THE UNIVERSITY OF CALGARY

THE EFFECT OF SELECTED LESIONS ON TYPE I AND II THETA GENERATION IN THE GUINEA PIG

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE

DEPARTMENT OF PSYCHOLOGY

CALGARY, ALBERTA

June 16, 1984

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate studies for acceptance, a thesis entitled, "The Effect of Selected Lesions on Type I and II Theta Generation in the Guinea Pig" submitted by Christopher Peter Montoya in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

In experiment I, the relationship between type I and II hippocampal theta activity and concomitant behavior was studied in guinea pigs with forebrain lesions. All subjects were observed under behavioral conditions known to reliably elicit both types of theta activity i.e. in the presence of snakes, opposite sexed conspecifics, and the sounds of birds of prey. Both atropine sensitive and atropine resistant theta was attentuated equally by medial septal (MS), diagonal band of Broca (DBB), and ventral fornix lesions above the level of the hypothalamus. Rostral lesions of the DBB recovered amplitude loss within ten days. MS lesions and lesions of the ventral fornix close to the decussation depressed both types of theta amplitude for the duration of the experiment. Medial mammillary body, and ventral fornix lesions near the level of the mammillary bodies decreased the frequency of type I theta by one hertz (Hz) concomitant with a decrease in motor responsiveness in all behavioral conditions. Bilateral nucleus accumbens lesions had an equivocal effect on both the amplitude and the frequency of theta generation. Bilateral posterior amygdala lesions that extended to the subiculum and partial bilateral entorhinal lesions disrupted the correlation between type I behaviors and theta for up to one week subsequent to surgery. In addition, these lesions reduced the frequency of theta that occurred during movement by 1 Hz

but had no apparent effect on the frequency of immobility theta. Results from experiment I, therefore suggested that at one point i.e. the entorhinal cortex, the type I and II theta systems may be anatomically distinct.

Subsequently, experiment II examined a total bilateral entorhinal lesion effect. Following lesioning theta accompanied walking 58% of the time compared to theta accompanying walking 100% of the time before lesioning. In addition, theta occurred 34% of the time during the presentation of stimuli that had prior to lesioning, reliably elicited type II theta 100% of the time. Subsequent to entorhinal lesions, the interperitoneal administration of atropine sulphate eliminated all theta activity suggesting a cholinergic base for the remaining theta.

Therefore, this thesis argues that the entorhinal cortex is a critical pathway for atropine resistant theta. In addition, the data demonstrate that although atropine sensitive theta was intact, type II theta was not. This suggests that cholinergic ennervation is necessary but not sufficient for the normal production of type II theta. Experiment II also suggests that both the medial septum and entorhinal cortex are necessary for the normal production of both type I and II theta, and that one function of the entorhinal cortex is the fine coordination of type I and type II theta and large amplitude irregular activity (LIA) generation during concomitant behavioral events.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Robert S. Sainsbury for providing research facilities and support, and for his patience during the preparation of this thesis. The author also expresses gratitude to Dr. Brian H. Bland for his suggestions and rapport.

The author also wishes to thank the members of his committee, Dr. B. H. Bland, Dr. G. Devins, and Dr. M. Brinkerhoff for their effort and interest.

Finally, the author is especially indebted to Laura Montoya for her continued support, encouragement, and tolerance throughtout the research and compilation of this thesis.

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INTRODUCTION

Two events in the 1950's highlighted the importance of the mammallian hippocampal formation. First, Green and Arduini (1954) discovered a correlation between the arousal or orienting response of the neocortex and hippocampal RSA (rhythmical slow wave activity). These investigators also demonstrated the necessity of brain stem afferents in maintaining the integrity of hippocampal RSA. Secondly, a series of papers was published, beginning in 1955, indicating that in humans, partial bilateral hippocampal removal left patients with severe anterograde amnesia (Milner & Penfield 1955; Scoville & Milner 1957; Penfield & Milner 1958).

Linking the hippocampus with attention and memory in the same decade culminated in the publication of hundreds of papers that spanned a myriad of species including rodents, canines, felines, reptiles, marsupials, old and new world monkeys, and man.

Hippocampal investigation has used four main methods:

(1) Lesion, using electrolitic, chemical, x-irradiation, radio frequency, scalpel, or aspiration techniques.

(2) Electrophysiological, principally utilizing the electroencephalograph (EEG) and either macro-bipolar, micro, or surface electrodes in mapping or stimulation studies.

(3) Pharmacological studies trying to determine putative transmitters involved in hippocampal function mainly involving the following substances: acetylcholine (Ach), noradrenaline (NA), serotonin (5-HT), dopamine (DA), histamine (HA), and gamma-amino butyric acid (Gaba). (4) Morphological studies using various dyes, cell degeneration transport and histoflorescence techniques to examine the anatomical basis of the electrophysiology of the hippocampus.

Most investigators have used various combinations of the previously outlined procedures. There are, however, inherent problems in each area which can cause difficulty.

ANATOMY

The hippocampus is an old cortical structure called allocortex, which forms a horn shaped semicircle around the thalamus. The hippocampus lies in the telencephalic region of the forebrain and is considered part of Papez's limbic circuit. The entire hippocampal formation is divided into two parts, the superior part called hippocampus proper or ammons horn and the inferior part called the dentate gyrus. Some authors include a third area called the subiculum.

The primary cell type in the hippocampus proper is the pyramidal cell. These cells form an interlocking gyrus with the granular cells of the fascia dentata.

Lorento de No (1934) defined four zones of the hippocampus proper: the dorsal fields of CA1 distinguished by small pyramidal cells arranged in two layers and having apical and basal dendrites; the CA2 subfield, largely a transition zone, but having a different ontogenetic maturational speed; the lower field CA3, a region with larger less densely packed pyramidal cells; and the CA4 layer designated by scattered pyramidal cells within the hilus or fold of the fascia dentata.

The hippocampal formation has also been divided into a number of architectonic layers, or strata based on cell morphology (Raisman, Cowan, & Powell 1965; Altman, Brunner, & Bayer 1973). The most dorsal layer is the alveus. This fiber bundle contains the axons of pyramidal cells going towards the fimbria rostrally and the subiculum ventrally and caudally. Fimbria fibers in turn run into the fornix or septum, while the subiculum adjoins the entorhinal cortex. The next layer is the stratum oriens. This layer contains the proximal parts of the pyramidal cell axons reaching towards the alveus, the beginnings of schaffers collaterals connecting CA3 and CA1, and the basal dendrites of the pyramidal cells. Beneath this is the stratum pyramidal. This layer contains the densely packed small pyramidal cell bodies. Pyramidal cells are surrounded by interneurons, called the basket cells of Cajal. The basket cell axon gives off collaterals which terminate near other pyramidal cells in a basket like plexus. Basket cells are

not projection cells i.e. they do not send axons out of the immediate area, but do receive innervation from the medial septum and diagonal band of Broca (Daitz & Powell 1954; Lewis & Shute 1967) which is thought to be inhibitory in nature (Buzsaki, Leung, & Vanderwolf 1983).

Rostrally, a putative layer extending through CA3 and touching CA2, named the stratum lucidum (mossy fibers) is formed by the axons of dentate granular cells. Next the stratum radiatum consists of the main shafts of the apical (most superficial) dendrites of the pyramidal cells. The following layer, stratum lacunosum, is apparent in the CA1 and CA2 layers. It consists of the association and commissural fibers called schaffers collaterals that connect the CA3, CA1, and CA2 systems both proximally and interhemispherically (Buzsaki & Eidelburg 1982).

The most superficial layer of the hippocampus proper is the stratum moleculare which contains cells that receive input from the terminal dendritic aborization of the pyramidal cells and the perforant path rising from the medial entorhinal cortex (Hjorth-Simonsen 1972). In addition, an efferent plexus of mossy fibers, the stratum lucidum extends from the granular cells of the fascia dentata to CA3.

Below the hippocampus proper is the dentate gyrus (Raisman, Cowan, & Powell 1965; Altman, Brunner, & Bayer 1973). The most superficial layer is the stratum

moleculare of the dentate gyrus. It contains the peripheral (apical) dendrites of granular cells and is continuous with the stratum moleculare and radiatum of the hippocampus proper. The primary cell layer of the stratum granulosum lies immediately ventral to the stratum moleculare. Densely packed granular cells fill this layer (Izquierdo 1967). Granular cells like pyramidal cells are also surrounded by interneurons. In contrast to pyramidal cells, granular cells only have apical dendrites. Beneath this layer is the stratum polymorphe, situated in the hilum of the gyrus. There is no clear distinction between this layer and the CA1 layer of the hippocampus proper. This layer contains segments of granular cell axons as they plexus to form the mossy fiber bundle. Other cells in this layer do not have the typical pyramidal shape and send their fibers to the alveus (Ramon y Cajal 1901; Lorento de No 1933; Blackstad 1958).

Early investigators believed that the hippocampus and dentate received input from the entorhinal cortex via the alvear bundle and perforant path and discharged via the fornix to the preoptic area, lateral hypothalamus, and mammillary bodies. More recently Hjorth-Simonsen (1972) has shown the perforant path originates from two anatomically distinct areas. First, a medial perforant path coming from the medial part of the entorhinal area and terminating in the medial portion of the molecular layer of the dentate and in the deep body of the lacunosum moleculare

of the CA3 hippocampal subfield. Second, a lateral perforant path originating from the lateral part of the entorhinal cortex that projects to a superficial part of the stratum lacunosum moleculare of CA3.

Chronister and White (1975) have noted two other avenues of input to the hippocampus. The other two sources are supracallosally i.e. the cingulum and stria Lancisii. This pathway would approach the hippocampus through extreme dorsal regions, possibly the septum or the fornix-fimbria system.

All areas of the hippocampal formation have commissural connections via the ventral and dorsal hippocampal commissures. The dorsal psalterium courses above the anterior portion of the hippocampal flexure at the point where the hippocampus dips ventrally. The larger ventral psalterium passes through the fimbria and crosses to the contralateral side beneath the fimbria-fornix. Not all connections are homotopic (Buzsaki & Eidelberg 1982). Usually strata will have both association fibers ipsilaterally and commissural fibers contralaterally. For example, the CA1 layer i.e. the apical dendrites of the pyramidal cells, receive input from contralateral and ipsilateral CA3 cells as well as the contralateral CA1.

While the major input to the hippocampal formation is the perforant path, the major output pathways emanate from the CA1 and CA3 subfields of the hippocampus proper. Both

areas flow out through lateral septum and subiculum (Meibach & Siegel 1977; Swanson & Cowan 1977).

PHYSIOLOGY

Green and Arduini (1954) provided the first extensive investigation into the electrical activity of the hippocampus by demonstrating that "the theta wave (rhythmic slow wave activity or RSA) response reflected the arousal or alerting reaction of the neocortex" (p. 533). Their investigation also highlighted the importance of fornical, septal and brain stem afferents in the generation of hippocampal theta activity.

Since then other investigators have reported that RSA is correlated with voluntary movements in the rat and other species (Sainsbury 1970; Kramis, Vanderwolf & Bland 1975; Winson 1976b) and have described two anatomically distinct theta wave generators in the hippocampus (Bland, Andersen & Ganes 1975; Bland & Whishaw 1976; Winson 1976a,b). The locus of one generator is the stratum oriens layer of CA1. The CA1 generator produces RSA that is 180 degrees out of phase with RSA generated in the molecular layer of the more ventral fascia dentata. In addition to RSA the hippocampal generators produce two other basic wave forms. The delineation of the three wave forms and the behaviors with which they most frequently appear has been well documented. (Stumpf 1965; Vanderwolf 1969;

Ranck 1973; Feder & Ranck 1973; Black 1975). The wave forms are as follows: (1) Rhythmical slow wave activity (RSA) which resembles a series of sinusodal waves that range in frequency from 6-12 Hz. Behaviors accompanied by RSA in this frequency range normally include walking, rearing, swimming, jumping, running and fighting. (2) Large amplitude irregular activity (LIA) is an irregular slow wave. Behavioral immobility, licking, eating, shivering, scratching the body with a hind paw, and grooming are usually concomitant with LIA. (3) Small amplitude irregular activity (SIA) appears as a sudden reduction in the amplitude of the electrical signal and lasts for only one or two seconds. No behavioral correlate has been shown to share temporal contiguity with SIA, although it usually appears during a sudden pause in the animal's ongoing behavior (Vanderwolf 1971).

Although RSA occurs most often during ambulatory movements in species like the rat, guinea pig, and hamster, in other species like the rabbit, RSA is also generated while the animal is essentially immobile (Kramis, Vanderwolf & Bland 1975). In the rabbit this immobility theta is correlated with the processing of sensory information (Sinclair, Seto & Bland 1982). Across all investigated species, theta generated during immobility is generally of a lower frequency and amplitude than theta generated during movement. Generally speaking,

theta activity that accompanies voluntary movements has a frequency range of 6-12 Hz, while theta that accompanies immobility has a range of 4-9 Hz (Bland, Sainsbury, Seto, Sinclair & Whishaw 1981). Earlier, Whishaw and Vanderwolf (1973) demonstrated that the amplitude of RSA could increase by a factor of 6 depending on the activity e.g., from small head movements to a jump. A strenuous activity therefore increases the frequency and amplitude of RSA in a linear fashion. As a result of this dichotomy, theta that appears during observable movement is called type I theta, whereas theta that coincides with non-movement is called type II theta. Rats, in contrast to rabbits, have a lower incidence of type II theta during spontaneous motor behavior in experimental settings. In rats, for example, just before a jump there is a continuous output of theta. The theta preceeding the time of the jump is type II theta. The frequency increases sharply at the time of the jump (type I) and interestingly the frequency and amplitude then positively correlate with the distance to be jumped. (Vanderwolf 1969; Whishaw & Vanderwolf 1973; Morris, Black & O'Keefe 1978). Although guinea pigs and rabbits demonstrate type II theta before movements they also produce type II theta that does not preceed ambulatory or exploratory behavior. For example guinea pigs, with good electrode placement, reliably produce type II theta in response to owl sounds and shadows or during a species typical defence reaction e.g. freezing in response to snakes or aggressive

conspecifics (Sainsbury & Montoya 1982).

PHARMACOLOGY

RSA occurring during behavioral immobility, tonic sleep, or volatile anaesthesia (i.e. ether, urethane, or alcohol) can generally be abolished by the application of anticholinergic drugs like atropine, scopolamine, and hemicholinium. RSA which accompanies movement is resistant to cholinergic blockers, but is sensitive to anaesthetics and large doses of morphine (Vanderwolf, Kramis, & Robinson 1978; Vanderwolf & Robinson 1981). In addition, Kolb and Whishaw (1977) demonstrated that orbital frontal lesions and lateral hypothalamic lesions transiently eliminated type I theta but unhindered or even amplified atropine sensitive type II theta. Such pharmacological and lesion findings led investigators to speculate that there were two theta systems, one driven by cholinergic afferents (type II) and the other driven perhaps by trace amine inputs (type I).

Chemical blockade of the type II theta system indicates cholinergic innervation. However, it may be misleading to look only at cholinergic pathways. Pepeu, Mantovani, and Pedata (1978) demonstrated that dopamergic agonists, such as amphetamines, release acetylcholine from the neocortex. Monmaur (1981) reported that clonidine, a dopamine agonist, caused

spontaneous atropine sensitive theta to appear in both the CA1 area and dentate gyrus.

The process by which type II theta is generated may be one or more synapses removed from the acetylcholine trigger neurons. There are two major pathways originating from the neocortex that have efferents terminating in the hippocampus (Leichnetz & Astruc 1975). They are the entorhinal cortex and the medial prefrontal cortex. Vanderwolf and Leung (1983) have demonstrated that entorhinal lesions selectively abolish type I theta and CA1 theta (theta generated in the stratum oriens layer). This procedure leaves atropine sensitive theta (type II) and dentate theta (theta generated in the molecular layer) intact.

AFFERENT AND EFFERENT THETA PATHWAYS

Three main fiber bundles directly affect hippocampal theta. These are the septum and diagonal band nucleui, the fimbria fornix system, and the subiculum. Lesions to the medial posterior septum or diagonal band of Broca eliminate all hippocampal theta (Green & Arduini 1954; Sainsbury & Bland 1981). Lesions to the lateral septum attentuate CA1 theta while leaving dentate theta untouched suggesting that the fibers that mediate CA1 and dentate are at one point anatomically distinct (Sainsbury & Bland 1981).

Rostral basal medial fimbria lesions disrupt all hippocampal theta (Andersen, Bland, Myhrer & Schwartzkroin 1979). More dorsal fimbria lesions disrupt ventral hippocampal theta while dorsal fornix lesions disrupt dorsal hippocampal theta. This leads one to adopt a dorsal to ventral organizational picture of the hippocampal formation (Rawlins, Feldon & Gray 1979) with a bottle neck afferent pathway at the septal fimbrial juction.

Lesions to the subiculum usually occur in conjunction with massive entorhinal cortical lesions. In the past, however, much debate has been raised as to the exact nature of the effect observed. Carreras et al (1955) reported that bilateral entorhinal lesions eliminated hippocampal RSA in curarized cats. A year later Adey et al (1956) demonstrated clear RSA following bilateral aspiration lesions to the entorhinal cortex of marsupials. Eighteen years later Chronister et al (1974) reported that increases in RSA were seen following lateral entorhinal cortex ablations in rats, but that subicular lesions caused over all depression of hippocampal RSA. Recently Vanderwolf and Leung (1983), working with rats, reported that entorhinal lesions eliminated all CA1 theta production and left the dentate generator with atropine sensitive type II theta intact.

SPECIES DIFFERENCES

There is a marked difference in the amount of type II theta produced by different mammals, especially in the restricted laboratory environment. Rats rarely produce type II theta in these settings, doing so only immediately prior to a jump or when faced with unavoidable foot shock (Whishaw 1972). Cats produce RSA which is correlated with arousal and REM sleep, but not with ambulatory activities (Sainsbury 1983, personal communication). Rabbits can sit motionless for extended periods of time while long trains of type II theta are elicited by a variety of stimuli (Sinclair, Seto & Bland 1982). Guinea pigs produce more type II theta than rats in the restricted experimental setting, yet display shorter runs of type II theta than rabbits. In addition, type II theta appearing in the guinea pig can be predicted from behavior. Freezing, a species typical defence reaction, results in a short burst of type II theta sandwiched between two segments of type I theta. Guinea pigs can also produce clear type II theta that neither preceeds nor follows type I theta, e.g. during the presentation of owl sounds.

Until recently most experiments examining hippocampal theta, in chronic preparations, have examined type I theta. Therefore the functional relationship between the two systems remains tentative. In studies that have examined

the two types of theta, type I and II theta have been delineated in three ways. First, and most important type I theta occurs during so called voluntary ambulatory activies such as swimming or walking, whereas type II theta occurs during immobility. Second, pharmacological manipulations suggest that cholinergic inputs are necessary for type II theta production, while no putative transmitter for type I theta has been found. Third, the mean frequencies of the two types of theta are different. Attempts have been made to further substantiate this position at the cellular level. Bland et al (1983) have demonstrated that at identical theta frequencies cell firing rates during type I theta are significantly higher than cell firing rates during type II theta.

Interestingly all three pieces of evidence that delineate the two types of theta have recently come under review. Vanderwolf and Leung (1983) suggest that bilateral entorhinal lesions in rats eliminate the CA1 generator and type I theta. These rats produced small amounts of atropine sensitive type II dentate theta while engaged in ambulatory activities. In addition the authors reported that the frequency of the type II theta remaining after entorhinal lesions does not vary significantly from the type I theta frequency observed during ambulatory activities prior to surgery. The entorhinal cortex, they argued, appears to be the hippocampal input for type I theta thus leaving the medial septum as the input for the

cholinergic type II theta pathway. Secondly, Sainsbury and Montoya (1984) argued that although the atropine effect, which eliminates type II theta, appears to be a strong drug effect, the site of action remains unclear. Atropine sulphate that eliminated type II theta in guinea pigs eliminated behaviors that are concomitant and perhaps necessary for type II theta production.

The purpose of the present study was to examine the effects of discrete lesions on the production of type I and II theta. The animal chosen was the guinea pig because both types of theta can be reliably elicited in the experimental setting (Sainsbury & Montoya 1984). To date, no RSA lesion data has been collected from the guinea pig . Therefore the first experiment attempted to parallel earlier electrophysiological lesion work in other species, specifically medial septal, diagonal band of Broca, and fornix lesions. These lesions in other species disrupt all theta and LIA activity recorded in the hippocampus.

In addition the first experiment also examined more controversial or unexplored lesion sites. The entorhinal cortex was the primary target for lesioning for two reasons. First, in the past, controversy surrounded the effects of entorhinal lesions, with some experimenters showing no effect, some showing total theta disruption and others reporting only type I theta disruption. Secondly, entorhinal lesions affect arousal in animals (Adey, Merrillees & Sunderland 1956). Arousal has been shown to

play a role in the production of type II theta (Sainsbury & Montoya 1984). In addition to entorhinal cortex lesions some animals received bilateral amygdala or medial prefrontal cortex lesions as these types of lesions also lower the arousal levels of experimental animals.

As septal-diagonal band and fimbria-fornix lesions disrupt all theta, two additional lesion conditions were added to experiment I. The two structures selected were the mammillary bodies located at the ventral continuation of the fornix and the nucleus accumbens, the rostral most continuation of the septum. These two members of Papez's Circuit, previously ignored in theta literature, are respectively involved in memory and place preference (Jahro, 1973; Spyraki et al 1982).

To monitor changes in arousal and activity levels, prior to and following surgery, all animals were observed in experimental settings that reliably elicited type I and II theta and that varied the arousal levels of the animals i.e. in the presence of snakes, birds of prey sounds, and opposite sexed conspecifics (Sainsbury & Montoya 1984). This procedure was implemented so that over all hyperactivity or depression of normal responses could be taken into account when interpreting EEG frequency and amplitude changes subsequent to lesioning.

The second experiment involved a closer examination of the effects of bilateral entorhinal lesions. The second

experiment increased the size of the bilateral entorhinal lesions. In addition, as atropine sulphate has proven effective in eliminating type II theta, the I.P. administration of atropine was added as an experimental condition.

EXPERIMENT I

METHOD

Subjects and Surgery

Twenty-three male and nineteen female mixed strain guinea pigs weighing between 600 and 900 grams at the time of surgery were used. Animals were housed in group pens of eight animals each. The pens measured 90 x 90 x 38 cm high. Commercial bedding covered the floor to a depth of 7 cm. Food and water containing vitamin C was available ad libitum.

Animals were anaesthetized with sodium pentobarbital (25 mg/kg I.P.) and topically with procaine. Movable recording electrodes, (Sainsbury et al 1983) and/or reference recording electrodes were bilaterally implanted. All recording electrodes were aimed at the dorsal or ventral dentate nucleus of the hippocampus and were implanted using standard stereotaxic techniques. No more than one electrode per animal was aimed at the ventral hippocampus. Coordinates for dorsal hippocampal recording electrodes were 5.5 mm posterior and 3.0 mm lateral to bregma and 3.7 mm

ventral from the dura. Coordinates for the ventral hippocampus were 5.5 mm posterior and 6.25 mm lateral to bregma and 11.0 mm ventral from the dura. Placements were made with lambda and bregma on the same horizontal plane. In addition, a ground electrode consisting of a male winchester subminiature connector soldered to a jewelers screw was fixed to the skull.

Electrical Recording Apparatus

Following a seven to ten day recovery period, movable electrodes were positioned to record the largest amplitude theta signal. Moveable electrodes were then cemented into position with dental acrylic. Control recordings were then taken from all electrodes. Animals were tested in a shielded observation box measuring 60 x 108 x 28 cm high while in the presence of snakes or opposite sexed conspecifics. Behaviors such as walking, grooming, and immobility were coded and recorded on the EEG chart paper concomitant with ongoing hippocampal electrical activity. Signals from the animals were passed through a nine pin commutator suspended 55 cm above the center of the box. The commutator was connected to a Grass Model 7D polygraph. The polygraph was set with the low range 1/2 amplitude filter at 1 Hz and the high range 1/2 amplitude filter at 35 Hz. Two sony video cameras, model AVC - 3260s/3260DX, were employed simultaneously. One camera

recorded from the behavioral box, the other recorded the pens of the polygraph. The two signals were merged via a Viscount model 1107 video production switcher. The end result was a split screen image of behavior and the brains' simultaneous electrical activity. Measurements of the frequency and amplitude of EEG activity were made using a clear plastic ruler on the polygraph records or by analyzing the recorded signal on a Cromemco System Three computer utilizing a Fast Fourier program.

An IAC 11242 isolation lab served as the second observation box to present birds of prey sounds to all subjects. The IAC observation space measured 40 cm x 40 cm x 35 cm high. A false unpainted plywood wall in the isolation lab housed an 11 cm 8 ohm 31S413 speaker connected to a Sony TC-399 stereo tape deck and a Sony TA-F35 integrated stereo amplifier. The amplifier was adjusted to the 2.5 volume setting. Birds of prey sounds produced by the stereo system were between 70-80 db in the observation box. Recording tecniques were identical to the first observation box except a direct line rather than a commutator connected the animals to the EEG.

PROCEDURE

Prelesion Testing

After a one half hour habituation session in the appropriate test box, all animals were presented with the

following stimuli on seperate sessions: one or more adult garter snakes; two opposite sexed conspecifics; the sounds of birds of prey; a 5 cm square of red tape waved on either side of the guinea pigs' head while the animal remained motionless for 5 seconds; a light stroke of a metal prod moving in a caudal direction along the animals flank while the animal remained motionless for 5 seconds (Whishaw and Dyck in press); and a push with a metal prod that caused the animal to walk across the length of the observation box.

Lesions

Following prelesion testing, subjects were randomly assigned to one of nine conditions. The first group received medial septal lesions, the second group received diagonal band of Broca lesions, the third group received ventral fornix lesions, the fourth group received bilateral entorhinal lesions, the fifth group received medial prefrontal cortex lesions, the sixth group received bilateral amygdala lesions, the seventh group received mammillary body lesions, the eighth group received bilateral nucleus accumbens lesions, and the nineth group was a control group. Electrolytic lesion electrodes were Plastic Products bipolar commercial MS303 electrodes. Four entorhinal and two medial prefrontal cortex lesions were aspiration lesions. In the aspiration groups large amounts of overlying skull had to be removed to gain access to the underlying structures. The dura was then parted and

the tissue removed by mild suction (5-10 psi). Bleeding was inhibited by periodic application of isotonic saline during aspiration.

The medial septal group (N=3) received one lesion (coordinates anterior bregma .5 mm on the midline, ventral from the dura 6 mm). Lesions were cathodal 3.0 ma for 15 sec.

The diagonal band of Broca group (N=2) received one lesion (coordinates anterior bregma .75 mm on the midline, ventral from the dura 8 mm). The electrode was moved 10 mm lateral from the midline and brought in at a 40 degree angle to avoid damaging the more dorsal medial septum. Lesions were cathodal 3.0 ma for 15 seconds.

The fornix group (N=4) received either a unilateral (N=2) or bilateral (N=2) lesion (coordinates posterior bregma 1.5 mm , lateral 1.25 mm from the midline, ventral from the dura 9 mm). The electrode was moved 10 mm lateral from 1.25 lateral and brought in at a 44 degree angle to avoid damaging the more dorsal fimbria-fornix structure. Lesions were cathodal 3.0 ma for 15 seconds.

The entorhinal cortex group (N=4) received bilateral entorhinal lesions whose center point was the most lateral and ventral aspect of the neocortex. One animal received bilateral electrolytic lesions (coordinates posterior to bregma three electrodes per side) 3 mm, 5 mm , 7 mm,

lateral to midline 7 mm, 7 mm, and 6 mm, respectively and ventral from dura 13 mm, 12 mm, and 10 mm, respectively. Two animals received bilateral aspiration lesions that had a midpoint 5.0 mm posterior to bregma and that extended approximately 3 mm anterior and posterior from the midpoint along the ventral surface of the cortex. One animal received one aspiration lesion and a contralateral electrolytic lesion, coordinates and methods as described above.

The medial prefrontal cortex group (N=2) received one aspiration lesion that extended anterior from bregma 8 mm along the midline. Laterally the lesion extended 3 mm on either side of the midline. Ventrally the lesion extended to the white matter of the corpus callosum.

The amygdala group (N=5) received bilateral amygdala lesions in the most posterior aspects of the nuclei (coordinates posterior bregma 2.5 mm, lateral from the midline 4.25 mm, and ventral from the dura 8.5 mm). The electrode was moved 8.5 mm lateral from 4.25 mm lateral and brought in at a 40 degree angle to avoid damage to more dorsal structures. Lesions were cathodal 3.0 ma for 15 seconds.

The mammillary group (N=8) received one lesion (coordinates posterior bregma 3.5 mm on the midline, ventral 12 mm from the dura). The electrode was moved 10 mm lateral to the midline and brought in at a 40 degree angle to avoid damaging the rostral hippocampus. Lesions

were cathodal 3.0 ma for 15 seconds.

The nucleus accumbens group (N=5) received bilateral lesions (coordinates anterior bregma 3.25 mm, lateral 1.75 mm from the midline, ventral 7.75 mm from the dura). Lesions were cathodal 3.5 ma for 15 seconds.

The control group (N=9) received no lesions but were anaesthetized with 25 mg/kg of sodium pentobarbital and put into the stereotaxic holder.

Post Lesion Testing and Histolgy

The entire testing procedure was repeated starting 24 hours post lesion and then once a week for one month. After one month all subjects were deeply anaesthetized with sodium pentobarital (75 mg/kg I.P.) and perfused with isotonic saline and 10% formalin solution. After three days the brains were transferred to a sugar and formalin solution. The brains were sectioned using the frozen technique at 50 microns and stained using cresylecht violett.

RESULTS

Histological Results

Histological results are reported here for the medial septal, diagonal band, ventral fornix, nucleus accumbens, mammillary bodies, bilateral amygdala, medial prefrontal

cortex, and entorhinal cortex lesioned animals that served as subjects in experiment I. All subjects exhibited good theta records in at least one recording electrode before lesioning. Five animals had operational dorsal and ventral hippocampal recording electrodes.

Medial Septum

No medial septal lesion destroyed the entire medial septum. No lesion extended rostrally enough to discolor the nucleus accumbens, but ventrally the lesions extended well into the diagonal band of Broca. The posterior extent of the lesions never extended far enough to touch the descending columns of the fornix, but dorsally the more medial aspects of the lateral septum were involved (fig. 1).

Diagonal Band of Broca

Due to the angled trajectory, lesions to the diagonal band of Broca also included unilateral damage to the overlying neocortex, caudate and putamen. Laterally the damage included the anterior commissure. Ventrally and caudally the lesion extended to the optic chiasm. Dorsally the lesion included the ventral parts of the medial septum in one animal. Rostrally the lesions were restricted to the diagonal band in all animals (fig. 1).

Ventral Fornix

Due to the angled trajectory, damage to the surrounding tissue included bilateral or unilateral damage

to the neocortex, corpus callosum, caudate-putamen nucleus, globus pallidus, internal capsula, entopeduncular nucleus, medial forebrain bundle, zona incerta, lateral hypothalamic area, and dorsal medial hypothalamic area (fig. 1).

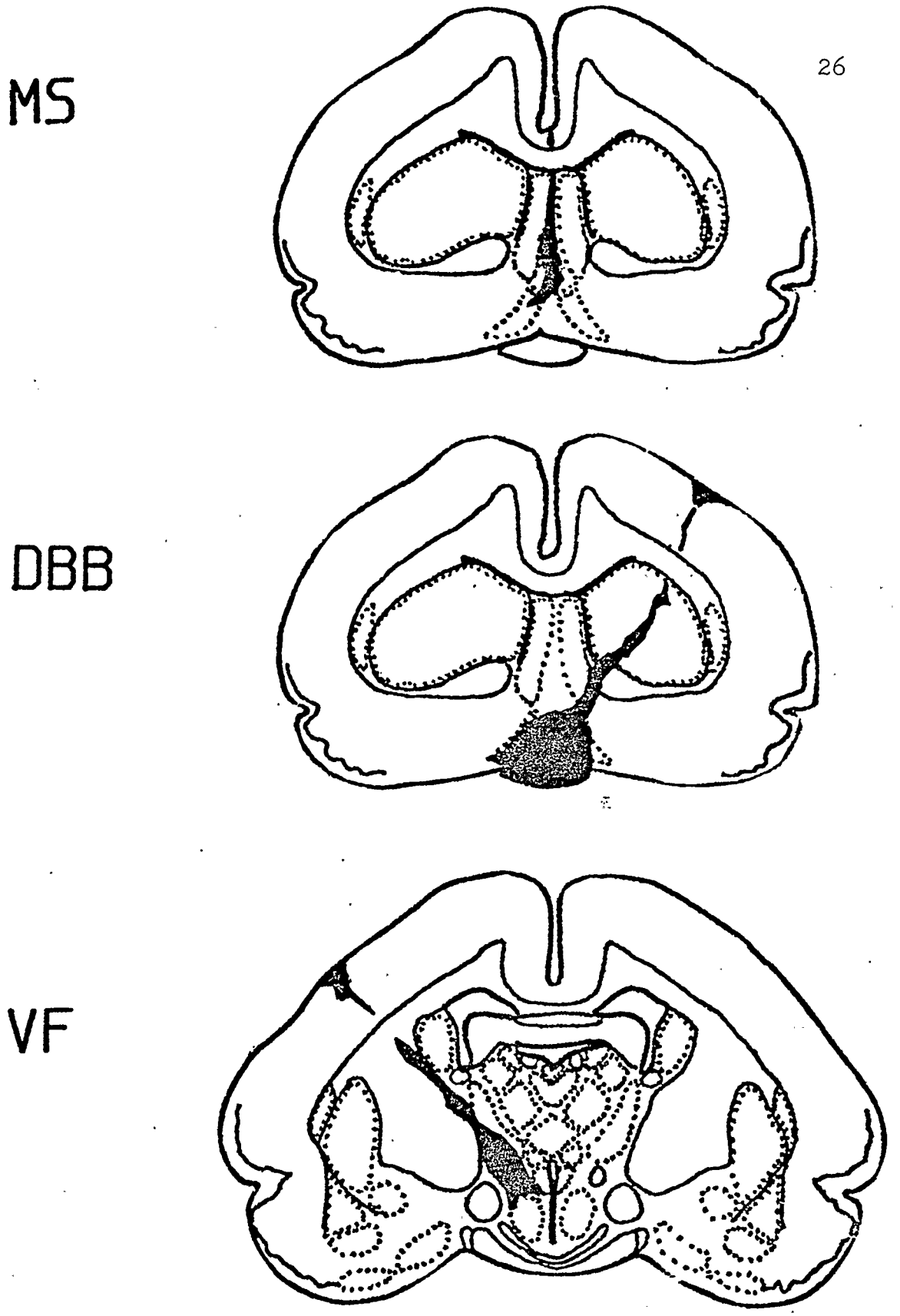


Figure 1. Schematic sections from guinea pigs 9, 38, and 53 of the medial septal (MS), diagonal band of Broca (DBB), and ventral fornix (VF) groups respectively, illustrating the midpoint of the electrolytic lesions.

Nucleus Accumbens

Generally lesions to the nucleus accumbens group included bilateral damage to the overlying neocortex, corpus callosum, caudate-putamen nucleus, and ventricle. Ventrally in two animals the lesion included unilateral damage to the anterior medial forebrain bundle, optic tract and rostral portions of the diagonal band of Broca (fig. 2).

Mammillary Bodies

Generally lesions to the mammillary bodies included unilateral damage to the neocortex, corpus callosum, medial lemniscus, crus cerebri, internal capsula, zona inserta, mammilothalamic fasciculus, and ventral fornix (fig. 2).

Amygdala

Generally the angled lesions to the amygdala included bilateral damage to the following areas: overlying neocortical areas, corpus callosum, lateral parts of the claustrum, optic tract and internal capsula. Caudally and ventrally the lesion in two animals extended to the ventral hippocampus, subiculums and entorhinal cortex (fig. 2).

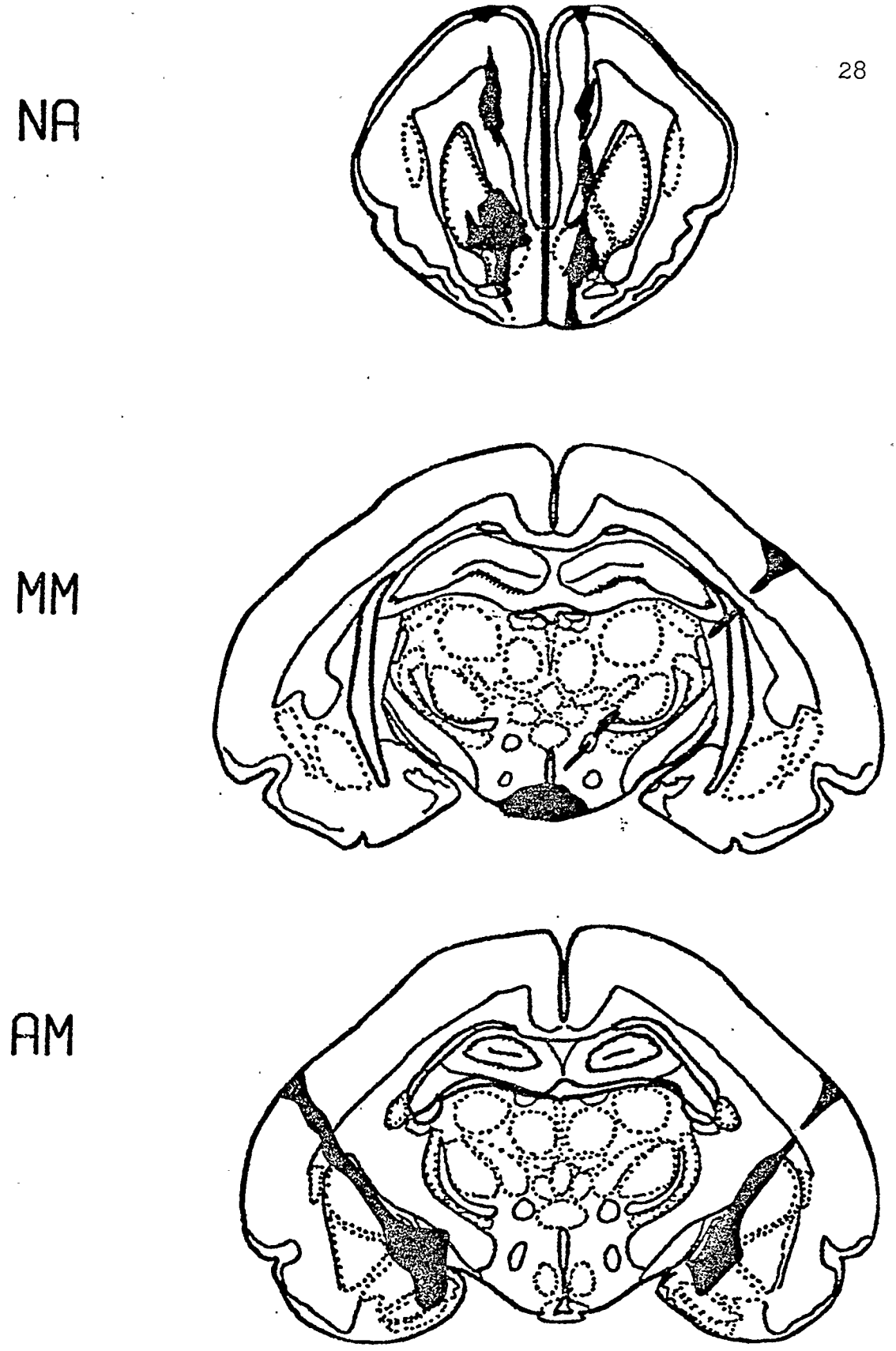


Figure 2. Schematic sections from guinea pigs 39, 41, and 63, of the nucleus accumbens (NA), mammillary body (MM), and amygdala (AM) groups respectively, illustrating the midpoint of the electrolytic lesions.

Medial Prefrontal Cortex

Aspiration lesions to the medial prefrontal cortex extended 8 mm anterior from bregma and 3 mm lateral from the midline bilaterally at time of surgery. Histological results demonstrated a complete reshaping of the forebrain. At the time of initial lesion the depth of the lesion extended to, but not into, the white matter of the corpus callosum. At the time of sacrifice the ventral surface of the lesion appeared to be at the dorsal surface of the brain (fig 3).

Entorhinal Cortex

Generally, lesions to the entorhinal cortex included partial bilateral damage to the following areas: neocortex, subiculum, para, pre, and pro subiculum, ventral dentate, piriform cortex, and corticoamygdaloid nucleus. The lesion extended dorsally to the rhinal fissure and medially to the amygdaloid fissure (fig. 4).

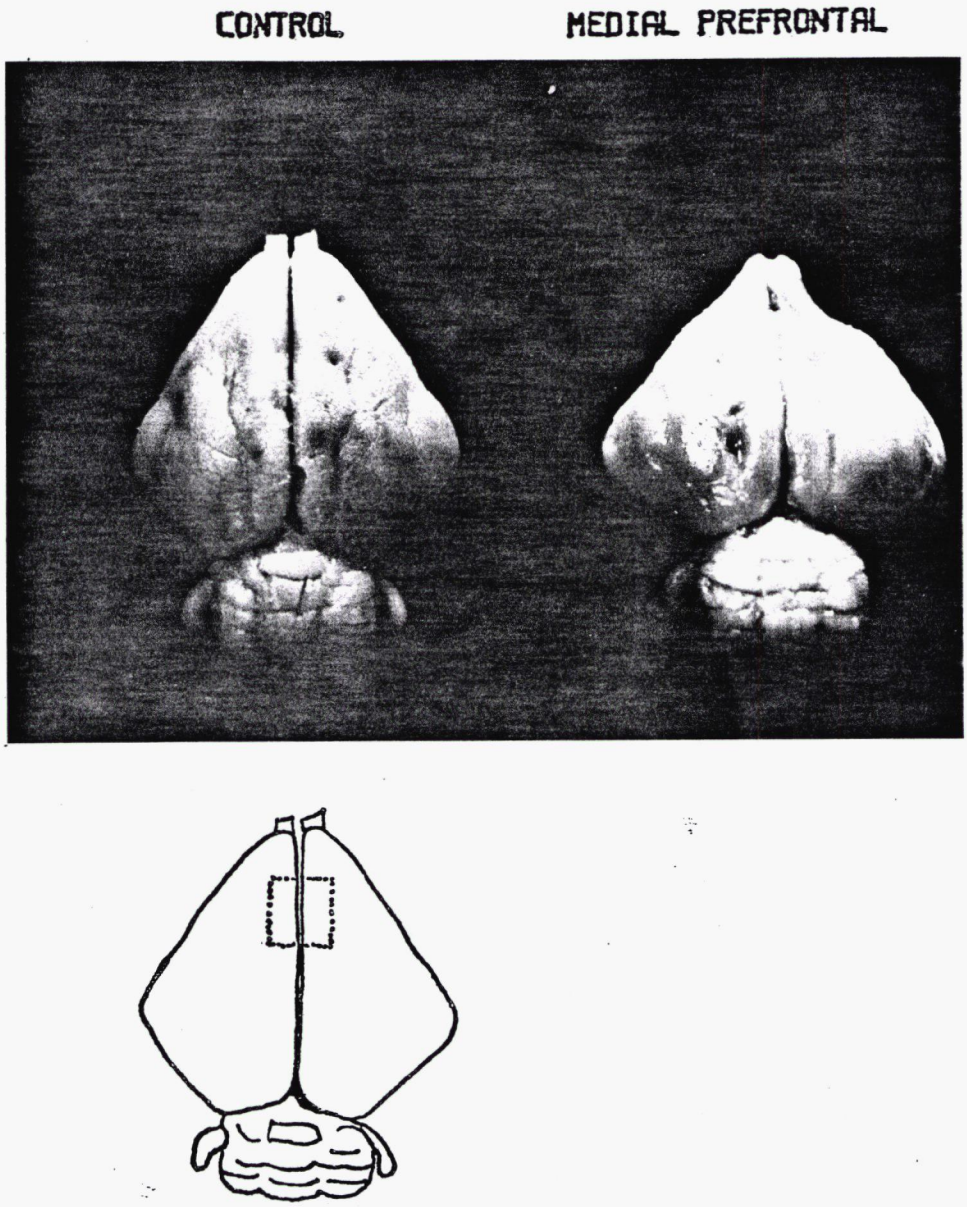


Figure 3. Photographs illustrating dorsal neocortical morphological changes subsequent to medial prefrontal lesion. Dotted line on schematic control brain illustrates the original extent of lesion in medial prefrontal brain.

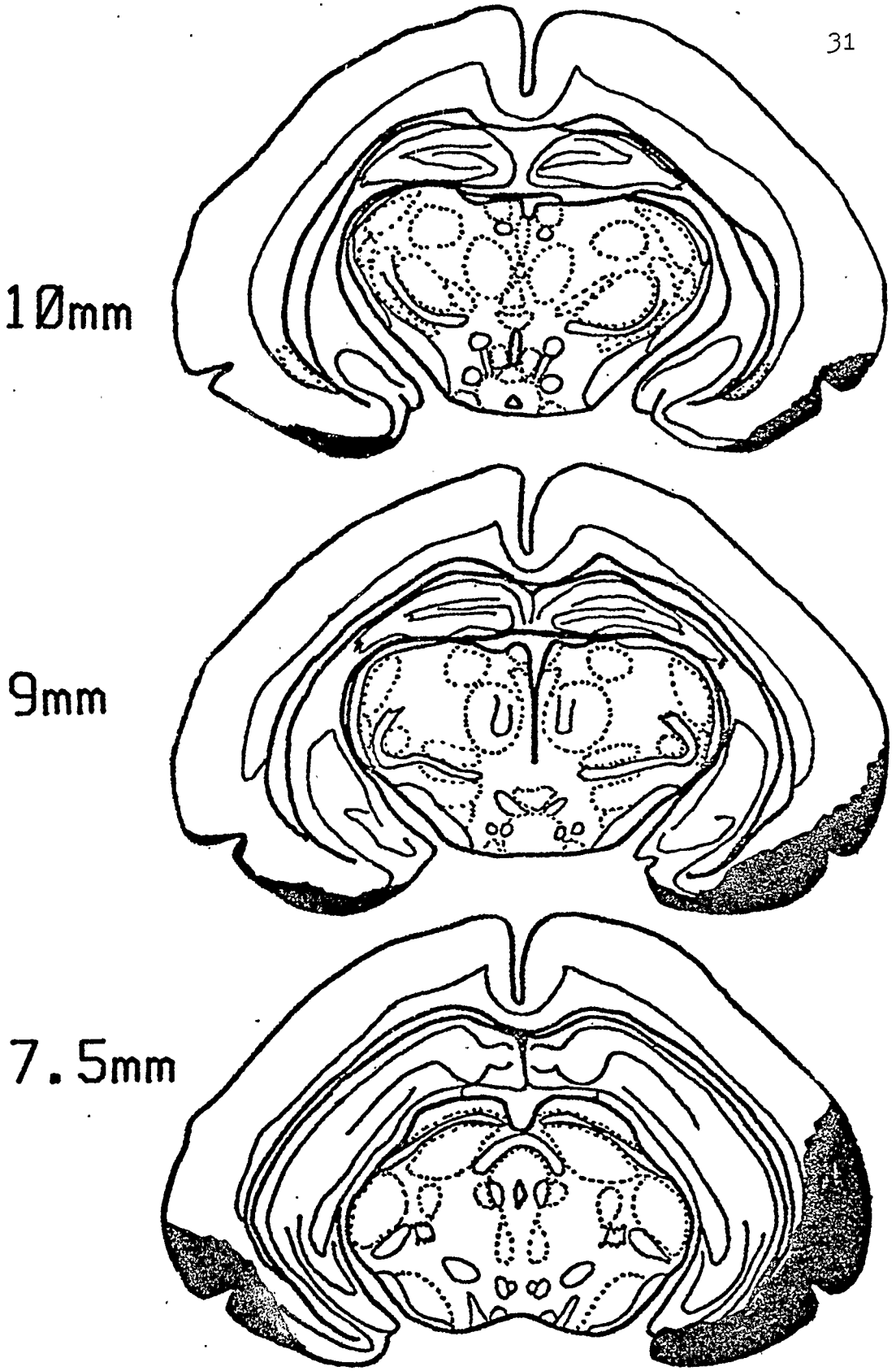


Figure 4. A schematic series of sections through the entorhinal cortex which demonstrates the extent of bilateral aspiration damage. Note not all of the entorhinal cortex was eliminated in this animal.

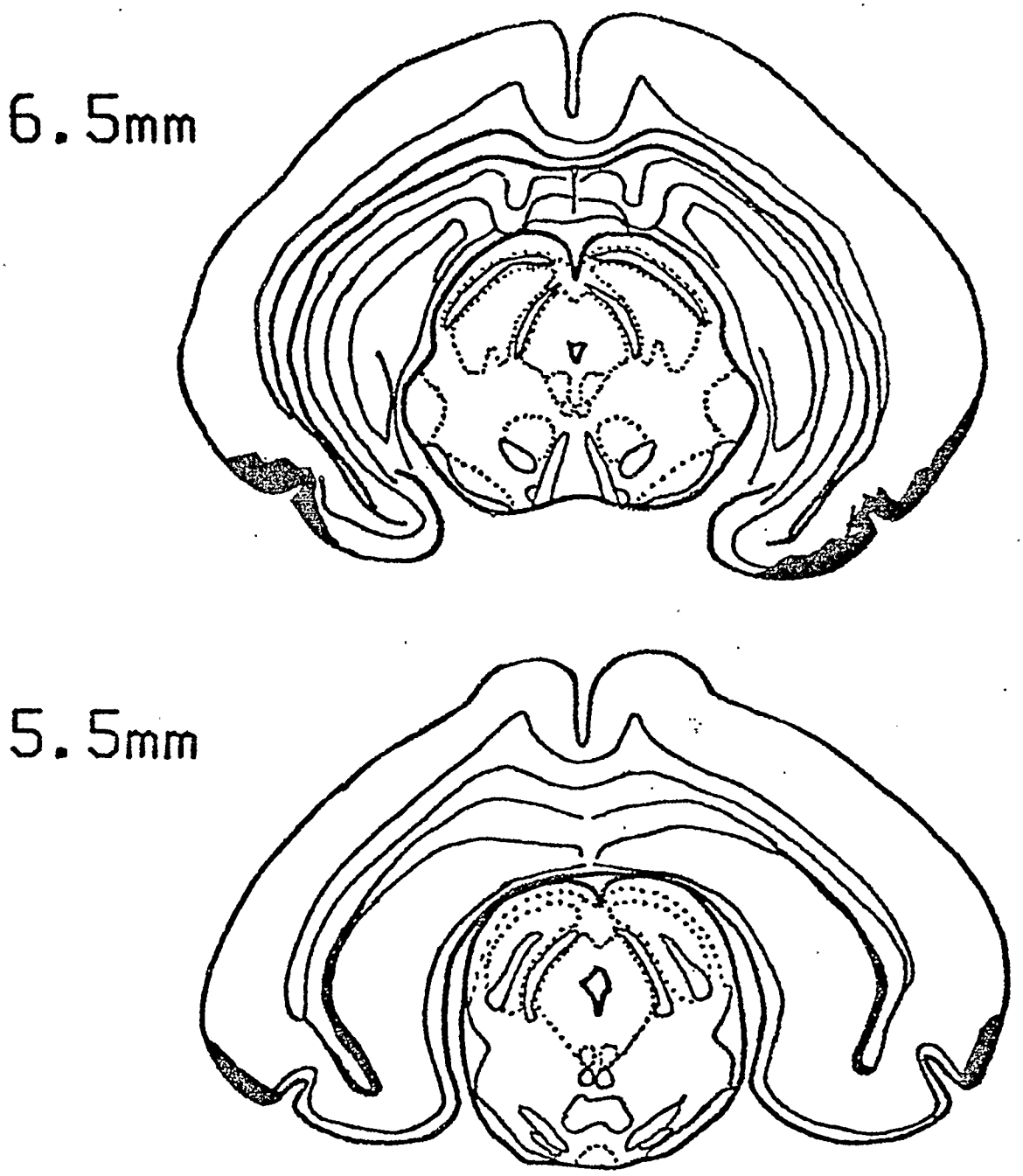


Figure 4. (continued)

Electrophysiological Results and Informal Behavioral Observations

Medial Septum

Lesions to the medial septum attenuated the amplitude of the EEG signal by 83% for the duration of the experiment (fig. 5). Although in some cases the amplitude of electrical activity in the generator zones appeared completely depressed, a Fast Fourier Program analysis found that during type I behaviors an average frequency of 8 Hz was present. In addition, although not as clear, during type II theta activities, an average frequency of 7 Hz was present. Behaviorally animals in this condition showed less arousal to the snake and owl sounds i.e. groomed in the presence of both for over 30 seconds. In the presence of the snake the guinea pigs would engage in excessive chewing behavior e.g., the guinea pigs would first nibble at the snake then perhaps groom, then nibble at the wood wall, then on some bedding then back to the snake with no apparent differentiation between the three.

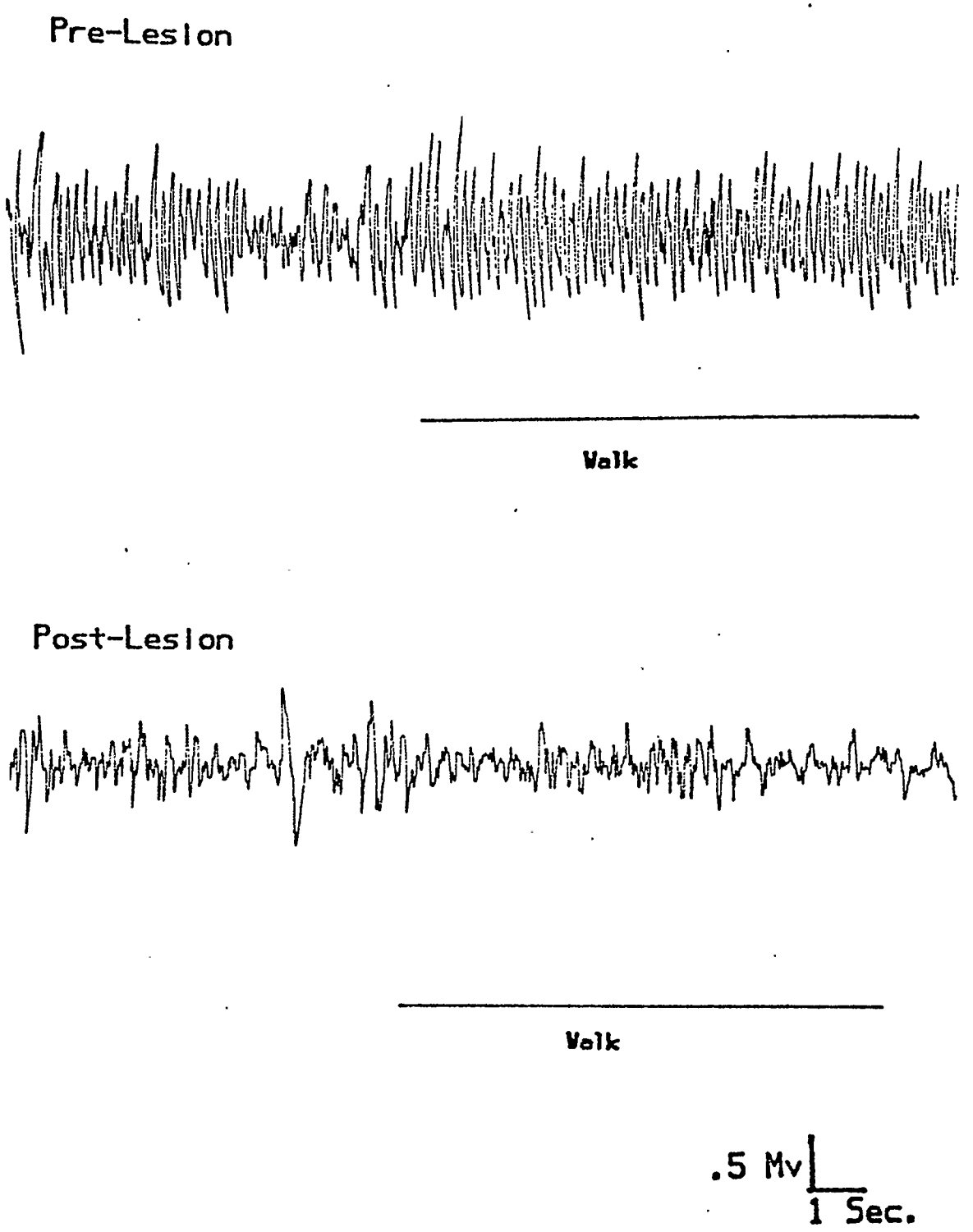


Figure 5. Pre- and post-lesion recordings from guinea pig 9 of the medial septal (MS) lesion group. Note the attenuation of amplitude subsequent to lesioning.

Diagonal Band of Broca

Lesions to the diagonal band of Broca attenuated the EEG amplitude by 54% for up to ten days. After ten days the amplitude increased until it reached prelesion levels. Although the amplitude of electrical activity in the generator zones appeared depressed a Fast Fourier Program analysis found that during type I behaviors an average frequency of 8 Hz was present and during type II behaviors, although not as clear, an average frequency of 7 Hz was present. Across all three behavioral conditions no discernible behavior change occurred subsequent to the lesions.

Ventral Fornix

Lesions to the ventral fornix caused attenuation of the EEG amplitude in both dorsal and ventral hippocampal generator sites by up to 75%. With unilateral lesions only the ipsilateral side was affected. As in medial septal and diagonal band animals, the frequencies of type I and II theta were unchanged even when the record appeared depressed. Subsequent to ventral fornix lesions above the level of the hypothalamus animals behaved in a manner similar to medial septal animals. Ventral fornix lesions at or slightly above the level of the mammillary bodies had no discernable affect on behavior, other than to produce an increase in chewing behavior.

Nucleus Accumbens

Lesions to the nucleus accumbens had an equivocal effect on theta activity. In two animals an increase in the amplitude of the theta signal was observed concomitant with an increase in the reactivity to the three behavioral situations i.e. animals would not stand still during tactile tests. In addition the animals seemed over reponsive to the snake and owl stimuli but responded normally to sexual stimuli. This effect was transient and was no longer measurable after 5 days. In two other animals whose lesions extended to the anterior tip of the diagonal band of Broca, transient attenuation of the theta and LIA signals was observed. This attenuation disappeared over a one month period. The attenuation of the theta signal had no apparent effect on the reactivity of the animals to the three behavioral situations. The remaining animal showed no effect after lesioning.

Mammillary Bodies

Subsequent to mammillary body lesions the mean frequency of both type I and II theta was 7 Hz (30 second samples). No other effect was apparent. However, these lesions included the ventral fornix and indicated that ventral fornix lesions at or around the level of the mammilliary bodies had no other effect on theta activity. Following mammillary body lesions the animals appeared sluggish. Two of the eight animals in this group died

within two days of lesioning. Behaviorally the animals reacted normally other than demonstrating a proclivity for chewing whether or not there was anything in the mouth.

Amygdala

Lesions to the posterior central nucleus of the amygdala had no apparent influence on theta activity unless the lesion extended into the entorhinal cortex and specifically the subicular fiber paths that project into the hippocampal formation. When subicular fibers were discolored during amygdala ablation the theta recorded by the chart pens no longer correlated with type I movements. The frequency of type I and II theta in these animals was 6.5 Hz as averaged over a 30 second sample of each type. Two of the four animals in this group died within 24 hours of surgery. Subsequent to lesioning the animals appeared very passive i.e. they could be handled very easily and had no rigidity as did normal animals. In addition they showed no reaction to any of the three behavioral situations following surgery, other than to take one or two steps when proded or displaced.

Medial Prefrontal Cortex

Lesions to the medial prefrontal cortex of guinea pigs had no effect on theta or LIA activity for periods of up to 10 months. Immediately following surgery the animals appeared to be very passive. For example, they would not respond to vigorous and loud hand claps i.e.

startle/orienting response, but did respond normally to presence of females, birds of prey sounds and snakes.

Entorhinal Cortex

Bilateral lesions to the entorhinal cortex initially reduced the amplitude of the theta signal. In addition, the EEG theta record no longer had a high correlation with type I movements during the first week following surgery. For example, although theta was more likely to occur during type I movements there were occassional omissions, where the animal would walk and there would be no theta. In addition, during a walk, stop, walk behavior there might be continuous theta on the EEG record. During this period, both type I and II theta had an average frequency of 7 Hz over a 30 second sample of each type. Two of the four animals in this condition died within 24 hours of surgery.

Control Animals

There was no systematic effect on behaviors, theta or LIA activity in the control animals upon the second presentation of the various stimuli in the experimental settings. All animals had a similar amplitude theta within subjects across sessions and similar frequency between subjects across sessions i.e. type I theta frequency was 8 Hz and type II theta frequency was 7 Hz.

DISCUSSION

The results indicate that no lesion was sufficient to selectively eliminate type II independently of type I theta. Medial septal, diagonal band of Broca, and ventral fornix lesions (around the level of the medial septum), however, attenuated the amplitude of all theta activity. This is in agreement with the findings in other species (Green & Arduini 1954; Andersen, Bland, Myhrer & Schwartkroin 1979; Rawlins, Feldon & Gray 1979; Sainsbury & Bland 1981).

Rawlins et al (1979) looked at the effect of medial dorsal fornix lesions on theta activity and found that these types of lesions disrupted dorsal but not ventral theta. The present findings demonstrate that at a more ventral level the fornix lesions affect both dorsal and ventral hippocampal theta. This suggests that there may be a reorganization of the fornix at the level of the septum.

Rawlins et al (1979), found a qualitative correspondence between AChe staining loss and theta loss in the hippocampus. At the flexure recording site, however both fimbria and fornix lesions depleted AChe but only the fornix lesion abolished theta activity. Thus these authors concluded that the septal cholinergic control of hippocampal theta was not conclusive. Fimbria lesions did deplete AChe in the ventral hippocampus concomitant with depression of

theta records. As the present findings indicate that ventral fornix lesions attenuate both ventral and dorsal hippocampal theta, the data suggests that some ventral fornical fibers may cross over to enter the fimbria near the level of the septum.

Subsequent to medial septal and ventral fornix lesions but not diagonal band of Broca lesions, behaviors that normally accompanied type I and type II theta wave activity were absent. For example, guinea pigs in the medial septal and ventral fornix conditions groomed and nibbled at bedding in the presence of the snake without showing classic arousal behaviors demonstrated by control animals (Sainsbury & Montoya 1984). Yet this is not to say that theta is necessary for these behaviors as diagonal band lesion animals (that also attenuate both theta and LIA activity) still demonstrated normal behaviors in the three behavioral conditions. What this data does imply is that a fiber path necessary for certain types of defensive responses runs through the fornix and septum but does not have a critical circuit through the diagonal band nuclei. As the diagonal band nuclei are one source of acetylcholine to the hippocampus and the medial septum the other source (Fibiger 1982), one might infer that acetylcholine from the diagonal band nuclei is not necessary for the performance of certain defensive behaviors. Along similar lines Gray et al (1981), demonstrated that septal lesions (and fornix, personal communication) disrupted defensive burying of a species

specific defence response in rats.

Both type I and II theta apparently share many common pathways. Lesions as far rostral as the anterior tip of the diagonal band attenuated both types of theta for up to 10 days. Lesions down the fornical columns to the level of the lateral hypothalamus also attenuated both types of theta rhythm. All lesions affected the production of theta in the same way either by attenuating both or by not attenuating both. One may infer from this that the two types of theta, although reputedly responding to different chemical transmitters, share many if not all common pathways. The one possible exception to this generalization were the lesions that affected the entorhinal cortex and subicular areas. These lesions will be dealt with at the end of the discussion.

The fact that a Fast Fourier program was able to detect an average frequency of 8 Hz during type I behaviors and an average frequency of 7 Hz (although not as clear) during type II behaviors in septal animals, may be due to the lesion not destroying all theta fibers, as shown by histological examination. Diagonal band of Broca lesions show a recovery of theta starting at about day 10. This may have occurred because the medial septal fibers of importance because of their proximity to the diagonal band lesion site were nonlethally impaired. What occurred over time then, was the reappearence of normal septal cell function. Fornical lesions showed no recovery of theta

activity over time. This may have resulted from the fornix fibers running into a theta bottle neck (Andersen et al 1979) posterior to the medial septum that abutts to the basal medial fimbria. If medial septal fibers are not monosynaptically connected to the theta generators (Kolher, Chan-palay & Steinbush 1982) they may presynaptically influence hippocampal generator sites via fornical fibers enroute to the hippocampus. There is, however, some controversy on this point (Segal 1979).

Following lesioning, the average type I and II theta frequencies across 30 second samples was altered in four of the eight groups. As type I theta is correlated with movement, increases and decreases in movement directly affect amplitude and frequency (Whishaw & Vanderwolf 1973). In addition, Sainsbury and Montoya (1982) demonstrated that arousal was one component in the production of type II theta, affecting both amplitude and frequency of the signal. Therefore, a theta frequency or amplitude change may indicate, for type I theta, an increase or decrease in motor output. For example, the amygdala group animals that demonstrated a drop in the frequency of type I theta displayed mainly head movements and postural adjustments. These animals also had a lower type II theta frequency and decreased arousal level, as reflected by grooming in the presence of snakes, not moving in the presence of owl sounds, and a lowered response to opposite sexed conspecifics. Therefore it is difficult to

determine whether the lesion sets limits on the behaviors or if the lesions are damaging frequency release or inhibit centers directly.

Entorhinal animals remain an enigma as these lesions cannot be explained behaviorally. Although the animals responded normally to the three behavioral conditions subsequent to lesioning, both type I and II theta were each averaged across 30 seconds to be 7 Hz. In addition, for the first 7 days type I theta did not correlate highly with type I behaviors i.e. there were times when the animal walked and type I theta was not present (omissions). The relationship between walking and concomitant theta production in intact animals is normally one to one (Sainsbury 1970). At other times when the animals walked, stopped and then walked again, the EEG record indicated continuous theta. Histology revealed that the lesions to the entorhinal area were partial, with the strongest effect being the more posterior lesion.

The present entorhinal results parallel those of Vanderwolf and Leung (1983) in that the type I theta record did not correlate highly with type I movements made by the animals. Vanderwolf and Leung (1983) argued that having eliminated the atropine resistant type I theta pathway to the hippocampus the only type of theta remaining after entorhinal lesions was atropine sensitive type II theta.

In the first study a number of lesions were performed

to demonstrate species generalization regarding anatomical areas involved in theta production. This was successful. Additional lesions were also performed to determine if the pathways that mediate type I and II theta are at one point anatomically distinct. Having found promising evidence with entorhinal lesions a second experiment was performed to test the theory that atropine resistant type I theta enters the hippocampal formation via the entorhinal cortex. As guinea pigs predictably produce normal type I and II theta in certain behavioral situations, the guinea pig was the ideal animal to determine not only if the theta produced was atropine sensitive but in addition, if it was still elicited by the same stimuli. That is, was the theta that remained following entorhinal lesions only atropine sensitive theta and if so, did it still serve the same function? Or was it also altered in some way by the entorhinal lesion as was type I theta.

EXPERIMENT II

Experiment II involved a more rigorous investigation of the entorhinal cortex lesion effect. To determine the pharmacological basis of the disturbance, atropine sulphate and physostigmine were administered I.P. in varying doses subsequent to total bilateral entorhinal cortex lesions. In addition a panel of naive judges categorically rated the behavior changes of the entorhinal and control animals, in the non-atropine snake and birds of prey conditions, to determine if there was a behavioral correlate to the altered EEG patterns.

METHOD

Subjects and Surgery

Nine male and one female mixed strain guinea pigs weighing between 800 and 900 grams at the time of surgery were used. Animals were housed in group pens measuring 77 cm x 45 cm x 30 cm high. Commercial bedding covered the floor. Food and water containing vitamin C was available ad libitum.

Animals were anaesthetized I.P. with sodium pentobarbital (25 mg/kg) and topically with procaine. Movable recording electrodes, described elsewhere (Sainsbury et al 1983) and/or reference electrodes were

implanted bilaterally, and were aimed at the dorsal hippocampal dentate generator. Coordinates for the dentate electrodes were 5.5 mm posterior and 3.0 mm lateral from bregma and 3.7 mm ventral from the dura. Placements were made with lambda and bregma on the same horizontal plane. In addition, a ground electrode consisting of a male winchester soldered to a jewelers screw was fixed to the skull.

Electrical Recording Apparatus

Test chambers and recording procedures were the same as those described for Experiment I, except that during some post lesion recordings the animals received an I.P. injection of atropine sulphate.

PROCEDURE

Prelesion Testing

After an half hour habituation session in the appropriate test box, all animals were presented with the following stimuli during seperate sessions: the sounds of birds of prey; a 5 cm square of red tape waved on either side of the guinea pigs' head while the animal remained motionless for 5 seconds; a light stroke of a metal prod moving in a rostral or caudal direction along the animals flank while the animal remained motionless for 5 or more seconds (Whishaw and Dyck in press); and a push with a metal prod that caused the animal to walk across the length

of the observation box.

Lesions

Following prelesion testing subjects were randomly assigned to one of two conditions. The first group received bilateral entorhinal aspiration lesions, the second group served as a control group.

The entorhinal cortex group (N=5) received bilateral entorhinal aspiration lesions whose center point was the most lateral and ventral aspects of the neocortex. Lesions extended 4 mm posterior and 3 mm anterior from the center point. The lesions also extended 4 mm dorsal and 5 mm ventral from the center point. Ventral from the dura the lesions averaged 2.5 mm.

The control group (N=5) animals received no lesions but were anaesthetized with 25 mg/kg of sodium pentobarbital and put into the stereotaxic holder.

Post Lesion Testing and Histolgy

The entire pre-testing procedure was repeated starting 72 hours post lesion but in addition, the snake and sexual conditions, as described in experiment I, were added. Entorhinal animals received either a 12.25 or 50 mg/kg I.P. injection of atropine sulphate, while control animals received a 50 mg/kg injection of atropine sulphate during atropine sessions. Atropine sessions were run according

to the following schedule. Three days following lesioning all animals were administered 50 mg/kg atropine sulphate I.P. and placed in the three behavioral settings. Three days subsequent to the atropine test all entorhinal animals were administered 12.25 mg/kg I.P. atropine sulphate and observed on video tape for 20 minutes, without being placed in the three behavioral conditions. Three days following the 12.25 mg/kg session all animals were run through the testing procedure without atropine being administered. At one month post lesion the entire sequence of sessions was run in the opposite order i.e. the no drug condition first. Following all testing one entorhinal and one control animal were injected with 3 mg/kg physostigmine every 10 minutes for 40 minutes. The behavior and concomitant EEG activity was monitored for the entire period. All subjects were then deeply anaesthetized with sodium pentobarbital (75 mg/kg I.P.) and perfused with isotonic saline and 10% formalin solution. After three days the brains were transferred to a sugar and formalin solution. The brains were sectioned using the frozen technique at 50 microns and stained using cresylecht violett.

Statistical Methods

Theta activity recorded from the hippocampus was visually inspected and edited during type I and II behaviors before being analyzed by either measuring the amplitude and frequency of individual waves with a clear plastic ruler or

using a Cromemco System Three computer using a Fast Fourier program. The first two seconds, of the first five behaviors, known to produce good type I theta e.g. walking (Sainsbury 1970), from each animal in each group, were viewed from the video taped record, both before and after lesioning (walking was defined as more than three leg displacements). The behaviors were then correlated with, each second, of the two second concomitant theta record according to the following criteria 1. Yes, theta is present 2. No, theta is not present. In a similar fashion type II theta was viewed from the video taped record, both before and after lesioning during behaviors that reliably produce type II theta in the guinea pig e.g. tactile test. The behaviors were then correlated with each second, of the two second concomitant theta record according to the following criteria 1. Yes, theta is present 2. No, theta is not present.

The numerical ratings were then collapsed across all animals in each lesion condition. The ratings were then subjected to a Chi Squared analysis.

Behavioral Data Analysis

Analysis of pre and post lesion behaviors recorded on video tapes were made by a panel of 5 naive judges in a blind test. A rating scale was created to predict the behavior of control animals when placed for 5-10 mins in behavioral settings containing garter snake(s) or birds of

prey sounds. The judges rated a 1 minute sample of owl and hawk sounds that contained the sound of the great horned owl, as this sound, on the tape recording, most reliably elicits type II theta in intact animals. The judges also rated the initial one minute of exposure of control and entorhinal animals to snake(s). Performance was compared both across and within groups for the birds of prey tape and across groups in the snake(s) condition. All data was then subjected to an analysis of variance using the Chi Squared statistic. The predetermined rating scale for these behavioral settings is as follows.

Snake and Guinea Pig Condition

A. One or more of the following behaviors normally occurs in the presence of snake(s).

1. Moves to far corner of cage and turns back to snake.
2. Jumps over wall.
3. Swagger movements while facing snake.
4. Attacks snake concomitant with piloerection.
5. Disruption of ongoing behavior includes behavioral freezing i.e. no movement at all for up to 60 seconds.

B. One or more of the following behaviors normally does not occur in the presence of snake(s).

1. Grooms in the presence of snake for more than 10 seconds.

2. Does not change ongoing behavior when snake(s) are placed in behavioral setting.
3. Allows snake to be draped over its' body for more than 5 seconds.
4. Eyes half closed or sleeps in presence of the snake.

If one or more behaviors in category A occurred a rating of 1 was given. If one or more behaviors in category B occurred a rating of 2 was given.

Birds of Prey and Guinea Pig Condition

A. One or more of the following behaviors normally occurs when the animal is presented with the sounds of birds of prey.

1. Animals demonstrate a behavioral freeze but respond to sounds of birds of prey with a slight head turn in the direction of sound i.e. orienting response.
2. Animals show increased movement during owl sounds.

B. One or more of the following behaviors normally does not occur when animals are presented with the sounds of birds of prey.

1. Animals show movement throughout the session.
2. Eyes appear half closed or animal lays down and sleeps during presentation i.e. no response to stimuli.
3. Cumulative grooming exceeds 10 seconds.
4. Animal eats bedding or chews walls continually i.e more than 15 seconds.

If one or more behaviors in category A occurred a rating of 1 was given. If one or more behaviors from category B occurred a rating of 2 was given.

The numerical ratings for behavior were averaged among animals of the same experimental condition. The ratings were subjected to a Chi Squared analysis. As all Chi analyses were on one degree of freedom all Chi data was corrected for continuity.

Sexual Behavior Analysis

Performance during the sexual sessions was rated according to the following criteria comparing the control and entorhinal animals. Total time spent grooming in the presence of opposite sexed conspecific and total time following other animal(s) was tabulated for the initial two minute exposure to the sexual stimuli. This data was subjected to a one by two completely crossed randomized factorial block design analysis of variance.

RESULTS

Histological Results

All experimental animals had total entorhinal cortex lesions that included partial bilateral but inconsistent damage to the following areas: Pre, para, and prosubiculum, ventral dentate, perirhinal cortex,

posterior amygdala and neocortex (figs. 6 & 7). All recording electrodes were located in the dentate gyrus of the hippocampus.

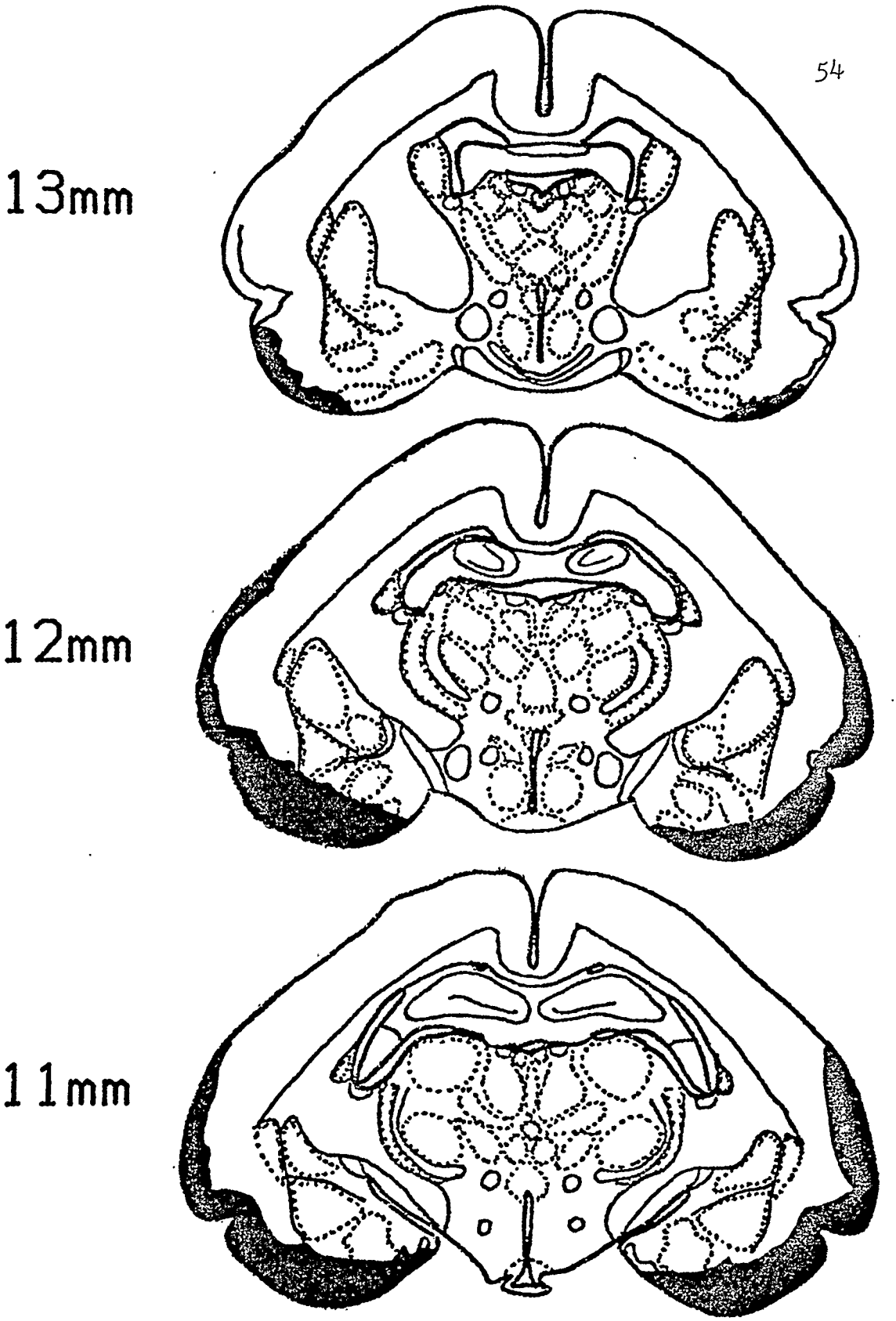
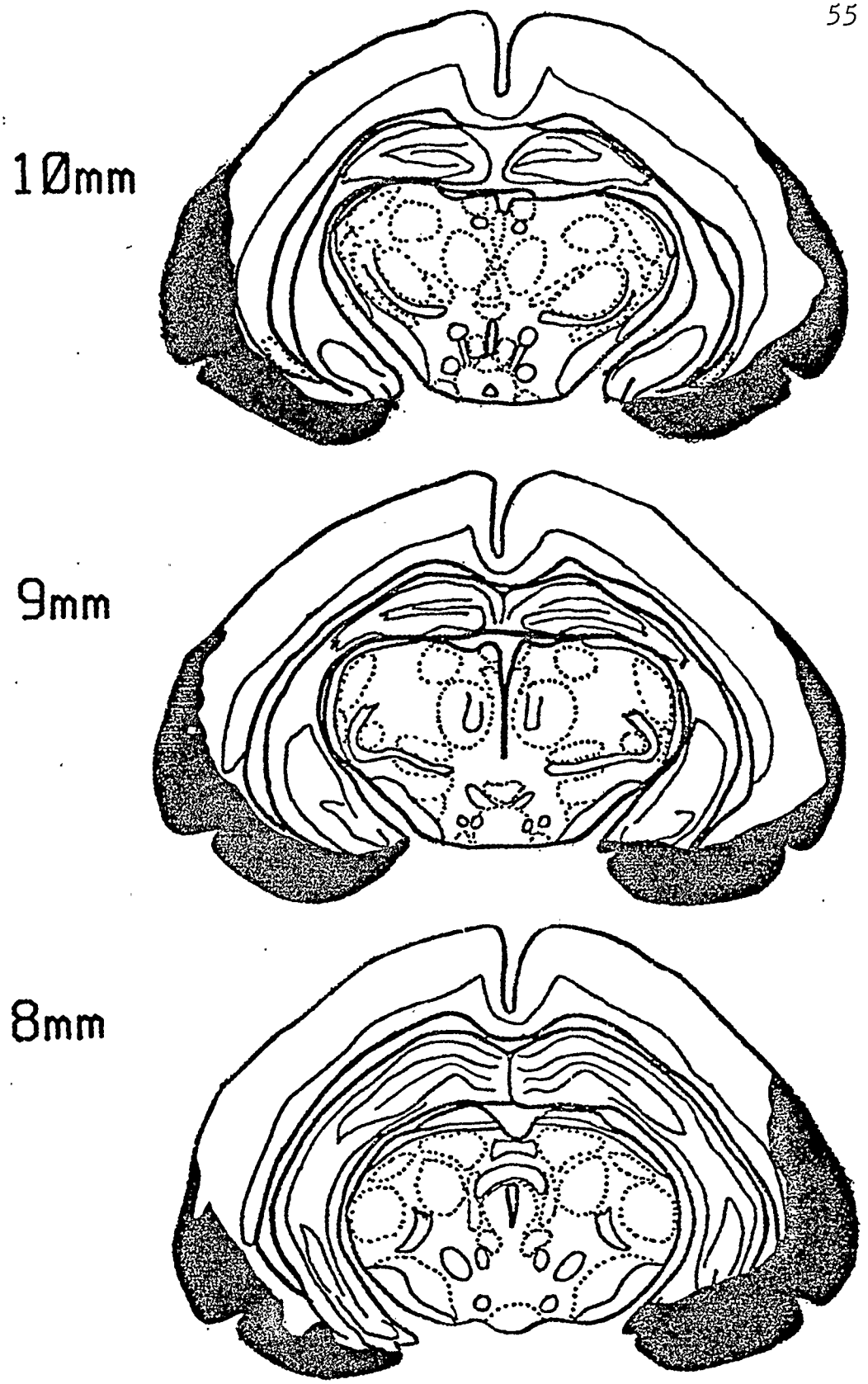


Figure 6. A schematic series of sections from entorhinal guinea pig 64 that illustrates the extent of the bilateral entorhinal lesion.



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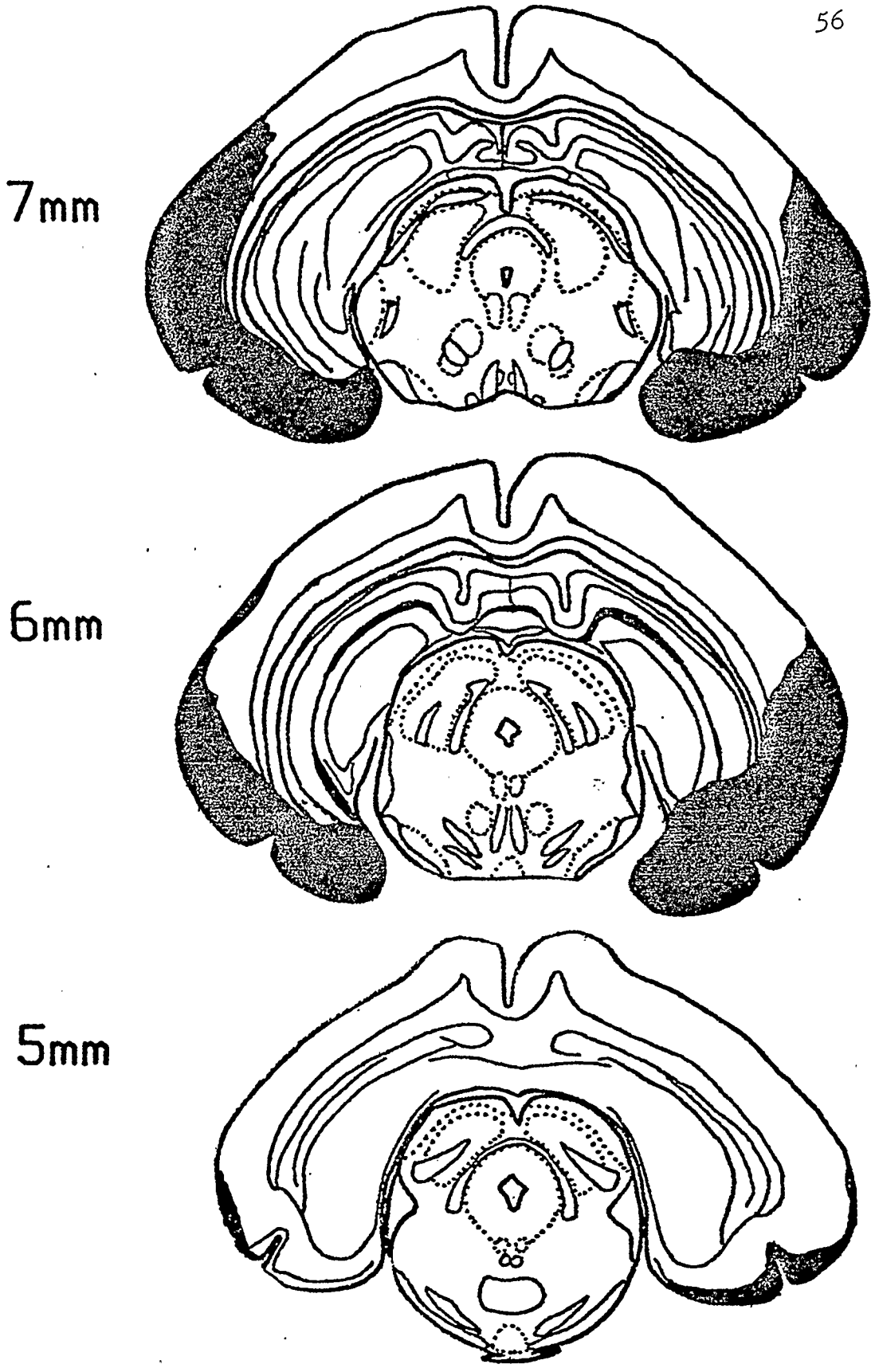

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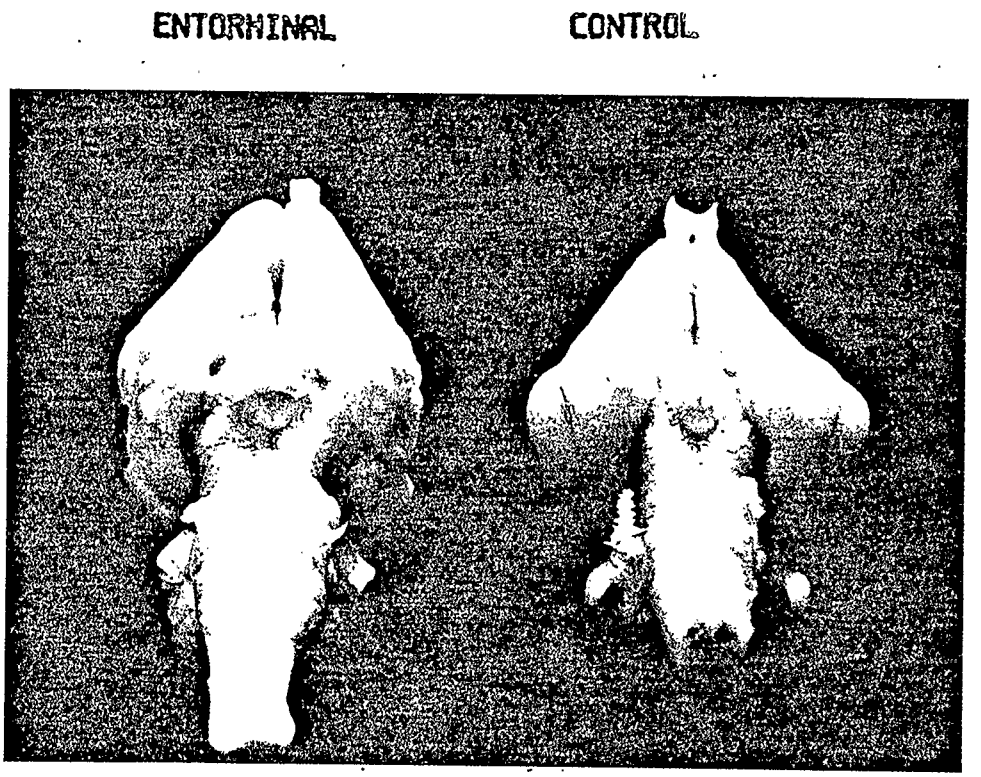

Figure 7. Photograph illustrating entorhinal aspiration damage compared to a control brain (ventral surface).

Electrophysiological Results and Formal Behavioral Observations

EEG Changes. Bilateral lesions to the entorhinal cortex resulted in a mean reduction in theta amplitude of 33% for the duration of the experiment. Amplitude reduction was determined by using a clear plastic ruler to measure 50, one second sample periods, of maximum theta amplitude both before and after entorhinal lesions [$F = 4.481$, $p < .05$, df, 1,98].

Type I Theta Effects. Subsequent to entorhinal lesions an analysis of 50 initial, two second, samples of walking behavior and concomitant theta activity indicated a significant reduction in the correlation between walking and theta activity [$X^2 = 24.10$, $p<.001$]. Theta was present in the 50, one second samples of walking behavior only 58% of the time, while it was present in 100% of the samples before lesioning. This effect may be seen in figure 8. From this figure one can see that the amplitude has diminished after the lesion. It is also evident from figure 8 that during occasion A the animal produces theta while walking; while on occasion B theta omissions occur.

The administration of 12.25 mg/kg of atropine sulphate significantly but transiently improved the correlation between motor behaviors and theta production. In the 50, 1 second, samples tested the actual amount of theta produced

increased from 58% to 90% during walking with three subjects showing a one to one relationship with walking [X^2 = 11.68, $p<.001$]. The time period for improvement lasted only 2-3 minutes post injection. The effect may be seen in figure 9. At approximately 4 minutes post injection of atropine sulphate an increase in the number of large irregular sharp waves (SPWs), was observed. These SPWs are thought to originate from an area of stratum radiatum where the apical dendrites of the CA1 pyramidal cells make connections with the schaffers collaterals (Buzsaki et al 1983). The amplitude of the SPWs was from 2-3 mv and their initial component was normally negative. At this point the SPWs had formed long spike trains that were abolished by motor behaviors e.g. walking or head movements. This effect may also be seen in figure 9. After 6-8 minutes post injection the SPWs became behavior independent showing only a slight reduction in amplitude during type I behaviors. In addition, no theta activity was observed during any behavior at this time (fig. 9).

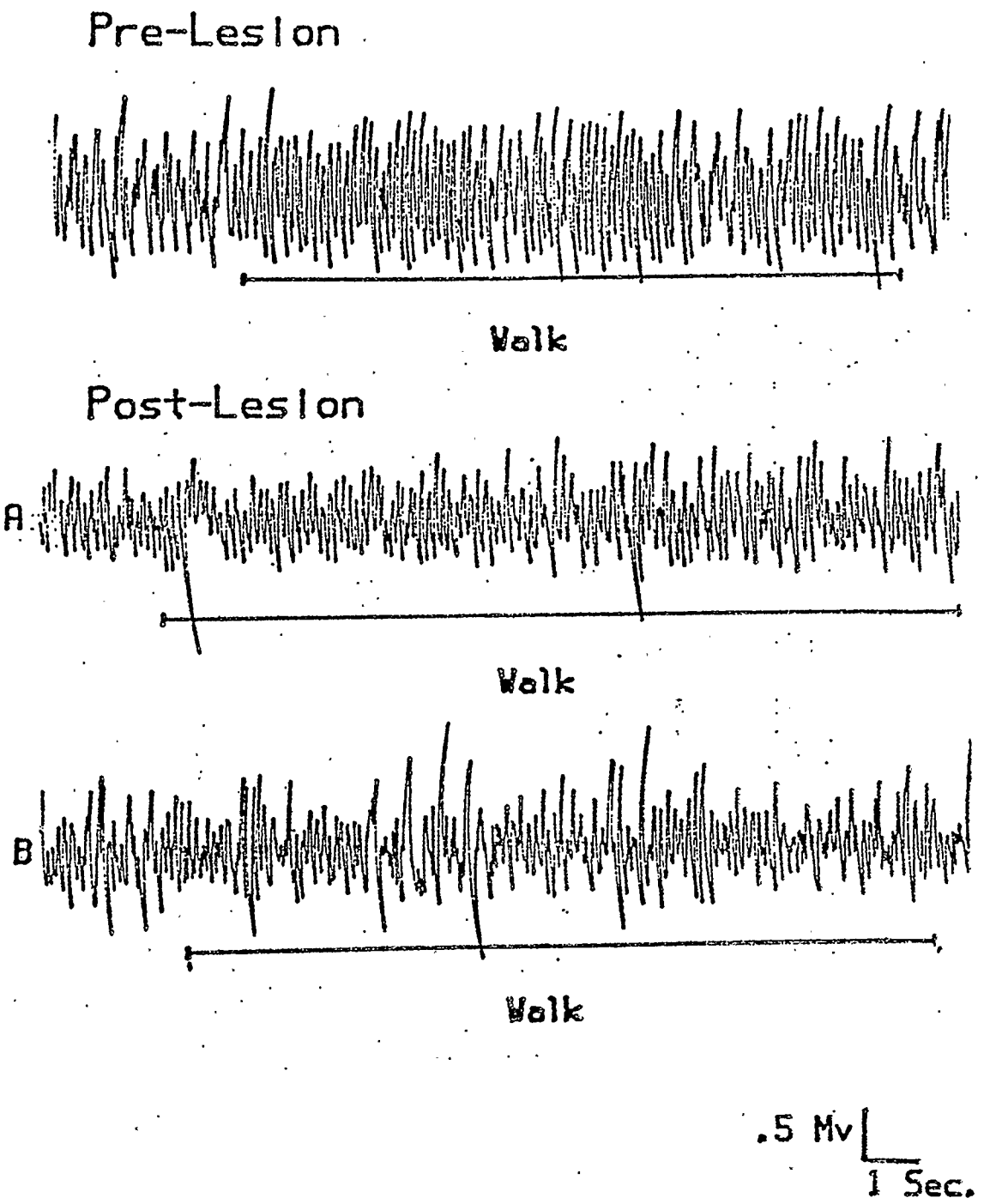

Figure 8. Pre- and post-lesion recordings from guinea pig 68. (A) Theta produced during walking behavior. (B) Note omission of type I theta activity that occurs in the post bilateral entorhinal lesion condition.

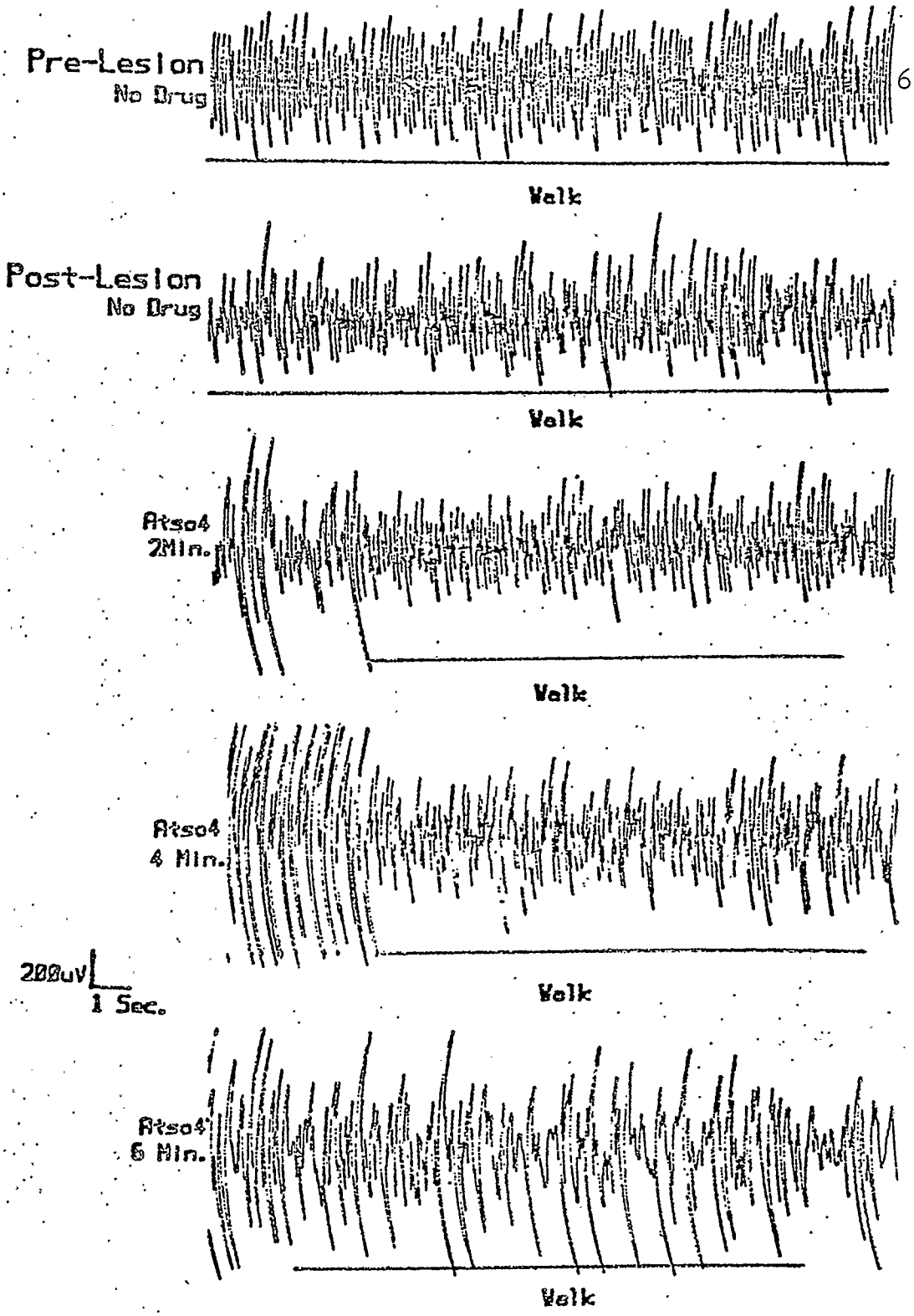

Figure 9. Pre- and post-lesion recordings from guinea pig 60. Note the omissions in type I theta activity from the pre- to the post-lesion condition. Some improvement is evident 2 minutes post ATSO4 injection; 4 mins. post injection spike activity is inhibited by walking behavior; 6 minutes post injection SPWs totally dominate EEG record independent of concomitant behavior (12.25 mg/kg dose of ATSO4).

These results are in direct contrast to those seen with non-lesioned control subjects. An example of the effects of atropine sulphate on type I theta production in a control subject may be seen in figure 10. From the top portion of figure 10 it is clear that atropine sulphate has little effect on theta production during walking in the normal animal. Injections of 50 mg/kg of atropine sulphate had the same effects on lesioned animals except that the time course of the effects was shortened.

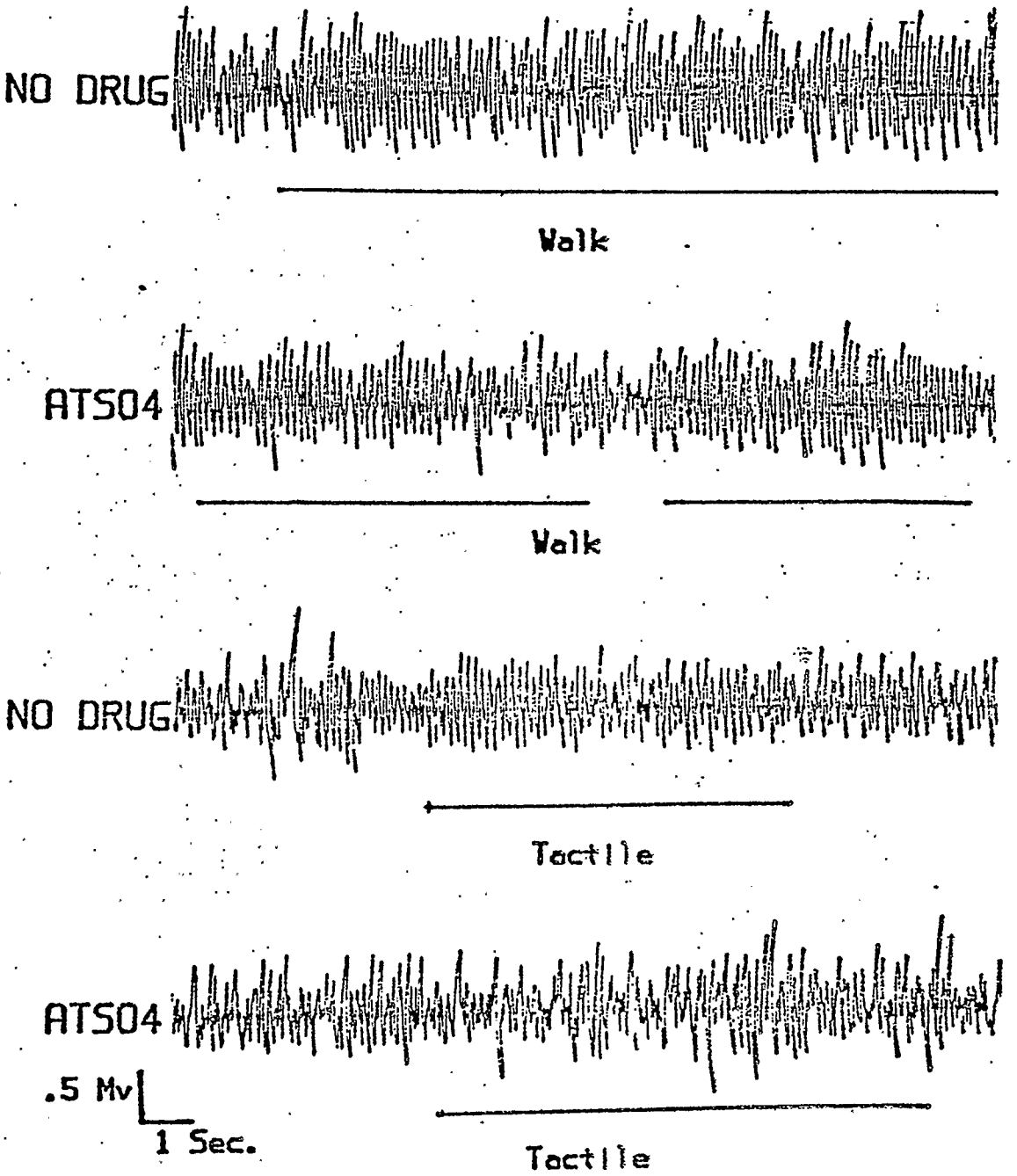

Figure 10. Drug and non-drug recordings from control animals. Top of figure illustrates an intact animal walking and then the same behavior with ATS04. Note type I still present during walking. Bottom of figure illustrates type II theta in an intact animal and the effect of ATS04 on type II theta.

Type II Theta Effects. Type II theta was affected in a similar fashion as type I theta was affected. During the first two seconds of the tactile immobility test, prior to entorhinal lesions, there was a 1.00 correlation with the production of type II theta. Following entorhinal lesions there was a significant drop in the correlation, to .34, across 50, one second test periods [$X^2 = 46.75$, $p<.001$]. This effect may be seen in figure 11. From this figure it can be seen that on occasion A tactile stimulation elicited theta while on occasion B tactile stimulation did not elicit theta. While I have chosen to show the effects of tactile stimulation, the results are the same with all the other type II stimuli i.e. snakes, sounds of birds of prey, and sexual stimulation, that were used.

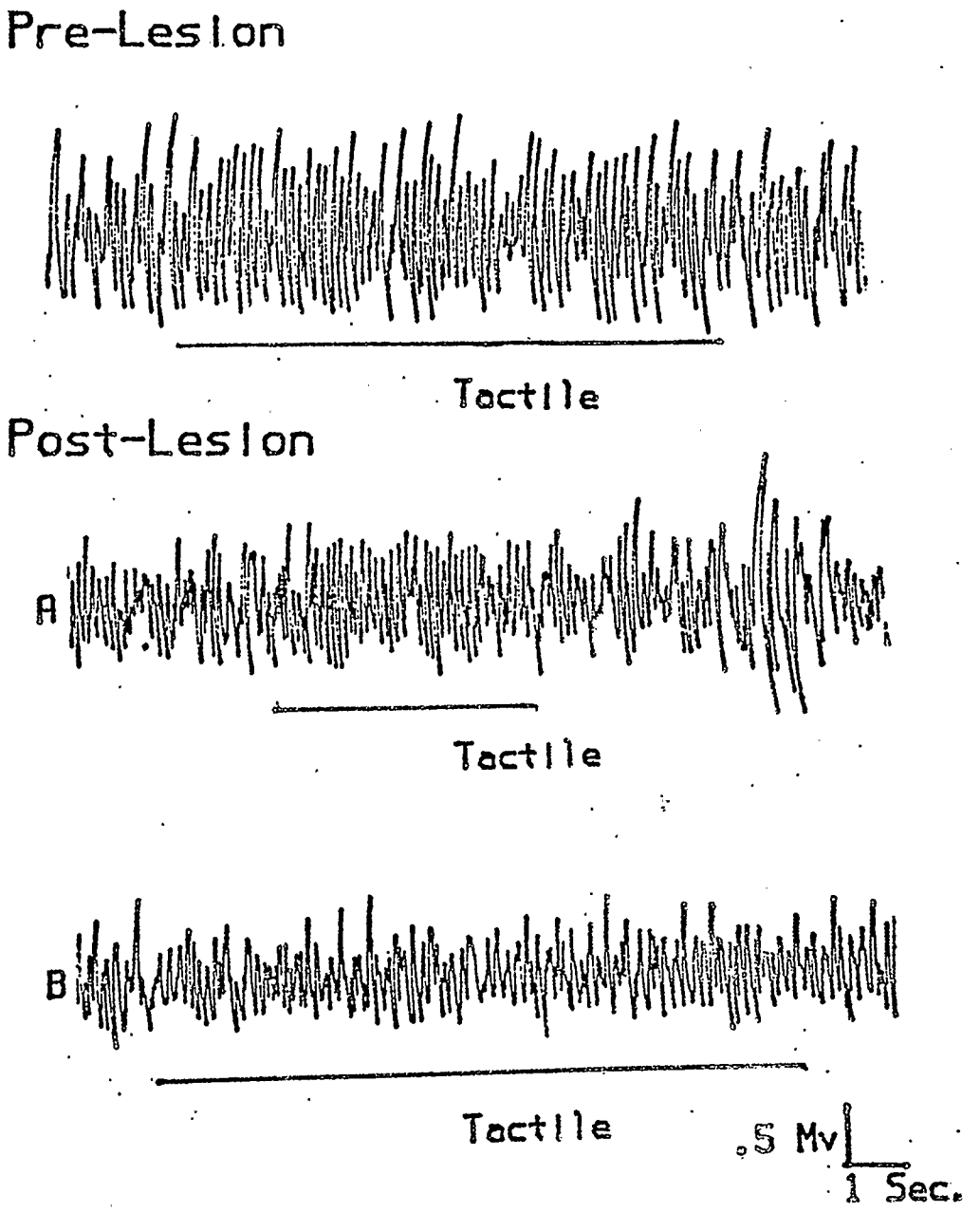

Figure 11. Pre- and post-lesion recordings from guinea pig 68. (A) Note tactile stimulation and concomitant theta. (B) Note tactile stimulation does not elicit theta activity.

The administration of 12.25 mg/kg of atropine sulphate significantly improved the relationship between type II theta activity and incoming sensory stimuli, for the fifty seconds tested. The improvement was from 34% to 56% with two animals producing theta 80% of the time during the presentation of stimuli [X^2 = 5.45, $p<.02$]. The improved type II theta relationship always occurred contemporaneously with the improved type I theta time period. The facilitory effect for both type I and II theta was transient, lasting approximately 2 to 3 minutes subsequent to injection (fig. 12).

After approximately 4 minutes post injection SPWs were observed in the theta record. As with motor movements type II stimulation produced a suppression of the SPW activity (fig. 12). As one can see from figure 12 the suppression is brief and only occurs at the beginning of stimulation. This is in contrast to the effects of motor movements which cause suppression throughout the movement. After approximately 6 minutes post injection theta is no longer visible and the EEG record is dominated by spiking activity. This too is in contrast with the effects of atropine on a non-lesioned control animal. From the bottom portion of figure 10 one can see that while atropine sulphate abolished type II theta the EEG record is not dominated by spiking activity. As with type I theta the use of 50 mg/kg of atropine sulphate simply reduced the time course of the atropine effects.

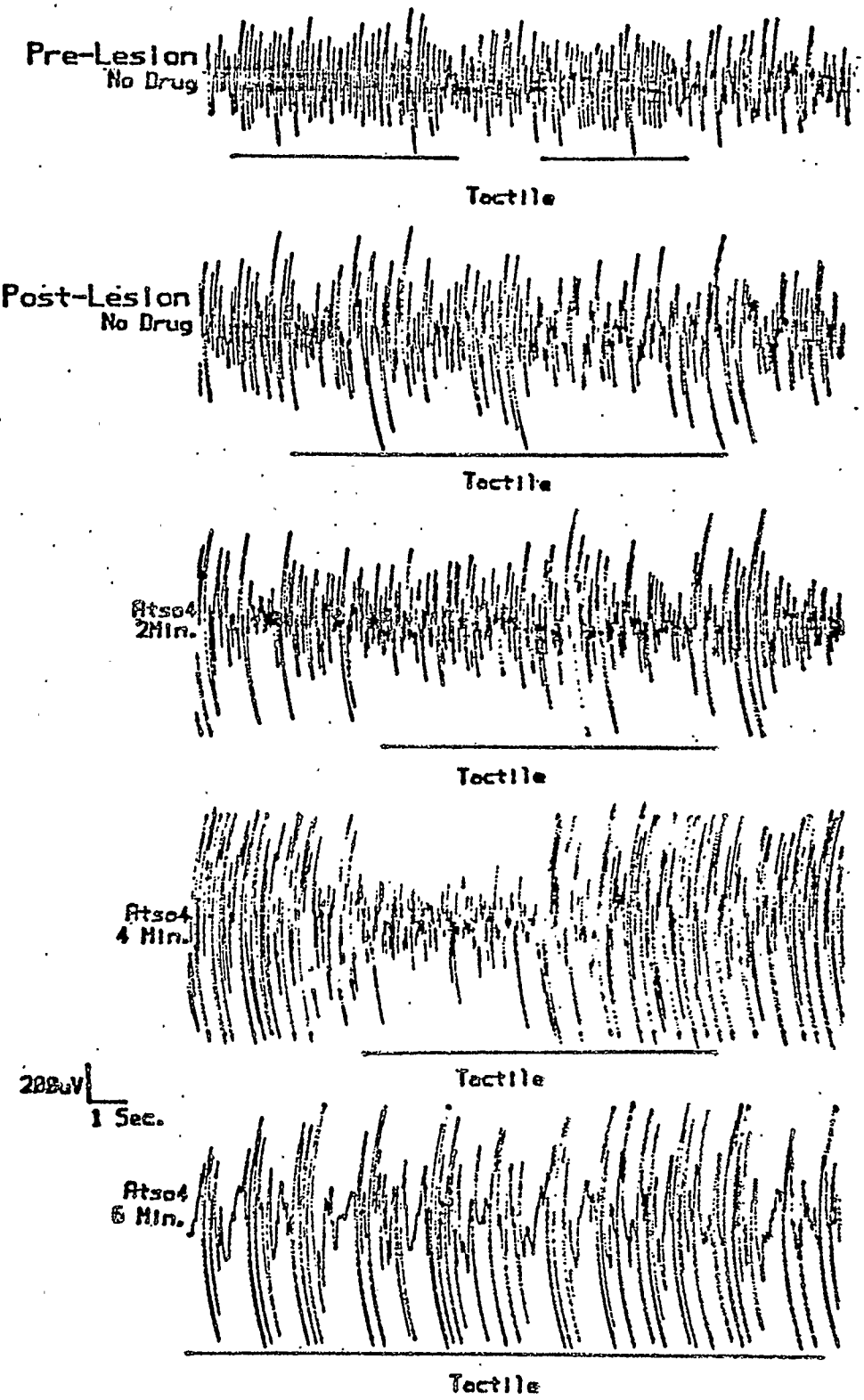

Figure 12. Pre- and post-lesion recordings from guinea pig 60. Note the absence of most theta activity during tactile stimulation. Some improvement is evident 2 mins post injection; 4 mins post injection stimulation initially inhibits SPWs but this effect appears to habituate rapidly; 6 mins post injection SPWs dominate record and are behavior independent.

Fast Fourier transforms of both type I and type II theta (60-1 second samples) revealed an average pre-lesion frequency of 8 Hz and 7 Hz respectively. After entorhinal lesions, theta which occurred during movement had an average frequency of 7.5 Hz while theta which occurred during immobility had an average frequency of 8.5 Hz. These results continued throughout the time course of the experiment (i.e. 40 days post lesion).

Judges rated behavior changes following entorhinal lesions as insignificantly different [X^2 = .000, $p>.05$]. Interjudge reliability was 76.7%. Reliability was determined by assuming that the majority of the judges were correct i.e. if three judges rated yes and two rated no, then the two no's would be considered in error and would be subtracted from the total score.

Sexual Behavior

The mean time spent following opposite sexed conspecifics subsequent to entorhinal lesions was 41.6 seconds compared to 60.4 seconds before lesioning for the initial two minutes tabulated. The difference, however, was insignificant [$F = 3.39$, df 1,8, $p>.05$]. In addition, total grooming time varied insignificantly between pre- and post-lesion sexual conditions [$F = 1.00$, df 1,8, $p>.05$].

Control Animals

The judges found no significant change in behaviors in the control group upon the second presentation of birds of prey stimuli [X^2 = .871, p>.05]. In addition, theta or LIA activity which occurred in the prensence of the various stimuli remained the same across the three experimental settings. All theta had a similar amplitude within subjects across sessions and similar frequency between subjects across sessions i.e. type I theta frequency was 8 Hz and type II theta frequency was 7 Hz. The 50 mg/kg of atropine sulphate increased the frequency of type I theta to 9 Hz, while eliminating theta activity during immobility.

The administration of physostigmine in increasing doses of 3 mg/kg to a total of 12 mg/kg in 40 mintutes did not elicit type II atropine sensitive theta, even at the highest dose level, in the control or entorhinal animal. The final injection of physostigmine was fatal. Although peripheral effects were already evident during the initial dose, the animals could sit and groom or walk supporting their weight if prodded, until the final injection. During the final injection the animals lost all postural support and respiration ceased.

DISCUSSION

The primary purpose of experiment II was to determine the organization of type I and type II theta pathways in the hippocampus. Vanderwolf and Leung (1983) suggested that immobility related RSA may result from theta being produced by a single input from the medial septum, while the RSA that accompanied walking and other type I behaviors in rats, was controlled by two inputs (one dependent on cholinergic synapses at some point) ascending via the medial septal nuclei; the other entering via the temporo-ammonic pathway from the entorhinal cortex. The present results suggest that the type II theta system is connected in an analogous fashion as the type I theta system in guinea pigs. Specifically, it appears that both systems require the entorhinal cortex to correlate incoming sensory information and movements in a normal manner concomitant with theta production. The present data indicate that the cholinergic input from the septum is necessary but not sufficient for normal type II theta production. Indeed the entorhinal cortex may play a role similar to the medial septum in some species. Carreras et al (1955), demonstrated that bilateral entorhinal lesions in the cat eliminated all theta activity. Recent personal observations in our laboratories have indicated that subsequent to atropine sulphate injections all theta is eliminated in cats. These findings strongly suggest that cats only have type II atropine sensitive theta and that it

is critically mediated, at least at one point, in the entorhinal cortex.

Apart from this discrepancy, the results of the present study were in essential agreement with the results of Vanderwolf and Leung (1983). Subsequent to bilateral entorhinal cortex damage there was a reduction in theta amplitude. In rats, the depressed theta record recovered, while in guinea pigs it remained depressed. In addition, type I behaviors such as walking, rearing and small head movements were no longer highly correlated with theta activity as recorded in the dentate gyrus of the hippocampal formation. In the past several authors have reported that the correlation between movements such as walking and theta production are 1.00 in intact animals (Vanderwolf 1969; Sainsbury 1970; Whishaw & Vanderwolf 1973). To date, no other lesion or drug manipulation has affected the correlation of type I theta and mobility or type II theta and incoming sensory information unless the lesion eliminated all type I or type II theta activity (Kolb & Whishaw 1977; Vanderwolf et al 1978).

The administration of 50 mg/kg of atropine sulphate following entorhinal lesions eliminated all theta activity regardless of behavior at approximately 6 to 7 minutes post I.P. injection. This suggests that the type of theta remaining subsequent to entorhinal lesions is cholingerically based. At 2 to 3 minutes post injection,

especially in the 12.25 mg/kg condition, there was a theta facilitory effect. During this period the correlation between walking and concomitant theta activity significantly improved (to 1.00 in some animals) within the first 2 to 3 seconds of a behavior. The correlation between type II theta and sensory processing during immobility also improved significantly during this period. This finding suggests, at least in entorhinal animals, that atropine sulphate may have a disinhibitory or facilitory first stage of action before blocking the muscarinic post synaptic membrane. This point remains unresolved however, as eserine failed to elicit atropine sensitive theta, in either control or entorhinal animals.

Four to five minutes subsequent to administering a 12.25 mg/kg atropine injection, periods of immobility were filled with LIA activity, specifically large irregular sharp waves (SPWs) whose initial component was normally negative. If the animals engaged in type I movements the EEG record was replaced by low voltage fast activity for the duration of the behavior. If the animals were presented with the sounds of birds of prey or other type II eliciting stimuli, the onset of the stimuli would be concomitant with the appearance of low voltage fast activity in the EEG record. As the animals became habituated to the stimulus, however, the SPWs returned even in cases where the stimulus was still present. In intact animals type II theta rapidly habituates to incoming sensory stimuli. It is apparent that

the mechanism responsible for type II theta habituation can function independently of atropine resistant or atropine sensitive theta.

Vanderwolf and Leung also reported that atropine sensitive theta observed during type I movements (this was the only time theta was produced in the awake entorhinal rat) was of a slower frequency than control animals. The present data supported these findings, but in addition, demonstrated that the theta produced during immobility was of a higher frequency than theta that occurred during type I behaviors within the same animals. This data suggests a pathway inhibition concept that will be dealt with in the general discussion.

Vanderwolf and Leung stated "Repeated, but entirely unsuccessful attempts were made to elicit RSA during behavioral immobility by various visual or auditory stimuli" (p. 19). The present findings demonstrated that subsequent to entorhinal lesions, certain incoming stimuli that prior to surgery reliably elicited type II theta, no longer reliably correlated with atropine sensitive theta production. This reduction in the amount of type II theta that was reliably produced before lesioning, might not be noticable in a species, like the rat, that rarely produces type II theta in the experimental situation, even prior to lesioning. This one species difference may also account for the different theoretical approach taken by Vanderwolf and Leung (1983).

As both frequency and onset of type II theta are altered following entorhinal lesions, this experiment demonstrates the importance of both the septal and entorhinal pathways to the normal function of not only type I but also type II theta production.

GENERAL DISCUSSION

Experiment I demonstrated pathway similarities between the guinea pig and other species, that were necessary for the generation of the theta signal in hippocampal generator sites. Specifically, the integrity of the medial septum, diagonal band and more dorsal fornix pathways must be maintained for the theta signal to be generated in the guinea pig. This data supports similar findings in other species (Green & Arduini 1954; Sainsbury & Bland 1981). The similarities between type I and II theta pathways were also highlighted as these lesions affected the two types of theta equally. From the rostral most continuation of the diagonal band of Broca to the columns of the fornix, a lesion that eliminated one type of theta eliminated both types.

The one condition that appeared to differentially affect type I and type II theta was the entorhinal lesion condition. The entorhinal lesion animals no longer produced theta which was highly correlated with walking. The entorhinal lesion results suggested that the fiber pathways that mediate type I and II theta, may at one point be, anatomically distinct. After experiment I several points remained unclear. First, histological results revealed only partial damage to the entorhinal cortex. As the

results demonstrated a transient effect, would total entorhinal lesions result in the permanent effect reported by Vanderwolf and Leung (1983)? Secondly, what was the pharmacological basis of the theta that remained following entorhinal lesions? Thirdly, and perhaps most important, were both types of theta equally disrupted by the lesions?

Experiment II demonstrated that the entorhinal lesions affected the production of type I and II theta in a similar but possibly not equal fashion. The administration of atropine sulphate eliminated all theta suggesting the post lesion theta was cholinergic. The administration of physostigmine, however, did not facilitate the production of atropine sensitive theta subsequent to entorhinal lesioning, or even prior to lesioning. In rats, the administration of 1 mg/kg of physostigmine produces long trains of atropine sensitive theta and is lethal without an atropine antidote. Guinea pigs received over ten times the dose and died without showing any atropine sensitive theta facilitation. This one species difference may explain some differences between the present papers' findings and others who work with species that are more susceptible to cholinergic agonists and antagonistic substances.

"Atropine sensitive" has, in the past, been one criterion for labelling a certain type theta "type II". Experiment II, however, demonstrated that although the medial septal cholinergic pathway to the hippocampus was intact, type II theta was not. The general utility of an

"atropine sensitive" category is weakened by the apparently unpredictable relationship between type II theta and the presence of stimuli that, before lesioning, reliably elicited the wave form. Thus, experiment II conluded that medial septal cholinergic fibers were necessary but not sufficient for the normal production of type II theta, in the presence of stimuli that reliably produce type II theta in the intact guinea pig. In addition, it was suggested that both medial septal and entorhinal inputs are necessary for the normal production of both types of theta activity.

Two hypotheses to date attempt to explain theta generation. The two models are the recurrent inhibition model and the feed forward model.

According to the recurrent inhibition model, medial septal pacemaker cells project rhythmical excitatory signals to the parallel laminar circuits of the hippocampus and impinge upon two primary cell types i.e. the pyramidal and granular cells in the generator zones. These cells in turn send out collaterals that excite interneurons (basket cells of Cajal) that are inhibitory in nature. These basket cells recurrently inhibit the primary cells (Buzsaki et al 1983).

The feed forward model is more complex and has four inputs, each capable of producing RSA activity, and each contributing to the theta profile. The entorhinal cortex projects excitatory signals via the perforant path to both CA1 pyramidal and dentate granular cell dendrites. The

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medial septum likewise, is the second source of input sending two excitatory signals via two seperate pathways to the interneurons of the CA1 and dentate generator zones. The interneurons are inhibitory in the hippocampus and make connections at the somata of the primary cells. The principle cells may then also excite interneurons through their collaterals. The model explains the CA1 and dentate generators being out of phase by supposing there are two types of pacemaker cells in the septum (Buzsaki in press). The arrival of somatic inhibition and dendritic excitation are assumed to arrive at the same time. Opposition to both models stems from studies that suggest that the medial septum impinges directly on both pyramidal cells and interneurons in the hippocampal formation (Segal 1978).

The results from the present experiment support the feed forward model of theta production for two reasons. First, according to the recurrent inhibition model, entorhinal lesions should only have an indirect effect on the frequency of type I or II theta, as no pacemaker cells are reputedly located outside the medial septum. Although behavior changes can affect the frequency of the theta signal, the panel of judges, in a blind test, found no significant change in the behavior of the guinea pigs after lesioning. The feed forward model on the other hand, assumes that any one of the four input pathways can affect, or even produce RSA and would therefore predict some change if two of the four inputs were to be lesioned. Second, subsequent to

entorhinal lesions in guinea pigs , a decrease in theta amplitude was observed in the EEG record that continued until the end of the experiment. Histochemical evidence has demonstrated that following perforant path deafferentiation, septal fibers relocate to the vacated spaces left by degenerating entorhinal fibers (Lynch, Mosko, Parks & Cotman 1973; McWilliams & Lynch 1978). According to the recurrent model of inhibition one might predict a larger amplitude signal after a period of regrowth, as septal pacemaker cells now have a "greater" influence on the pyramidal and granular cells in the generator zones. The feed forward model predicts that the profile of the theta record will be altered following bilateral entorhinal lesions as the inputs determine the generator maximas. Therefore, with fixed electrodes the feed forward model would predict a drop in amplitude of theta signal concomitant with a loss of clarity, while if using roving electrodes, a shift in the theta profile would be observed.

When intact guinea pigs were immobile SPWs were occasionally seen during LIA activity. However, SPWs disappeared during the presentation of stimuli that elicited type II theta or during type I behaviors. Therefore, type I and II theta generation inhibited the SPWs by some unknown mechanism. Accepting the argument that type II theta is cholinergically based, the feed forward model would agrue that type II fibers excited interneurons which in turn inhibited the primary cells at the level of the soma, and

somehow blocked the SPWs. On the other hand, the feed forward model would argue that type I inhibition came entirely from the entorhinal cortex i.e. the inhibition of SPWs would be in the form of dendritic EPSP's that may then excite interneurons.

As it is reasonable to assume that neither the septal or entorhinal input suppresses SPWs all of the time, then the elimination of either septal or entorhinal influence should increase the probability of SPWs. In the case of atropine administration and following bilateral entorhinal lesions this effect is observed in guinea pigs. The model would then predict that subsequent to the elimination of both inhibitory systems (entorhinal lesions plus atropine sulphate) a larger number of SPWs would occur, and be behavior independent. This is indeed the case.

The 12.25 mg/kg administration of atropine sulphate demonstrated that the type I and II theta inhibition of SPWs was not the same. One or more habituating mechanisms was present in the type II circuit. Subsequent to theta facilitation, long spike trains occurred in entorhinal animals. These SPWs were eliminated by type I movements that did not produce theta but instead elicited a low voltage fact activity. The presentation of a stimulus that in intact animals elicits type II theta produced low voltage fast activity that eliminated the SPW trains. During this period, for as long as the animals engaged in type I

behaviors the SPWs were suppressed. This was not the case with the low voltage fast activity that occurred during sensory processing. If the animals were allowed to habituate to the stimulus the SPWs returned even if the stimulus was still present.

The present findings lead to some interesting speculation concerning the feed forward model. One, habituation type circuits are linked to the soma of the primary cells via interneurons while non-habituation input comes through the dendrites of the pyramidal and granular cells. In addition, rather than just looking at cellular inhibition, this circuitry appears to indicate pathway suppression via adjacent pathways. For example, although type I and II theta pathways were shown to suppress SPWs, perhaps they also actively inhibit each other. If this was the case, the elimination of type I or II theta might increase the frequency of the wave form, the amplitude of the signal, or the sheer amount of time it is observed.. In the rat the application of atropine sulphate that eliminates type II immobility theta causes the animal to walk, almost incessantly, and therefore produces significantly more type I theta that is of a higher frequency than the predrug condition. In the rat, following entorhinal lesions, atropine sensitive theta was the only theta produced and Vanderwolf and Leung (1983) stated, "Data of this type are not available for the other rats since they did not walk about spontaneously very often" (p. 19).

In intact guinea pigs, keeping in mind the cholinergic species difference, an application of atropine sulphate that eliminates all immobility theta, concomitantly increases the frequency of the type I theta by one cycle per second. Following entorhinal lesions that eliminate atropine resistant theta, the immobility theta frequency increased 1.5 cycles per second above prelesion values i.e. from 7 Hz to 8.5 Hz. Interestingly, during ambulatory behaviors the frequency of atropine sensitive theta decreased to 7.5 Hz, back to within prelesion range even without the atropine resistant theta pathways being present.

These data may imply that a central mechanism regulates the pattern and occurrence of type I and II theta and LIA, even in the absence of theta or LIA electrographic activity. There are three other examples of this amorphous central mechanism. One, subsequent to medial septal lesions occasional SPWs can be inhibited by tactile stimulation during immobility or type I behaviors in the guinea pig. Two, during the 12.25 mg/kg atropine test subsequent to entorhinal lesions, type I behaviors eliminated SPWs in the absence of type I theta. Three, during these same sessions stimuli that elicited type II theta in intact animals but in entorhinal animals produced low voltage fast activity, supressed the SPWs. However, as the animals habituated to the stimuli the SPWs reappeared in the EEG record along the same time course as LIA reappears in the type II theta record when an intact animal

habituates to an incoming stimulus.

The proposition of pathway suppression in the hippocampus is not new. The trisynaptic pathway can be activated via the perforant path. Activation goes to the dentate gyrus that causes a volley in the schaffer collaterals that leads to a negative field potential (sink) in stratum radiatum and a positive field potential in the cell layer (source) of CA1. This entire process, while functional in the immobile animal, is virtually non-functional during ongoing type I behaviors (Buzsaki et al 1983).

In the type II system, pathway tonic inhibition has been suggested. Dajas et al (1983) postulated that a nicotinic acetylcholine blockade was the pharmacological mechanism used to elicit theta rhythm, although (admittedly) other factors could also be at work. Dajas et al stated that there was a balance between the nicotinic and muscarinic central actions. Blocking the nicotinic receptors with a nicotinic ligand would depress a tonic inhibition, thus allowing the manifestation of a muscarinic excitatory effect i.e. theta rhythm.

Future studies might investigate the effects of lateral or medial entorhinal lesions on theta production as the first experiment found a better, albeit transient effect with a more medial entorhinal lesion. A study disconnecting the entorhinal cortex from the rest of the

association cortex as Buzsaki et al (1983) performed in a species that reliably produces both type I and II theta also seems worthwhile.

In conclusion, as both frequency and onset of type II theta are altered following entorhinal lesions, this thesis demonstrates the importance of both the septal and entorhinal pathways in the normal function of not only type I but also type II theta production.

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