exposure) (Neafsey, 1981) was lowered into the hippocampus with the aid of a micromanipulator. Electrical stimulation was delivered using a 60 second train of 0.25 msec pulses at 25 Hz at a current intensity of 50 uamps. A Grass S88 stimulator with a PSIU6C photoelectric constant current stimulus isolation unit was used to deliver the stimulus; current intensity was read off the S88 VOLTS dial (accurate to \pm 20%). Points were considered responsive if a 50 uamp stimulus elicited a clear decrease in heart rate.

Once a responsive point was located, the microelectrode was withdrawn and a glass pipette (tip diameter 25-30 μ m) filled with 1% WGA-HRP fastened to a 1.0 ul Hamilton syringe was lowered to that point in the hippocampus with the aid of a micromanipulator. A pressure injection was made in the hippocampus of 0.01-0.02 ul of WGA-HRP, and the pipette left in place for 10 minutes to insure complete delivery and uptake of WGA-HRP. Following the injection, the pipette was withdrawn and the wound sutured. The animals were allowed to recover and survive for two days. After the survival period the rats were sacrificed by an overdose injection of sodium pentobarbital and perfused transcardially with 0.9% saline, 1.25% paraformaldehyde-1% glutaraldehyde, and, finally, with 10% buffered sucrose according to

the technique of Rosene and Mesulam (1978). The brains were removed, allowed to sink in 10% buffered sucrose, cut into 50 um sections on a freezing stage microtome, and reacted for HRP histochemistry according to the tetramethylbenzidine procedure of Mesulam (1978). Two sets of alternate sections were mounted on glass slides; one set was left unstained while the other set was counterstained with 1% pyronin Y. The sections were viewed under brightfield and polarized light microscopy to determine the presence and location of anterograde label in the medial frontal cortex.

Electron microscopic studies

Long-Evans hooded rats (n=3) were anesthetized with Ketamine HCl (100 mg/kg, intraperitoneally) and placed in the stereotaxic apparatus. The skull and neck musculature were exposed by a surgical incision and the cisterna magna was opened to gain access to the dorsal medulla. The animal's neck was flexed slightly and part of the occiput was removed to expose obex which was used as a reference point.

A glass micropipette (tip diameter 25-30 um) filled with 1% WGA-HRP fastened to a 1.0 ul Hamilton syringe was positioned at 0.3-0.5 mm lateral to obex and 0.3-0.5 mm ventral to the brainstem surface using a micromanipulator. Pressure injections of 0.01 to 0.03 ul of WGA-HRP were made in the brainstem over a 5-10 minute period. Following the injection, the micropipette was withdrawn and the neck musculature sutured.

A small craniotomy was performed over the ventral hippocampus

ipsilateral to the solitary nucleus injection site. The micropipette filled with WGA-HRP was lowered into the ventral hippocampus using the stereotaxic coordinates of Paxinos and Watson (1986). A pressure injection was made in the ventral hippocampus of 0.01-0.03 ul of WGA-HRP over a 5-10 minute period. Following the injection, the micropipette was withdrawn, the hole in the skull packed with Gelfoam and the skin sutured. The animals were allowed to recover for 24 hours and then were sacrificed by an overdose injection of sodium pentobarbital. The animal was perfused transcardially with 0.9% saline followed by a solution of EM grade 1% paraformaldehyde-1% glutaraldehyde in 0.1M phosphate buffer (pH 7.4), then 2% paraformaldehyde-2% glutaraldehyde, and, finally, 10% buffered sucrose. The brain was removed and stored in 10% buffered sucrose overnight.

The brain was blocked with a razor blade and the frontal cortex was cut on a vibratome into 50 um sections into cold 0.9% saline. The sections were then reacted for HRP histochemistry over ice and in the dark according to the tetramethylbenzidine stabilization technique of Rye et al. (1984) and the presence of reaction product determined. The sections were then placed in small vials containing cold cacodylate buffer (0.15M, pH 7.4) overnight.

The next day the sections were osmicated with 1% OsO₄ in 0.15M cacodylate buffer for 30 minutes. They were then stained with 2% uranyl acetate in maleate buffer and subsequently dehydrated through a graded series of ethanol to propylene oxide. The sections were

infiltrated with a 50%-50% mixture of propylene oxide and Epon followed by 100% Epon and mounted from warmed Epon onto clean, spray silicon lubricant-greased glass slides, coverslipped with silicon-greased glass coverslips, and polymerized overnight at 57°C.

Following light microscopic examination, the coverslips were removed, and the sections of the medial frontal cortex with retrogradely labelled cells and anterogradely labelled terminals were bonded to the face of an Epon block. Sections of the cortex contralateral to the hippocampal and solitary nucleus injection sites with only retrogradely labelled cells were also prepared for electron microscopic analysis as controls. (It should be noted that the projection to the medial frontal cortex from the hippocampus is strictly unilateral [Swanson, 1981; Ferino et al., 1987]; the projection from the medial frontal cortex to the solitary nucleus is bilateral [van der Kooy et al., 1982, 1984; Terreberry and Neafsey, 1983, 1987].) The blockfaces were trimmed with a razor blade and ribbons of 70-80 nm sections were cut and placed on Formvar coated slot grids. The grids were stained with uranyl acetate and lead citrate. Observations were made with an Hitachi H600 transmission electron microscope operated at 75kV.

RESULTS

Hippocampal injections

Injections of WGA-HRP into the ventral hippocampus involving the CA1 cell field, which, upon low threshold electrical stimulation, elicited marked decreases in heart rate, result in anterograde terminal-like labelling in the ipsilateral nucleus accumbens, the dorsal peduncular cortex, layers I through V of the ventral portion of the infralimbic area of the medial frontal cortex, and a concentrated band of labelling extending dorsally in layer V of the infralimbic area to the prelimbic area of the medial frontal cortex (Figure 1). No anterograde labelling was observed in the contralateral medial frontal cortex. In addition, no retrograde labelling in the medial frontal cortex following hippocampal injections was observed. Injections of WGA-HRP into the dorsal hippocampus did not label any part of the medial frontal cortex.

A typical ventral hippocampal injection site is illustrated in Figure 2B. The injection site shown here involves a small portion of the overlying cortex and the dentate gyrus. Control injections into only the overlying cerebral cortex or only the dentate gyrus did not label the medial frontal cortex, therefore the anterograde labelling observed in the medial frontal cortex was due specifically to the involvement of the hippocampus and its cell layers in the injection.

Solitary nucleus injections

Injections of WGA-HRP into the dorsomedial medulla retrogradely labelled cells bilaterally in layer V of the infralimbic and prelimbic areas of the medial frontal cortex (Figures 1&2). The brainstem injection site illustrated in Figure 2A illustrates that the solitary nucleus is clearly involved in the injection as well as a number of other brainstem structures. Previous anterograde tracing studies in our laboratory have shown that injections of WGA-HRP into the medial frontal cortex label terminals only in the solitary nucleus in this region of the brainstem (Terreberry and Neafsey, 1987), therefore, the retrograde labelling seen in the medial frontal cortex in the present study is due specifically to the involvement of the solitary nucleus in the injection. In addition, no anterograde labelling was ever observed in the medial frontal cortex following injections of WGA-HRP into the dorsal medulla (Terreberry and Neafsey, 1983).

Overlap of anterograde and retrograde labelling

Figures 2C & D show that the dense terminal-like labelling in the medial frontal cortex from the hippocampal injection appeared, at the light microscopic level, to overlap the retrogradely labelled cells ipsilateral to the hippocampal injection site. The camera lucida drawing in Figure 1 shows that the anterograde labelling (stippling) in layer V of the infralimbic area from the ipsilateral hippocampal injection is entirely coextensive with the retrogradely labelled cells (black dots) in layer V of the infralimbic area from the ipsilateral

solitary nucleus injection.

Ultrastructural observations

Ultrastructural examination demonstrated that the anterograde labelling observed in the medial frontal cortex following hippocampal injections consisted of HRP-labelled, vesicle-filled terminal boutons. There were an average of 154 terminal boutons (\pm 31 S.D.) per 130,000 μm^2 section (n=5) of which an average of 32 \pm 3 (21%) were observed making synaptic contact on presumed dendritic processes in any particular section; the area of a section is indicated by the dashed line box in Figure 2C. Observations of axo-somatic synapses between anterogradely labelled axon terminals and retrogradely labelled cell somas were rare, but could be found. Figure 3A illustrates an anterogradely labelled terminal in contact with a retrogradely labelled cell soma. Terminal vesicles are seen in the bouton associated with the presynaptic membrane. The synaptic cleft is clearly delineated, and there appears to be a specialization of the plasma membrane of the retrogradely labelled cell. HRP reaction product is clearly identifiable in the terminal and the reaction product contained within the labelled cell is confined to the perikaryon; in this case, the HRP reaction product crystals are sufficiently large as to penetrate the nuclear envelope (circled). Figure 3B shows an example of the more commonly observed axo-dendritic synapse. Labelled terminals commonly contacted small to intermediate sized dendritic profiles characteristic of the more distal portions of dendrites in relationship to the cells

of their origin. In no cases were there any clearly labelled terminals seen synapsing on clearly labelled dendritic profiles. No anterogradely labelled terminals were seen anywhere in the medial frontal cortex contralateral to the injection sites.

DISCUSSION

The results we have obtained indicate that points in the ventral CA1 region of the hippocampus which, upon electrical stimulation, evoke decreases in heart rate, project ipsilaterally to the infralimbic and prelimbic regions of the medial frontal cortex, a cortical area which, in turn, projects directly to the solitary nucleus. This hippocampal projection very closely overlaps the cells which send their axons to this cardiovascular control center in the brainstem. At the light microscopic level, the terminal-like labelling from the hippocampal injections is densest in layer V, overlapping cell bodies of cortical cells which project to the solitary nucleus. Electron microscopic analysis indicates that these terminals do, albeit rarely, synapse directly on the somas of cells retrogradely labelled from the solitary nucleus. Many other synaptic contacts from the hippocampal projection are made primarily on dendritic processes adjacent to these somas.

The combined use of the anterograde and retrograde tracing properties of horseradish peroxidase and electron microscopy has previously been successful in determining the convergence of a neural

projection onto a discreet collection of neurons (Kosinski et al., 1988). In this study, the use of WGA-HRP for both anterograde and retrograde tracing of neuronal connections is justified for two reasons. Firstly, injections into the ventral hippocampus labelled only terminals ipsilaterally, and no retrograde labelling was seen in the medial frontal cortex following injections of only the ventral hippocampus. Secondly, injections into the solitary nucleus only retrogradely labelled cells in the medial frontal cortex; there was no evidence of anterograde transport of HRP from solitary nucleus injections because the contralateral cortex contained no terminals. In addition, the absence of labelled terminals in the contralateral cortex rules out the possibility that retrogradely labelled cells communicate between each other in the medial frontal cortex. Therefore, the labelled cells in the medial frontal cortex are only from solitary nucleus injections whereas the labelled terminals in the the medial frontal cortex are only from hippocampal injections.

The high sensitivity of the tetramethylbenzidine (TMB) procedure (Mesulam, 1978) and the increased stabilization of the TMB reaction product for electron microscopic processing afforded by incubation of the sections in diaminobenzidine (DAB) and cobalt chloride (Rye et al., 1984) allows for the demonstration of the presence of HRP where it may be present in very small amounts, e.g. distal dendrites and synaptic terminals (Carson and Mesulam, 1982). With increased sensitivity, the size of the reaction product increases misrepresenting the actual

extent of HRP distribution. In this study, crystals of HRP reaction product were seen to penetrate plasma membranes of retrogradely labelled cells, as well as those of dendrites and axon terminals in the neuropil. In a number of cases, large amounts of reaction product damaged tissue to such an extent where undisputed identification of the cellular profile as a labelled dendrite or terminal was not possible. In addition, although at the light microscopic level the cell soma and proximal portions of its dendrites appeared to be filled with reaction product, electron microscopy shows that reaction product does not entirely fill the cytoplasm throughout the cell soma and dendrites. The results of this study may therefore be an underestimation of the direct contact the hippocampus has on cells projecting to the solitary nucleus; the origin of the dendritic processes on which many labelled terminals synapse may either be those of retrogradely labelled cells which project to the solitary nucleus or those of cortical interneurons which influence the activity of the solitary nucleus projection neurons.

The fact that no labelled dendrites were observed receiving synaptic input from labelled terminals also argues against the possibility of trans-synaptic transport of HRP. Survival times not greater than 24 hours were sufficiently long to allow for adequate anterograde and retrograde HRP-labelling of terminals and cells, but not trans-synaptic transport of the enzyme.

The existence of synaptic contacts between hippocampal terminals

and cells in the medial frontal cortex which project to the solitary nucleus anatomically reinforces conclusions from previous physiological experiments which suggest that the medial frontal cortex is a relay between the hippocampus and the solitary nucleus (Ruit and Neafsey, 1988a), and that the hippocampus does have a strong linkage with central cardiovascular control.

Figure 1.

Camera lucida drawing of a coronal section through the frontal cortex of the rat illustrating the distribution of anterograde terminal labelling (stippling) resulting from injections of WGA-HRP into the ipsilateral ventral hippocampus and retrograde cell labelling (black dots) resulting from injections of WGA-HRP into the brainstem. PL = prelimbic area of medial frontal cortex; IL = infralimbic area of medial frontal cortex; tt = tenia tecta; acb = nucleus accumbens; ac = anterior commissure; cc = corpus callosum; scale bar = 1.0 mm.

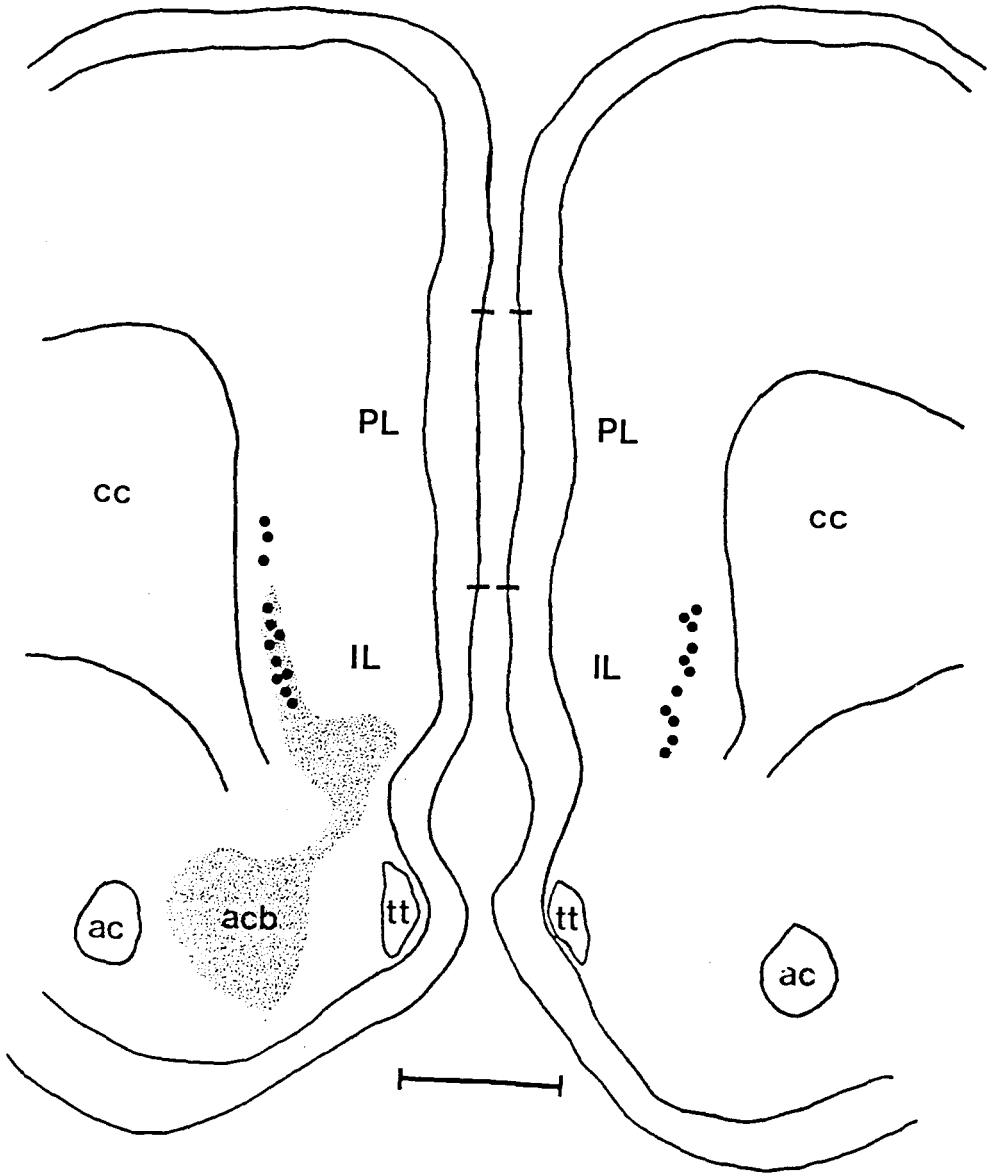


Figure 2.

A. Photomicrograph of solitary nucleus injection site. Note labelling of contralateral vagal complex, but no spread of the injection to the contralateral hypoglossal nucleus. XII = hypoglossal nucleus; pt = pyramidal tract; spV = spinal nucleus of trigeminal nerve; NTS = nucleus of the solitary tract; arrow points to location of micropipette penetration; scale bar = 1.0 mm.

B. Photomicrograph of hippocampal injection site involving CA1 cell region and dentate gyrus (DG). Scale bar = 1.0 mm.

C. Photomicrograph of nissl-stained coronal section of the medial frontal cortex. Cells retrogradely labelled by solitary nucleus injection are confined to the infralimbic (IL) and prelimbic (PL) regions. Cells in the prelimbic region are circled. Boxed region is represented in further detail in Figure 1D. Region enclosed within the dashed-line box represents the size of a section examined at the electron microscopical level. tt = tenia tecta; na = nucleus accumbens; cc = corpus callosum; scale bar = 0.5 mm.

D. Photomicrograph of unstained coronal section of the medial frontal cortex illustrating the relationship of anterograde terminal-like labelling from hippocampal injections to retrogradely labelled cells from solitary nucleus injections. Boxed region is represented in further detail in Figure 1E. Scale bar = 0.1 mm.

E. Detail of the relationship of anterograde terminal-like labelling (arrowheads) to retrogradely labelled cells. Scale bar = 50 μ m.

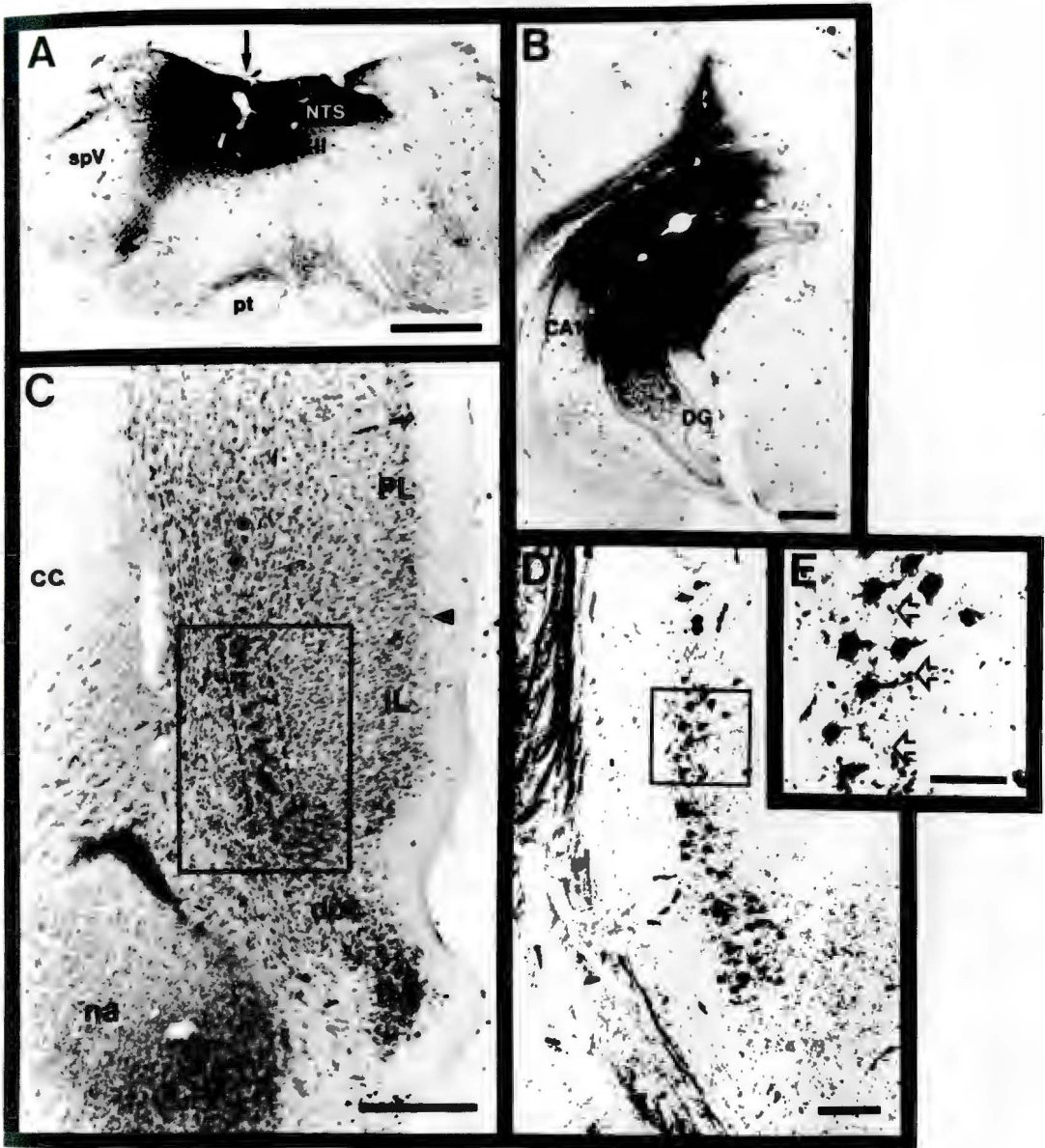
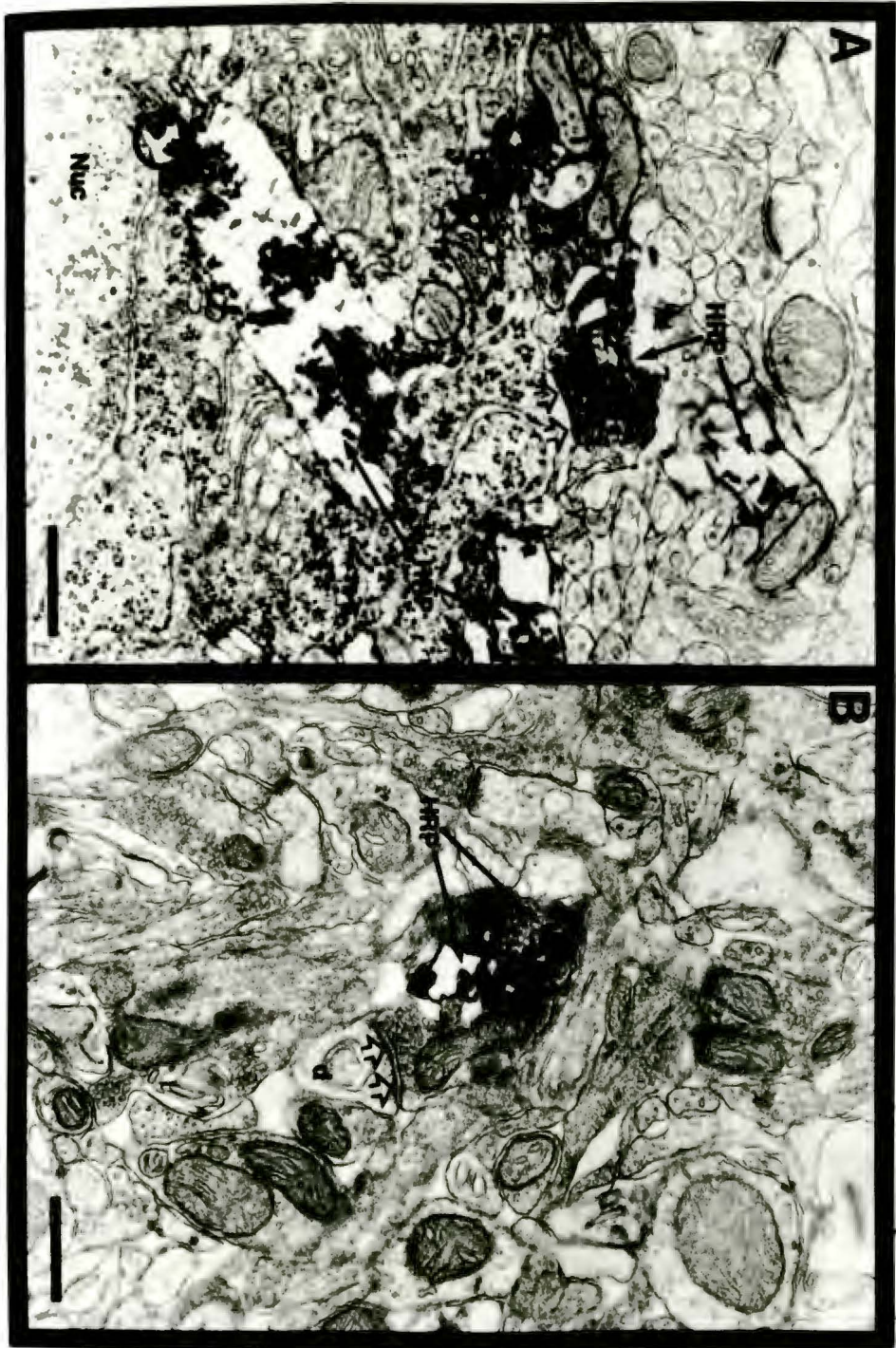


Figure 3.

A. Electronmicrograph illustrating an anterograde labelled terminal (t) making synaptic contact with a retrogradely labelled cell. HRP is clearly seen within the cytoplasm of the cell and within the terminal (arrows). The crystals of HRP are large enough to impinge on and penetrate the nuclear envelope (circled). The arrowheads point out the subsynaptic density in the labelled cell. Nuc = nucleus of labelled cell; scale bar = 0.5 μ m.

B. Electronmicrograph illustrating the more commonly seen synaptic contact between a labelled terminal (t) and a dendritic process (d). HRP is clearly seen within the terminal (arrows) and the arrowheads point out the subsynaptic density within the dendritic process. Scale bar = 0.5 μ m.



CHAPTER VI

CONVERGENCE OF HIPPOCAMPAL INPUT TO
THE MEDIAL FRONTAL CORTEX ON
NEURONS WHICH PROJECT TO THE SOLITARY NUCLEUS:
AN ELECTROPHYSIOLOGICAL STUDY

ABSTRACT

The hippocampus is strongly linked anatomically with the medial frontal cortex, a region of the brain associated with central cardiovascular control via its direct projection to the solitary nucleus. The present study was undertaken to determine electrophysiologically the degree of convergence of hippocampal input on neurons in the medial frontal cortex which have been previously identified as projecting to the solitary nucleus. Electrical stimulation of the solitary nucleus antidromically activated cells in the infralimbic and prelimbic regions of the medial frontal cortex. The average latency of antidromic activation was 30 msec, corresponding to a conduction velocity of approximately 0.7 m/sec. Electrical stimulation of the ventral hippocampus orthodromically activated cells in the medial frontal cortex. With an appropriate delay between the hippocampal and solitary nucleus stimuli, the orthodromic and antidromic potentials could be made to collide, establishing a functional link between the hippocampus and solitary nucleus projection-neurons in the medial frontal cortex. In addition, this study describes the characteristics of the pathway from the medial frontal cortex to the solitary nucleus, a pyramidal tract pathway which exhibited very slow conduction velocities compared to the myelinated, fast conducting fibers of the pyramidal tract which have been studied previously. The possibility that this pathway may comprise a portion

of the small diameter, unmyelinated fibers which make up 60% of the pyramidal tract, whose function was previously unknown, is discussed.

INTRODUCTION

The connections of structures in the central nervous system often provide clues to the functions of those structures. The principle efferent pathway from the hippocampus is the fornix which terminates in the lateral septum (Swanson and Cowan, 1979) and hypothalamus (Swanson and Cowan, 1977), structures that have been traditionally associated with the Papez circuit hypothesized to form a substrate for emotion (Papez, 1937) and more recently associated with cardiovascular activity (Calaresu and Mogenson, 1972; Loewy and McKellar, 1980; Gelsema and Calaresu, 1987). The hippocampus also projects heavily to the medial frontal cortex (Swanson, 1981; Ferino et al., 1987), a cardiovascular control region (Kaada, 1951; Lofving, 1961; Burns and Wyss, 1985; Terreberry and Neafsey, 1984, 1988) which projects directly to the nucleus of the solitary tract (van der Kooy et al., 1982, 1984; Terreberry and Neafsey, 1983, 1987).

The medial frontal cortex has been implicated as a cortical relay for hippocampal control of cardiovascular activity because ablation of the medial frontal cortex attenuates or abolishes cardiovascular responses to stimulation of the hippocampus (Ruit and Neafsey, 1987, 1988a). The hippocampal projection to the medial frontal cortex

terminates, in some cases, directly on the somas of cells in the medial frontal cortex which project to the solitary nucleus (Ruit and Neafsey, 1988b), a finding which strengthens the linkage of the hippocampus with cardiovascular control. The present electrophysiological study was undertaken to study the functional status of this pathway by determining the responses to hippocampal stimulation of medial frontal cortical neurons that had previously been shown to respond antidromically to stimulation of the solitary nucleus, a brainstem cardiovascular control region.

MATERIALS AND METHODS

Experiments were performed on ten Long-Evans hooded rats. The animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally), and a tracheal cannula was inserted for artificial ventilation. The animals were then placed in the stereotaxic apparatus, and the skull and neck musculature exposed by a surgical incision. The cisterna magna was opened to gain access to the dorsal medulla. The animal's neck was flexed slightly, part of the occiput removed, and the posterior part of the cerebellum removed by aspiration in order to fully reveal obex, which was used as a reference point. A craniotomy was then performed over the hippocampus and the ipsilateral frontal cortex, and the dura mater reflected.

A glass insulated tungsten microstimulation electrode (Neafsey,

1981) (tip exposure 75-100 μm) was positioned 0.3 to 0.5 mm lateral to obex and 0.3 to 0.4 mm ventral to the medullary surface using a micromanipulator. Another stimulating electrode was positioned in the ventral hippocampus according to the stereotaxic coordinates of Paxinos and Watson (1986) (5.0 to 6.0 mm posterior to bregma, 5.0 to 5.5 mm lateral to the midline, and 3.0 to 6.0 mm ventral from the cortical surface). Finally, a glass insulated tungsten recording electrode (tip exposure 15 to 25 μm) was positioned over the medial frontal cortex (between 3.0 and 4.0 mm rostral to bregma and 0.5 to 0.75 mm lateral to the midline). Before stimulating and recording began, the animal was paralyzed with an intraperitoneal injection of succinylcholine chloride (20 mg/kg) and put on a respirator (70 breaths per minute, 2.0 cc per breath). Every sixty minutes the animal was given supplemental doses of chloral hydrate to maintain a stable level of anesthesia and succinylcholine to maintain paralysis.

Electrical stimulation was delivered in the solitary nucleus as the medial frontal cortical recording electrode was lowered through the cortex using a microdrive. Solitary nucleus stimulation parameters were single shocks of 0.20 msec negative pulses at current intensities of 300 to 500 uamps. The recording electrode monitored extracellular neuronal activity in the medial frontal cortex, and the signal was fed into a Grass P511 Series amplifier (bandpass 300-10000 Hz). Series of stimulus trials were recorded using raster/stepper oscilloscope displays. When an antidromically activated cell was identified, the

current intensity delivered to the solitary nucleus was gradually reduced to determine the threshold current for the response. Threshold was considered to be the lowest stimulation current intensity at which the antidromically activated cell fired every time. Criteria for antidromic activation were constant latency and high frequency (>200 Hz) following (Lipski, 1981; Zarzecki, 1982). Collision was also used if the cell's spontaneous or evoked activity permitted.

When an antidromically activated cell was identified, a collision test was used (Waters et al., 1985) to determine if the antidromically activated cells also received input from the hippocampus. Electrical stimulation was delivered to the hippocampus using single shocks of 0.20 msec negative pulses at current intensities between 300 and 800 uamps, threshold being the lowest current which resulted in the firing of medial frontal cortical neurons. By varying the delay between the stimulus in the hippocampus and the stimulus in the solitary nucleus, the antidromic potential from the solitary nucleus could be made to collide with the orthodromic potential from the hippocampus, confirming the functional connection of hippocampal pathways to neurons projecting to the brainstem. Three to five electrode tracks were made through the medial frontal cortex, and the positions of the stimulating electrodes were adjusted to elicit optimal medial frontal cortical neuronal responses.

During the experiments, small marking lesions (10 uamps DC for 10 seconds) were made at responsive points in the medial frontal cortex,

and following the experiments, lesions were made in the solitary nucleus and the hippocampus to facilitate histological reconstruction of the electrode tracks. The animals were then sacrificed by an overdose injection of sodium pentobarbital and perfused transcardially with 0.9% saline followed by 10% buffered formalin. The brains were removed and allowed to sink in a 30% sucrose/formalin solution. The medial frontal cortex, hippocampus, and brainstem were sectioned at 50 μ m on a sliding microtome with a freezing stage, mounted on gelatin-coated glass slides, stained with 0.1% cresyl violet, and coverslipped. The slides were examined under the light microscope, and the exact location of the marking lesions noted in order to reconstruct the electrode tracks.

RESULTS

Solitary nucleus stimulation

In ten experimental animals, 123 medial frontal cortical cells were antidromically activated by electrical stimulation of the solitary nucleus. The cells responded with an average latency of 30 msec (\pm 10 msec S.D., range: 11-60 msec), with an average conduction velocity of 0.75 m/sec (\pm 0.29 m/sec S.D., range: 0.33-1.82 m/sec calculated using 20 mm as the distance between the medial frontal cortex and the solitary nucleus). All cells followed high frequency stimulation trains at 200-300 Hz. The threshold current for antidromic activation

was 409 uamps (\pm 181 uamps S.D., range 50-800 uamps). Figure 1(I) shows an oscilloscope tracing of four solitary nucleus stimulus trials. The medial frontal cortical cell fires each time at a constant latency, and the last two trials illustrate how the cell follows high frequency (300 Hz) stimulus trains (note the presence of only the initial segment of the second antidromic spike following the high frequency stimulus train). These medial frontal cortical neurons were generally not spontaneously firing and could only be detected by their response to stimulation of the solitary nucleus. When spontaneously firing cells which also responded antidromically to stimulation in the solitary nucleus were encountered, an attempt was made to follow a spontaneous spike with a single shock stimulus in the solitary nucleus in order to establish collision as a third criteria for antidromic activation. Figure 1(II) shows an oscilloscope tracing in which four solitary nucleus stimulus trials are observed. In trials A through C there is no spontaneous spike, and the antidromic spike appears at a constant latency. However, in trial D the cell fires spontaneously, and the delay between the spontaneous spike and the solitary nucleus stimulus is such that the antidromic and orthodromic potentials collide and the antidromic spike does not appear. The average depth of the responsive cells was 3.50 mm (\pm 0.71 mm S.D.) ranging between 1.70 and 4.90 mm ventral to the cortical surface and 3.3 to 3.5 mm rostral to bregma (Figure 3).

Hippocampal stimulation

Single cells in the medial frontal cortex were also activated orthodromically by electrical stimulation in the ventral hippocampus. Of the 123 cells activated antidromically by stimulation in the solitary nucleus, 97 were tested for responses to hippocampal stimulation. Of the 97 neurons tested, hippocampal stimulation resulted in the firing of the same cell or cells in the vicinity of the antidromically activated cell 15 times. Figure 1(III) illustrates an oscilloscope tracing in which a 700 uamp stimulus in the ventral hippocampus succeeded in driving a medial frontal cortical cell. The orthodromic latencies were variable and averaged 26 msec (\pm 7 msec S.D., range: 15-40 msec). None of the responses in the medial frontal cortex to stimulation in the hippocampus were characterized as being antidromic.

Collision testing

Evidence for an input from the hippocampus to the antidromically activated cortical cells was demonstrated by the ability to collide the antidromic and orthodromic potentials. Of the 15 medial frontal cortical neurons responding to hippocampal stimulation, collision of the orthodromic and antidromic potential was observed in 4 cases. As shown in Figure 1(IV), in trials A and C the stimulation in the hippocampus did not evoke an orthodromic firing of the cell, and the antidromic spike to solitary nucleus stimulation was seen in both cases. However, in trials B and D, hippocampal stimulation elicited an

orthodromic spike in the medial frontal cortical cell, and, with a delay of 40 msec between the hippocampal and solitary nucleus stimuli, the orthodromic potential collided with the antidromic action potential elicited by the stimulus in the solitary nucleus, and the antidromic spike did not appear. Figure 2 illustrates the positions of the electrodes in the different areas of the brain for the case shown in Figure 1. Panel A shows the electrode location in the solitary nucleus, panel B the hippocampus, and panel C the medial frontal cortex. The arrow in panel C marks the position of the cell recorded in Figure 1(IV). Figure 3 is a line drawing of a sagittal view of the rat brain illustrating the locations of all the antidromically activated cells (in some cases one dot equals more than one cell). The responsive cells were confined to the prelimbic and infralimbic regions of the medial frontal cortex, in agreement with previous anatomical findings (Terreberry and Neafsey, 1983; Neafsey et al., 1986; Ruit and Neafsey, 1988b).

DISCUSSION

There are two important results of this study. Firstly, the hippocampus influences the activity of neurons in the medial frontal cortex which project directly to the solitary nucleus. Previous studies have presented electrophysiological evidence that the hippocampus projects to the medial frontal cortex. The cells in the

CA1 region of the hippocampus respond antidromically to electrical stimulation of the medial frontal cortex with mean latencies of 15 msec (estimated conduction velocities of 0.6 m/sec) (Ferino et al., 1987). Their conduction velocities are very similar to those observed in this study between the medial frontal cortex and the solitary nucleus. Electrical stimulation of the hippocampus elicits both increases and decreases in the spontaneous firing rates of single units in the medial frontal cortex, the earliest latencies averaging 15 msec (Ruit and Neafsey, 1986).

The results of this study also suggest that the hippocampus/medial frontal solitary nucleus-projection neuron pathway is not primarily monosynaptic. This finding is consistent with the results of our previous anatomical study which found many hippocampal terminals in the vicinity of the somas of solitary nucleus-projection neurons but only few terminals on the somas themselves (Ruit and Neafsey, 1988b). Cortico-cortical connections such as the hippocampal projection to the medial frontal cortex may therefore not be specific to cortical neurons projecting to the solitary nucleus. Instead, the specificity may lie in cortical local circuit interneuronal connections with the cells that project to the brainstem.

The relatively small number of successful "collision" tests is not surprising in view of the technical difficulties involved in this type of experiment. Correct electrode placement, particularly in the hippocampus, is critical in determining the ability to orthodromically

activate medial frontal cortical neurons. In any particular case, although a cell in the medial frontal cortex may fire antidromically by activation of the solitary nucleus, it may not respond to stimulation at the present location of the hippocampal electrode, but may require a systematic search of the hippocampus for the optimum stimulation point. Such a search requires that the antidromically activated cell be held for possibly quite some time before adequate testing can be done, and this cannot always be accomplished.

The second important result of this study is the elucidation of the characteristics of the pathway from the medial frontal cortex to the solitary nucleus in the dorsal medulla. Previous anatomical and physiological studies in our laboratory have shown that this projection is via the pyramidal tract (Terreberry and Neafsey, 1987). In this work, injections of horseradish peroxidase into the medial frontal cortex label fibers in the decussating pyramidal tract which could be traced into the solitary nucleus. The extremely long antidromic latencies and slow conduction velocities of these pyramidal tract neurons, as observed in the present study, are remarkable. Previous electrophysiological studies of pyramidal tract neurons have focused on the fast conducting pyramidal tract pathway which originates from the large cells in layer V of the sensorimotor cortex. For example, the early work of Woolsey and Chang (1948) and subsequent studies showed that following stimulation of the medullary pyramids, electrical potentials could be recorded extracellularly at the cortical surface

and from single units approximately 1.0 mm ventral to the cortical surface at latencies ranging between 0.5 to 3.6 msec, corresponding to conduction velocities of 20-55 m/sec (Porter, 1955; Landau, 1956; Jabbur and Towe, 1961; Porter and Sanderson, 1964; Bannister and Porter, 1967; Elger et al., 1977; Harrison and Towe, 1986). Following epicortical stimulation, spinal cord surface potentials could be recorded at cervical, thoracic, and lumbar levels with conduction velocities on the order of 60 m/sec (Elger et al., 1977; Janzen et al., 1977), results which are very similar to those observed in the antidromic studies. These corticospinal/pyramidal tract electrophysiological studies distinguished two types of pyramidal tract neurons; the fast conducting pyramidal tract neurons (conduction velocities of 20-60 m/sec) and the slow conducting pyramidal tract neurons (conduction velocities between 11 to 18 m/sec) (Phillips and Porter, 1977). No conduction velocities below 10 m/sec were ever reported.

The fiber composition of the pyramidal tract of the rat has been described as a collection of primarily small diameter, unmyelinated processes which make up approximately 59% of the tract (Leenen et al., 1982), in addition to the large diameter and small diameter myelinated fibers. Both the "fast" and "slow" conducting fibers described above are myelinated. However, the functions of the numerous unmyelinated fibers of the pyramidal tract are still unknown (Thomas et al., 1984; Leenen et al., 1985; Biedenbach et al., 1986).

In contrast to the antidromic studies described above, our findings show that the neurons located in the infralimbic and prelimbic areas of the medial frontal cortex which send processes directly to the solitary nucleus via the pyramidal tract respond antidromically to medullary stimulation at an average latency of 30 msec, equivalent to a conduction velocity of about 0.7 m/sec. The very slow conducting fibers observed in this study are not at all comparable to those described in previous studies. Figure 4 is a summary graph illustrating the results of the work of Porter and Sanderson (1964) who described pyramidal tract neuron antidromic latencies recorded in rat somatomotor cortex (SMC) following stimulation of the medullary pyramids. Their results are compared to the results of the present study in which antidromic latencies were recorded in the medial frontal cortex (MFC) following stimulation of the solitary nucleus. Our findings may provide evidence for a function of at least a portion of the numerous, small diameter, unmyelinated fibers which make up approximately 60% of the pyramidal tract. In addition, although many authors have described short latency (< 5 msec) antidromic responses to stimulation of the medullary pyramids in motor and parietal cortex, very long latency (> 10 msec) cells have never been reported. This suggests that these unmyelinated fibers may not originate from these areas but rather from other regions of the cerebral cortex such as the infralimbic and prelimbic areas of the medial frontal cortex.

In general, descending pathways associated with autonomic

functions have been shown to have relatively slow conduction velocities. Projections from the paraventricular nucleus of the hypothalamus to the dorsomedial medulla and thoracic cord of the rat have been shown by antidromic activation studies to have conduction velocities averaging less than 1.0 m/sec (Kannan and Yamashita, 1983; Zerihun and Harris, 1983; Rogers and Nelson, 1984; Lovick and Coote, 1988). Similar studies have shown that reciprocal connections between the central nucleus of the amygdala and the solitary nucleus have conduction velocities of approximately 0.2 m/sec (Rogers and Fryman, 1988). Pyramidal cells in layer V of the medial region of the frontal cortex projecting to the lateral hypothalamic area have conduction velocities, based on antidromic activation studies, of 3.8 m/sec (Kita and Oomura, 1981). Our findings are very similar to the conduction velocities observed in hypothalamic and amygdaloid projections to the dorsomedial medulla.

In conclusion, anatomical and electrophysiological evidence exists to support the hypothesis that the hippocampus is in a position to influence cardiovascular functioning. Although the hippocampus is generally associated with emotion and learning and memory (Papez, 1937; Siefert, 1983), processes which involve very complex neuronal networks which are not fully understood, the present study emphasizes that there is also a relationship between the hippocampus and a well-defined visceral motor system necessary for the basic survival of an organism.

Figure 1.

I. Oscilloscope tracings of medial frontal cortical neuron antidromic responses to stimulation in the solitary nucleus. Trials A through D illustrate constant latency of the antidromic response. Trials C and D illustrate following of high frequency stimulation. The arrows point out the presence of only the initial segment of the second antidromic spike in the high frequency stimulation trials. Calibration = 100 uV and 10 msec.

II. Oscilloscope tracings of medial frontal cortical neuron antidromic responses to solitary nucleus stimulation illustrating collision of the antidromic potential with a spontaneously occurring spike. Arrow indicates delivery of solitary nucleus (NTS) stimulus. Calibration = 100 uV and 10 msec.

III. Oscilloscope tracings of medial frontal cortical neuron orthodromic responses to stimulation in the hippocampus. Calibration = 100 uV and 10 msec.

IV. Oscilloscope tracings of medial frontal cortical neuron orthodromic and antidromic responses to stimulation in the hippocampus (HIPP) and solitary nucleus (NTS), respectively. The same cortical cell activated antidromically by NTS stimulation is activated by HIPP stimulation. Collision of the spikes is illustrated in trials B and D. Calibration = 100 uV and 10 msec.

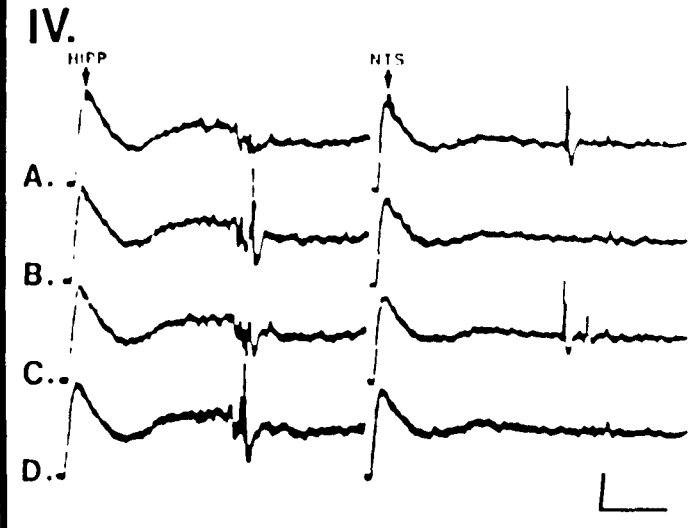
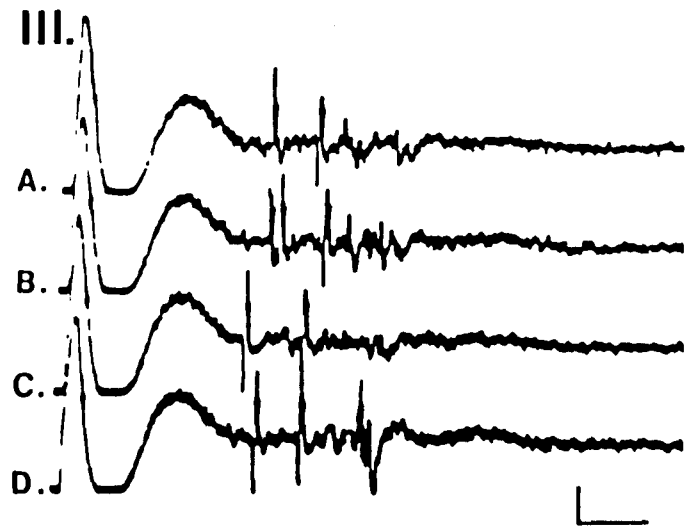
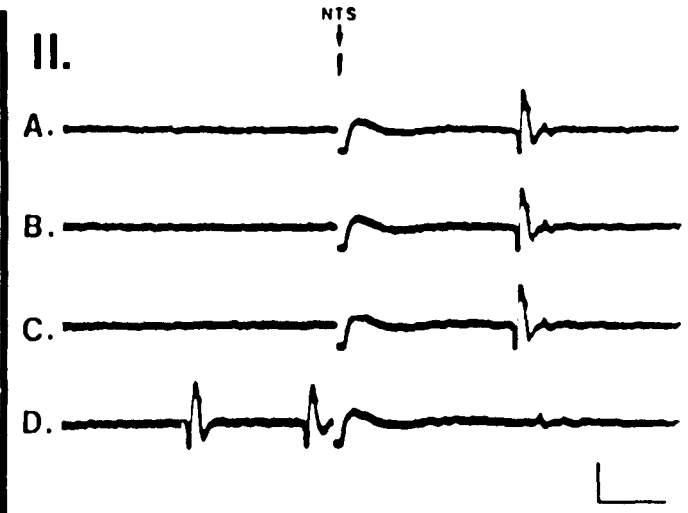


Figure 2.

A. Nissl-stained coronal section of brainstem illustrating the lesion (*) marking the position of the stimulating electrode in the solitary nucleus (NTS). DMN = dorsal motor nucleus; XII = nucleus of hypoglossal nerve; scale bar = 0.5 mm.

B. Nissl-stained coronal section of hippocampus illustrating the lesion (*) marking the position of the stimulating electrode in the dendritic region of the CA1 cell layer. DG = dentate gyrus; CA3 = CA3 cell region of hippocampal formation; scale bar = 1.0 mm.

C. Nissl-stained coronal section of medial frontal cortex illustrating an electrode track along which the cell, whose activity is shown in Figure 1(IV), was located (arrow). The asterisk marks the extent of the electrode penetration. The cortical cytoarchitecture is delineated. tt = tenia tecta; IL = infralimbic area; PL = prelimbic area; AC = anterior cingulate cortex; AgM = medial agranular cortex; cc = corpus callosum; scale bar = 1.0 mm.

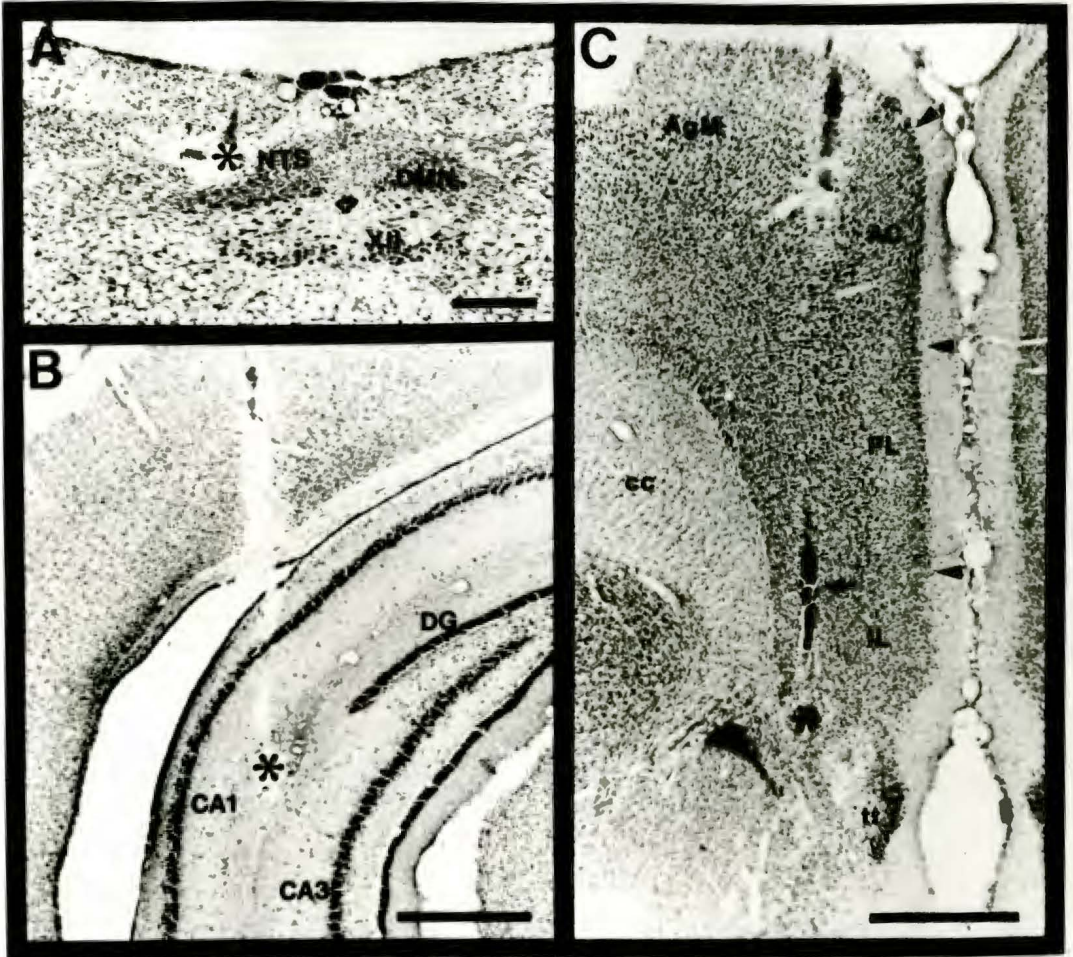


Figure 3.

Line drawing of sagittal section of rat brain illustrating the location of all cells in the medial frontal cortex which responded antidromically to stimulation of the solitary nucleus. Due to overlap some dots represent more than one cell. The cytoarchitecture of the cortex is delineated and the cells are confined to the prelimbic (PL) and infralimbic (IL) regions. MO = medial orbital cortex; AC = anterior cingulate cortex; AgM = medial agranular cortex; cc = corpus callosum; tt = tenia tecta; Olf = olfactory bulb; ac = anterior commissure; arrow points out the position of bregma; scale bar = 1.0 mm.

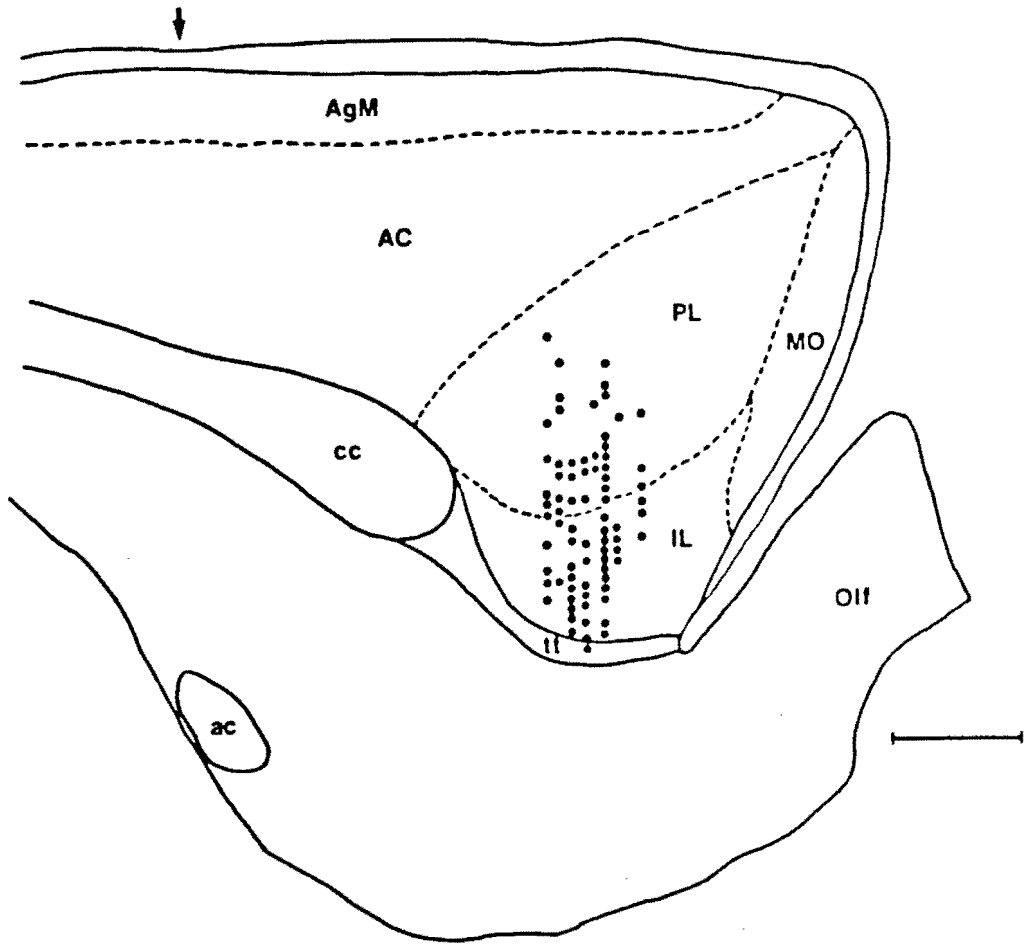
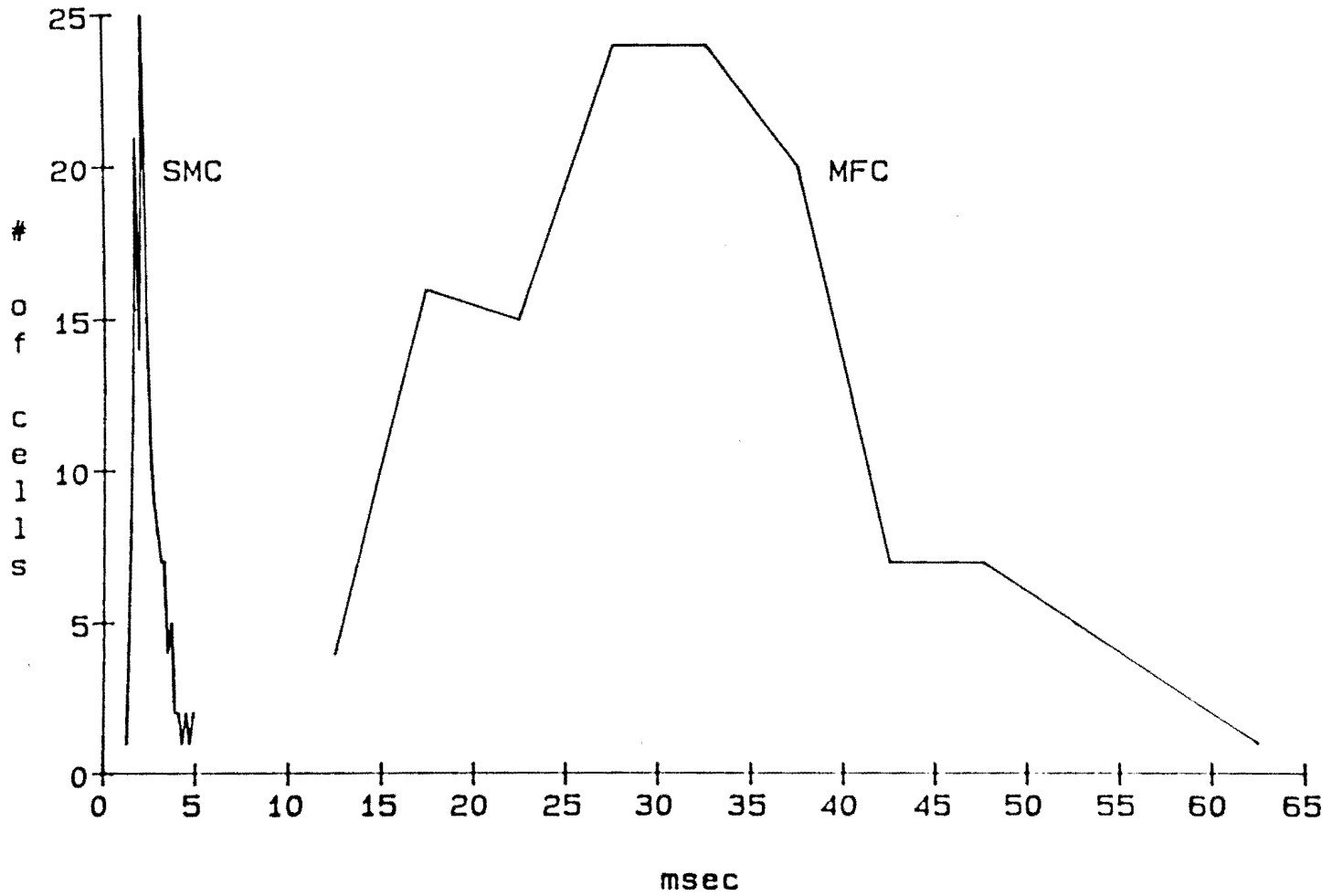


Figure 4.

Summary graph comparing the pyramidal tract neuron latencies observed by Porter and Sanderson (1964) from stimulating the medullary pyramids and recording in somatomotor cortex (SMC) and pyramidal tract neuron latencies observed in this study from stimulating the solitary nucleus and recording in the medial frontal cortex (MFC). Inflections in the line graph of SMC latencies represent the number of cells recorded in intervals of 0.2 msec. Inflections in the line graph of MFC latencies represent the number of cells recorded in intervals of 5 msec.

PTN Antidromic Latencies



CHAPTER VII

GENERAL DISCUSSION

The experiments of this dissertation have studied hippocampal influences on cardiovascular and respiratory activity and the role the medial frontal cortex plays as a relay for this function. Electrical and chemical stimulation of the hippocampus evoked decreases in heart rate, blood pressure, and respiratory frequency; ablation of the ventral medial frontal cortex attenuated or obliterated the responses. The projection from the hippocampus to the medial frontal cortex closely overlaps cell bodies in the medial frontal cortex which project to the solitary nucleus. At the electron microscopic level the hippocampal terminals did, in some cases, make synaptic contact on cells that project to the brainstem. Collision experiments showed that hippocampal input to the medial frontal cortex does "excite" neurons which project directly to the solitary nucleus. All of these findings confirm that this hippocampal projection to the medial frontal cortex is a functional pathway consistent with the concept that the limbic system functions as the "visceral brain" (MacLean, 1949).

The results of this dissertation clearly associate the hippocampus with autonomic functions, yet the hippocampal literature today focuses almost universally on the role of the hippocampus in learning and memory. The question is raised, how do visceral motor control and learning and memory interrelate? The father of classical conditioning, Ivan Pavlov, by correlating a conditioned stimulus (bell) with an unconditioned stimulus (food), ultimately conditioned animals to exhibit an autonomic or visceral conditioned response (salivation)

by the presentation of only the conditioned stimulus (Pavlov, 1927). Pavlov also described learned responses to novelty or an "orientation response" as involving somatic changes (eye movements, body movements, etc.) followed by vegetative or autonomic changes (respiration, heart rate, blood pressure, etc.) (in Hinde, 1970). Since that time, though, experiments in learning situations have focused primarily on more easily measured somatic responses without measuring respiratory or cardiovascular responses which are clearly occurring but are underappreciated (Anokhin, 1961).

An interesting dichotomy existed between the classical conditioning paradigm of Pavlov and the operant or instrumental conditioning paradigm based on learning by reward elaborated by Skinner (1938). Some suggested that classical conditioning was inferior to operant conditioning because classical conditioning was able to influence only simple, involuntary or visceral acts (e.g. salivation) whereas operant conditioning influenced overall behavior by voluntary, skeletal muscle responses. It followed then that the autonomic nervous system was in some way inferior to the voluntary central nervous system.

N.E. Miller drew the two paradigms together as manifestations of the same process rather than two distinct phenomena when he published his work describing how operant conditioning techniques could cause the learning of any visceral responses that could be learned through classical conditioning (Miller, 1969). He went on to suggest that the

autonomic nervous system is not inferior to the voluntary control nervous system, and that the ability to learn visceral as well as somatic responses had important applications to maintenance of a homeostatic environment for an organism.

Although complex, integrative functions such as learning and memory seem far removed from more specific functions such as visceral motor control, the fact that these functions are together associated with the hippocampus is not surprising given the fact that complete expression of a learned response involves autonomic as well as somatic components.

If the hippocampus is indeed involved in learning and memory and in visceral control, then what is the significance of the observed cardiac and respiratory deceleration and hypotension presumably mediated by the influence of the hippocampus on the brainstem via the medial frontal cortex? Teleologically, the lowering of heart rate, blood pressure, and respiratory rate represent a "cooling down" or relaxation process telling the animal that his environment is in order and no immediate threat exists. This type of response is consistent with the results of studies which have shown that the hippocampus is intimately associated with responses to stress. Hippocampal neurons contain the highest number of corticosterone receptors compared to all other regions of the brain (McEwen, 1982), and the hippocampus exerts an inhibitory influence over adrenocortical activity participating in glucocorticoid feedback on plasma corticosterone levels (Wilson and

Critchlow, 1973-74; Casady and Taylor, 1976; Frankel et al., 1978; Dunn and Orr, 1984; Sapolsky et al., 1984a). The number of corticosterone receptors in the hippocampus has been shown to decline in the aged rat; this observation is associated with cell loss in the hippocampus due to aging (Sapolsky et al., 1983). The decline in the number of receptors has been correlated with the observation that aged rats, in response to stress, are unable to exert negative feedback on increased plasma corticosterone levels and, hence, exhibit a prolonged corticosterone hypersecretion following the end of stress compared to young animals which return their plasma corticosterone levels to baseline shortly after the removal of stress (Sapolsky et al., 1984b) (Figure 1).

The influence the hippocampus has on cardiovascular functioning suggests that the hippocampus may also play a role in autonomic as well as hormonal responses to stress. The hippocampus functions in "turning off" the organism's response to a stressful stimulus. It may be possible, therefore, to substitute "Heart Rate" or "Blood Pressure" for "Plasma Corticosterone Levels" on the graph in Figure 1 where young subjects may be able to bring down their heart rate and blood pressure as well as their plasma corticosterone levels following the removal of stress whereas aged subjects may have a more difficult time doing so.

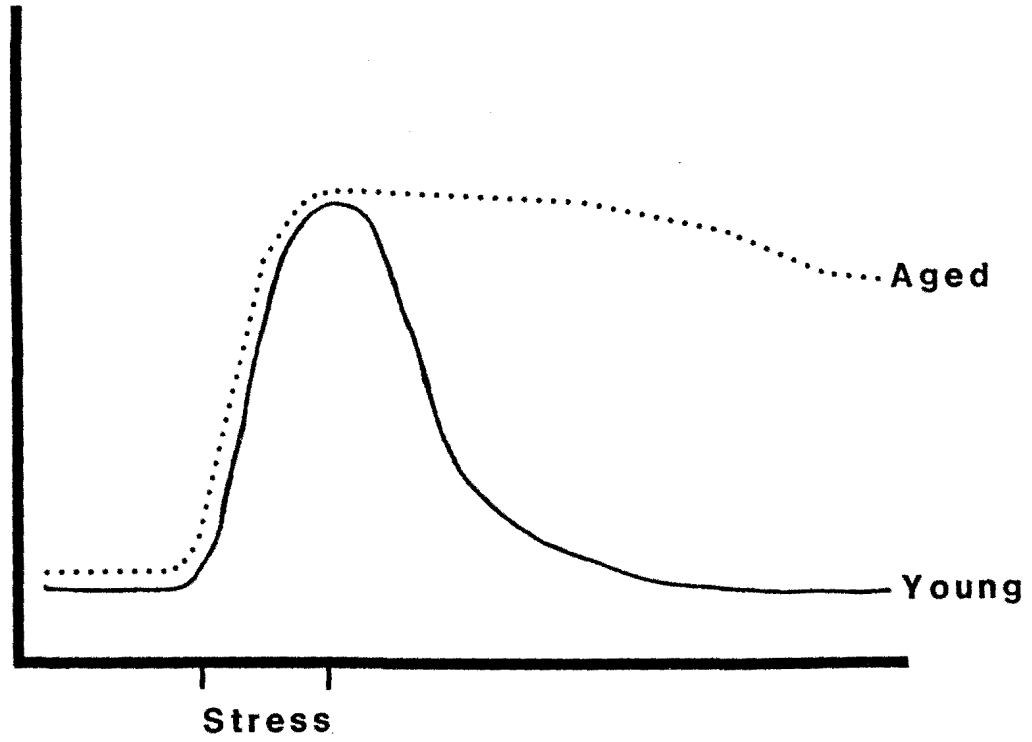
The association of a cerebral cortical structure with the ability to learn, to form new memories, or bring down heart rate or blood pressure when they are too high suggests a diversity of cortical function which can be explained by a novel approach to cortical

organization outlined by Diamond (1979). He suggests that since the pyramidal cell layer of the entire neocortex (and I venture to include the pyramidal cell layer of the allocortical hippocampus) projects to regions which ultimately effect motor (somatic or visceral) output, therefore all cortical regions can be considered "motor," not just the precentral gyrus. In addition, all regions of the cortex can be considered "associational" because of integrative cortico-cortical connections. Finally, all regions of the cortex can be considered "sensory" because of the fibers they receive, including the hippocampus, which arise in the thalamus. Such a view of telencephalic organization provides support for specific, e.g. visceral motor control, as well as global, interactive functions, e.g. learning and memory, of cortical regions such as the hippocampus and medial frontal cortex.

Figure 1.

A graph illustrating the results of the work of Sapolsky et al. (1984) in which aged animals, in response to a stressful stimulus, are unable to terminate the adrenocortical response to stress whereas young animals' plasma corticosterone levels quickly return to normal following the removal of stress.

Plasma
Corticosterone
Levels



CHAPTER VIII

SUMMARY

The experiments of this dissertation were designed to examine physiologically and anatomically the organization and function of hippocampal connections involved in cardiovascular control. The findings of this study were:

1) Electrical and chemical stimulation of the hippocampal formation of the rat evoked marked decreases in heart rate, blood pressure, and slower, more regular respirations. Artificial ventilation had no effect on the cardiovascular responses indicating these effects were not secondary to respiratory changes. Methyl atropine eliminated the bradycardia response suggesting that these responses were mediated by the vagus nerve. Ablation of the medial frontal cortex attenuated or obliterated the cardiovascular and respiratory responses to stimulation of the ventral hippocampus but not the dorsal hippocampus suggesting that the medial frontal cortex may be a relay by which the hippocampus influences cardiovascular function.

2) At the light microscopic level, terminals in the medial frontal cortex anterogradely labelled by injections of WGA-HRP in the ventral hippocampus appeared to closely overlap neurons in the medial frontal cortex retrogradely labelled by injections of WGA-HRP in the solitary nucleus. Electron microscopic analysis revealed that the hippocampal terminals make synaptic contact primarily on dendritic processes in the neuropil adjacent to the cells. In addition, labelled terminals did,

in some cases, make synaptic contact on the somas of labelled cells. These results suggest that the hippocampus is strongly linked anatomically with a region of the brain involved in cardiovascular control.

3) Electrical stimulation of the solitary nucleus antidromically activated cells in the medial frontal cortex while electrical stimulation of the ventral hippocampus orthodromically activated cells there. Collision of the antidromic and orthodromic potentials established a functional link between the hippocampus and solitary nucleus-projection neurons in the medial frontal cortex which influence cardiovascular function; this finding suggests that the limbic system is indeed the "visceral brain" (MacLean, 1949). The pathway between the medial frontal cortex and the solitary nucleus is a very slow conducting pyramidal tract pathway which may comprise a portion of the small diameter, unmyelinated fibers making up 60% of the pyramidal tract.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and the dissertation is now given final approval by the committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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