James Ronald Norman VISUAL AFFERENTATION IN THE TURTLE. (Under the direction of Dr. S. J. Putnam) Department of Biology, June 1973.

Electrophysiological studies were undertaken to solve the following problems.

(a) What is the spontaneous electrical activity of the cerebral cortex and the optic tectum of the turtle?

(b) What connections exist between the cerebral hemispheres and the optic tecti?

(c) What are the electrical characteristics of the visual input to the cerebral cortex and optic tectum?

The brain was exposed and the appropriate stimulus, i.e. electrical in "b" and stroboscoptic in "c" above, was delivered while the cortex and/or the tectum was recorded from with an electrode resting on the surface pia layer.

The studies demonstrated a concentration of the largest amplitude cortical response in the case of spontaneous activity, tectal input to the cortex and visual input, in the rostral regions of both hemispheres while the spontaneous activity and visual response appeared homogeneous in the optic tectum. Visual input to the cortex had a longer latency and was of greater amplitude than that to the optic tectum. Extirpation of the optic tectum had a "releasing" effect on the cortical response to photic stimulation. The visual response was then recorded over the entire surface whereas before extirpation, the response was limited to rostral cortical areas.

VISUAL AFFERENTATION IN THE TURTLE

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

by

James Ronald Norman

June 1973

VISUAL AFFERENTATION

IN THE

TURTLE

by

James Ronald Norman

APPROVED BY:

SUPERVISOR OF THESIS

Dr. Serpas J. Putnam

CHAIRMAN OF THE DEPARTMENT OF BIOLOGY

Dr. Graham J. Davis

DEAN OF THE GRADUATE SCHOOL

Dr. Joseph G. Boyette

ACKNOWLEDGMENT

Grateful acknowledgment is made to my friend and adviser, Dr. S. J. Putnam, who shared with me his abundant knowledge and experience at a time in both our lives when these assets were most valuable.

This thesis is lovinging dedicated to my wife, Susan, who provided the inspiration needed to complete this work.

TABLE OF CONTENTS

INTRODUCTION

In the evolution of the visual system, there is a very striking transition from mesencephalic to telencephalic afferentation. In fish and other lower forms, the retino-tectal connections constitute the primary visual projection system. Amphibians and reptiles are the first to exhibit a secondary retino-thalamo-cortical visual system, with the retino-tectal system predominating. In higher mammals, the transition is complete with the retino-thalamo-cortical system being the primary visual projection system and the retino-tectal system secondary.

This research involves one of the transition species, the turtle, and more specifically, Pseudemys scripta elegans (Schoepff). An unanesthetized animal was used to determine:

1. the spontaneous electrical activity of the cerebral cortex and optic tectum
2. connections between the two cortical hemispheres and the two optic tecti
3. visual input to the turtle brain.

REVIEW OF LITERATURE

The receptor layer of the turtle is sclerad, as is common among reptiles. The retina is non-vascular, and there is no conus (Underwood, 1970). Brown (1970) found P. scripta elegans possesses a linear area centralis. The ability to demonstrate this linear concentration of receptors depended on characteristic multi-colored oil droplets present in the turtle cone visual cells. Droplet colors demonstrated so far include bright red, orange, yellow, light green, and colorless (Forbes et al, 1958; Granda and Stirling, 1966; Walls by Underwood, 1970). Forbes and Deane (1958) suggested that three of these colored oil droplets may be filters responsible for the characteristic peak sensitivities of 645 mm (red), 620 mm (orange), and 575 mm (yellow) found in the turtle. But Granda and Stirling (1966) maintain, after compiling previous data, that more research is needed before assigning this function to the droplets.

Some authors believe that two receptor types are present; one responsible for photopic vision and the other for scotopic vision. They all agree that the former are cones but the identification of the latter has caused controversy. "In 1942, Walls summarized the evidence to date and concluded that turtles possess some non-cone structures which are probably rods" (Granda, 1962). Granda also indicated that in 1959, Verriest termed them "cone-like structures." Granda believes that the duality theory may not hold true for lower vertebrates, and that other systems may be responsible for the apparent photopic and scotopic vision.

The receptor types Walls found in the turtle, Chelydra serpentia, include major and minor single cones, double cones, and "rods"

(Underwood, 1970). He found that visual receptors have the outer receptor segments, oil droplet, elipsoid, parabloid, and nucleus, in that order (with "rod" type lacking oil droplet).

Verriest found that 50% of the outer nuclear layer is made up of displaced bipolar nuclei in Pseudemys (Underwood, 1970). These bipolar cells synapse on the ganglion cells and the axons of these cells exit from the retina.

The axons of the ganglion cells in reptiles project medio-caudally as the optic nerve after leaving the retina. Armstrong (1950) enucleated the diurnal lizard, Lacerta vivipara, and observed the degeneration using a Nonidez modification of the Cajal technique (a silver impregnation method). In 1951, Armstrong did a similar degeneration study on the snake, Natrix natrix, using the same stain. Kosareva (1967) observed degeneration of the optic pathway in Emys orbicularis, a turtle, when staining the material with both a modification of the Nauta method and the Marchi technique. In all three cases, the experimental material was compared with undamanged control tissue using various staining techniques including Nissl and Weigert-Pal.

Armstrong (1950) found that the entire optic nerve degenerates following enucleation which indicates only afferent fibers. Upon reaching the optic chiasma, the nerve splits into bundles which interdigitate with the other optic nerve bundles. The size and number have been investigated extensively and are found to be "very variable" (Armstrong, 1950, 1951). Decussation at the chiasma is incomplete, but the number of uncrossed fibers is very small, (Armstrong, 1950, 1951; Kosareva, 1967). Armstrong (1951) found that in a snake, these uncrossed

fibers continue on to the outer surface of the ipsilateral geniculate nucleus. In his degeneration studies, Kosareva (loc. cit.) found only fibers of passage in the ipsilateral geniculate nucleus. No site of termination has been found for these few uncrossed fibers.

The bundles of crossed fibers then rejoin as the optic tract and continue dorso-laterally on the ventral hypthalamus. Immediately after decussation, a small number of fibers separate from the dorsal tract and pass through the supraoptic region of the hypothalamus without synapsing, and rejoin the tract (Armstrong, 1950; 1951). As the tract continues toward the lateral forebrain bundle, a group of coarse fibers, the basal optic root or "posterior accessory optic tract," leaves the optic tract to pass onto the ventral surface of the subthalamus and tegmentum and then rostrally to enter the ventral nucleus opticus tegmenti, "slightly rostral to the exit of the oculomotor nerve" (Armstrong, 1950).

In his discussion, Armstrong (1950, 1951) suggests that this tract may function in reptiles as the rapid reflex pathway by which extrinsic eye muscles are influenced by retinal impulses since a similar tract is known to mediate this reflex in mammals. The tract is made up entirely of contralateral retinal fibers in Lacerta (Armstrong, 1950) and mixed with supraoptic fibers in snakes (Armstrong, 1951).

The optic tract continues dorso-caudally on the lateral thalamus towards the tectum of the midbrain. At a level dorsal to the lateral forebrain bundle, two groups of fibers separate from the optic tract. One group synapses in the lateral neuropil of the dorsal and ventral lateral geniculate nuclei. In the snake, some of these fibers pass

through the Nucleus Ovalis without synapsing to reach the geniculate nuclei (Armstrong, 1951). The other group passes through the geniculate to join and course with the ventral-lateral tectothalamic path to deep layers of the tectum, via the lateral pretectal region as in Lacerta and Natrix (Armstrong, 1950, 1951).

The main body of the optic tract continues on the lateral thalamus and then proceeds medially toward the tectum of the midbrain geniculate nucleus. Some fibers within the tract then proceed medially, being stem fibers and collaterals, to synapse in the lateral neuropil of the geniculate nucleus. Kosareva (1967) found fibers of passage present in almost every thalamic nucleus in the turtle, Emys orbicularis. He found the greatest amount of termination in the lateral geniculate nucleus. The rostral and midthalamic regions had more termination when considering the dorsal lateral geniculate than the ventral, while caudally there was more termination in the ventral region. He also found terminal degeneration to a lesser degree in both the Nucleus Ovalis and Suprapeduncularis of the turtle; Armstrong did not observe any terminations in these nuclei for either Lacerta (1950), or Natrix (1951).

The optic pathway has now been followed from the retina to an area where a bundle has split from the major trait to synapse in the relay structure, the thalamus. The major tract, the retino-tectal pathway, will be traced after a brief consideration, both anatomically and electrophysiologically, of the retino-thalamus-cortical system.

There is disagreement as to whether there is visual input to the ipsilateral cortical hemisphere in reptiles. Kruger & Berkowitz (1960) recorded visually evoked responses only in the contralateral cortical

hemisphere in Alligator mississippiensis. Karamian & Vesselkin (1966) received similar results using a turtle, Emys orbicularis, after both photic stimulation and electrical stimulation of the optic nerve. But in the same year, using the same species of turtle, Mazurskaya & Smirnov (1966) recorded responses in the ipsilateral as well as the contralateral cortical hemisphere after photic stimulation and electrical stimulation of the optic nerve.

The characteristics of the visual evoked responses obviously varies widely from animal to animal. In general, the following observations may be made:

(a) Photic stimulation of one eye elicits a contralateral cortical response having a latency from 60-100 msec. and amplitudes as great as 1 mv.

(b) Photic stimulation elicits a cortical response in the central rostral area corresponding to the area of the greatest amplitude of spontaneous activity.

(c) Electrical stimulation of the optic nerve elicited a contralateral cortical response in the same region as seen in photic stimulation, having a latency from 22-35 msec and amplitudes up to 1.5 mv.

(d) The initial waveform seen in the cortical evoked response in a negative deflection indicating that the initial synapse from relay enters in the dendrite layer just under the surface.

The previous four statements come from electrophysiological data in studies by Kruger & Berkowitz (1960) using Alligator mississippiensis;

Orrego (1961) using Pseudemys scripta elegans; Karamian & Vesselkin (1966) and Mazurskaya & Smirnov (1966) using Emys orbicularis.

In order to study thalamic efferents anatomically, an area of the thalamus must be lesioned and the brain stained after the degeneration has occurred. One problem encountered in such studies is that the reptilian thalamus is so small that specifically located lesions are difficult to produce. But more importantly how can fibers receiving visual information be specifically lesioned when there is such an admixture of input to the various nuclei? Therefore, the nucleus receiving the greatest visual input, i.e., the lateral geniculate nucleus, will be lesioned and the efferent tract followed with the understanding that the fibers are not carrying just visual information.

Using Pseudemys scripta, Hall & Ebner (1970) electrolytically destroyed areas of the thalamus to observe antereograde degenerations. Staining with Fink Heimer Procedure I, the pathway was seen after lesioning the dorsal lateral geniculate. Fibers project rostrally through the dorsal peduncle to join the lateral forebrain bundle. They continue rostro-laterally to terminate just under the cortical surface of the lateral portion of the rostral general cortex. After observing various other lesions, Hall & Ebner (loc. cit.) found that the dorsal lateral area of the thalamus was the only one that projects to the general cortex and that lesioning various areas of this dorsal lateral area produced degeneration which was specially organized. That is, a lesion just dorsal to the one mentioned in the dorsal lateral geniculate nucleus produced degeneration in the rostral-medial general cortex.

This work seems to support work done by Orrego (1961) on Pseudemys scripta, as his conclusion also pointed to a special orientation of thalamic input to cortex. Orrego found that visual, olfactory and somatic modalities project to discrete, well circumscribed areas. On the other hand, Karamian & Vesselkin (1966)emphasized the non-specific nature of cortical input in the reptile. They demonstrated the probability that cortical afferentation comes from two levels in the turtle; one from the dorsal thalamus with a latency of 13 to 15 msec, and one from the ventral thalamus and pretectal nucleus with a latency of 27 to 46 msec.

As was mentioned earlier, the primary visual projection system in reptiles is the retino-tectal system. This tract predominates over the retino-thalamus-cortical pathway both structurally and functionally. Karamian & Vesselkin (1966) and Mazurskaya & Smirnov (1966) using the turtle, Emys orbicularis, compared electrophysiologically visual input to the cortex and to the optic tectum. As mentioned above, the visual evoked response in the cortex has an initial negative waveform, a latency from 60-100 msec, amplitudes up to 1.0mv, and is localized in rostral areas of the cortex. Both the above mentioned teams observed that the visual evoked response in the optic tectum is of a shorter latency and duration and a smaller amplitude than that response recorded in the cortex. The latency of the response ranges from 25 to 35 msec and amplitudes may reach .4mv. An additional finding by both teams was that the tectal response is more stable than cortical response. Further, Heric & Kruger (1965) demonstrated a precise topographical organization of the retina to the optic tectum by delivering punctate

stimuli to the retina. Such organization has not been clearly demonstrated for the cortex.

As we have already followed the retino-tectal pathway to the level of the thalamus anatomically, the next area that the tract encounters as it progresses caudally is the pretectal region.

An anatomical study done by Curwen and Miller (1939) gave both spatial orientation and relative size of the nuclei involved in the turtle, Pseudemys scripta troostii. The most rostral, nucleus posterodorsalis, is immediately caudal to the nucleus rotundus and nucleus dorsolateralis of the thalamus. The largest, nucleus pretectalis, is divided into dorsal and ventral aspects and is lateral to nucleus posterodorsalis and the posterior commissure. The most lateral is the nucleus lentiformis mesencephali. Finally, Area pretectales and Nuclei of the posterior commissures are mentioned and each with various subdivisions. Curwen and Miller (1939) pointed out the "relative, primitive, undifferentiated arrangement."

As the optic tract proceeds caudally, at the rostral tectum, a group of stem and collateral retinal fibers turn medially, passing dorsally to the caudal end of the lateral geniculate nucleus and split into two groups. One group enters the nucleus lentiformis mesencephali, and the other terminates in the nucleus geniculatus pretectalis. The former group passes dorsally to the nucleus geniculatus pretectalis, forming the brachium tecti medialis. This nucleus also receives retinal fibers leaving the optic tract as it ascends on the anterior aspects of the tectum. Kosareva (1967) found much more termination in pretectal areas. These included termination in contralateral dorsal pretectal nucleus,

dorsal pretectal nucleus, entopeduncular nucleus, dorsal pretectal nuclei, nucleus geniculatis pretectalis, pretectal ventral nucleus, lentiform mesencephalic nucleus, and posterior commissural nucleus. The caudal portion of the pretectal nuclei with pretectalis ventralis and nucleus commissural posterioris also had terminals. Armstrong said that pretectal centers function in purple light reflex in animals.

Although many retinal fibers have left the tract to enter tegmentum, thalamus and pretectal centers, the great majority reach the tectum. As the tract nears the tectum it expands into a broad sheet of fibers. The rostral tract reaches the anterior and medial tectum and the caudal tract reaches the lateral and posterior tectum. There is disagreement as to the major layer innervated. Armstrong (1950 & 1951) found the majority of the tract enters layer 2 (stratum opticum) but a few entered rostrally to layer 1 (stratum zonale) and layer 3 (stratum fibrosum et grisium superficiale). Those fibers entering layer 1 descended to layer 3 to synapse. But he found the greatest termination in layer 2, especially in the rostral tectum. But using the turtle, Kosareva (1967) found that stratum opticum and stratum zonale were almost anatomically inseparable. He found the densest termination involving the largest fibers in layer 2 (stratum fibrosum et griseium superficiale). He also found termination of varying sized fibers on layer 3 (stratum griseium centrale), and fibers of passage in layer 4 (stratum album centrale). Armstrong pointed out that there was no evidence of cross over of retinal fibers to the opposite tectum.

To determine any possible electrophysiological interactions between the retino-tectal system and the retino-thalamo-cortical system,

Mazurskaya and Smirnov (1966) applied an electrical stimulus to the surface of the optic tectum while recording from the ipsilateral cortical hemispheres. They recorded an evoked potential in the rostral cortex of 16.5 msec latency which was less complex than the visual evoked response recorded from similar sites. To demonstrate that the cortical response recorded was not simply an antidromic firing, Mazurskaya and Smirnov (loc. cit.) applied a c.n.s. stimulant, Picrotoxin, to the tectal roof, and were able to record potentials in the cortex. They then set out to see if this input from the tectum to the cortex influences the cortical visual evoked response. After extirpation of the tectal roof, they found that the cortical response was still present, which provides evidence for the presence of both tecto-pallial and retino-pallial pathways. In their discussion, Mazurskaya and Smirnov (loc. cit.) suggested the possibility of the two pathways possessing common links, e.g., a tecto-thalamic connection.

MATERIALS AND METHODS

Operative Procedure

Both male and female *Pseudemys scripta elegans* were used in the experiments. They were kept in a tank of running tap water about 2.5 feet deep, and fed on a diet of hamburger and liver. To complete the vitamin requirement, an ultraviolet lamp shone down on the water's surface. Twelve hours before surgery, a turtle was removed from the tank and placed in a refrigerator at 6°C. After about eleven hours in the refrigerator and after intramuscular injection of Flaxedil (10 mg/kg) a skeletal muscle blocking agent, it was placed in the freezer compartment (-12°C) for one hour, or until the body temperature reached +1°C. Following this cold anesthesia, the turtle was placed on the operating table and the skin of the neck was infiltrated with 1 cc. of 0.5% Xylocaine. A longitudinal incision was made through the skin and the trachea was separated from underlying tissue. A horizontal incision was then made in the trachea and an "L" shaped cannula was inserted and tied with thread. The skin incision was closed using wound clips. The animal was placed in the stereotaxic apparatus and artificially respired. The respirator (Phipps and Bird) had a stroke rate of forty per minute, and a stroke volume of 12 cc.

The skin over the temporal muscles on either side of the skull was infiltrated with 0.5 cc. of 0.5% Xylocaine. Two incisions were then made; one midline incision extending from 2 mm. caudal to the tip of the nose to the posterior border of the supraocipital bone, and the second at right angles to the first, extending laterally 4 mm. on each side of

the midline and 1 mm. caudal to the posterior extent of the orbit. The four flaps of skin were reflected to expose the superior surface of the skull using the curved edge of a periosteal elevator. The flaps were held in a reflected position with small alligator clips and pulled to the side with weights. The temporal muscles were then reflected from the postfrontal and squamosal bones to procure a more complete exposure of the skull. The cranial roof was removed using rongeurs that exposed the dura from the posterior olfactory bulbs to the cerebellum. The dura was reflected using iridectomy scissors. The blood vessels attached on the ventral surface of the dura were left intact along the midline. The pia-arachoid was removed over the tectum only when this structure was to be utilized in the experiment. During the operation, exposed surfaces were covered with Ringer's solution containing:

117 mM NaCl

4 mM KCl

2 mM $CaCl_2$

1 mM $MgSO_4$

2 mM Sørensen Phosphate buffer, and

water added to make one liter and pH adjusted to 7.2 (Dawson & Bartholomew 1958). Previous to recording, the Ringer's solution was replaced with Paraffin Oil, N. F.

Experimental Procedures

At the beginning of each experiment the stereotaxic coordinates were taken for the midline of the brain, the most anterior extent of the cortex, and the most posterior extent of the tectum. These coordinates were then transferred to a piece of graph paper and the brain was indirectly gridded into square millimeters (Plate 1). The various operations took place in the center of each square. Even though the surgery was intended to expose the entire cortex and tectum, the presence of blood vessels would not allow extreme lateral or medial exposure in some cases, expecially in the medial-tectal areas. These deficiencies appear as empty areas on the plates and are indistinguishable from areas in which no response was recorded. All electrical stimulation was delivered by a Grass S-8 Stimulator (Grass Instruments, Quincy, Mass.). The output was fed through a Grass SIU-5 Stimulus Isolation Unit (Grass Instruments, Quincy, Mass.) into the stimulating electrodes. Potentials recorded by the recording electrode were fed through a Grass P-15 A. C. Preamplifier into a Tectronex Type R564B Storage Oscilloscope with Type 3A3 Dual-Trace Differential Amplifier and Type 2B67 Time Base plug-in units.

The oscilloscope beam was internally triggered when the stimulator was not used in the experiment and by the stimulator "S_1, S_2 sync. out" when it was. A bioelectric RCM-1 Reflexor was mounted on the face of the oscilloscope and a Grass C4N Kymograph Camera mounted vertically on the Reflexor. When light stimulation was required, the light flash was delivered by a Grass PS-2 Photo Stimulator. The output from the S-8 entered the "sync-in" terminal on the back of the photo stimulator and a lead from "external monitor out" entered the oscilloscope in the "positive

input" terminal. This acted to produce a stimulus artifact in the oscilloscope trace to indicate in the records the onset of the light flash. The oscilloscope trace was initiated by the S-8 stimulator thrity milliseconds before the flash occurred.

In one experiment a Birtchev Model 755 Blendtome was used to lesion the right optic tectum.

Instrumentation:

Electrode types:

a. recording electrode - NE-300 - Stainless Steel Monopolar

shaft diameter - 0.25 mm

shaft length - 10 to 150 mm

lead diameter - 0.20 mm

contact diameter - 0.25 mm

contact length - 0.50 mm

b. stimulating electrode - NE-200 - Stainless Steel Bipolar

shaft diameter - 0.5 X 1.0 mm (flattened)

shaft length - 20 mm to 150 mm

lead diameter - 0.2 mm

contact diameter - 0.2 mm with 0.5 mm separation

contact length - leads protrude from shaft 2.0 mm and are exposed 0.5 mm

c. stimulating electrode - NE-100 - Stainless Steel Concentric Bipolar

shaft diameter - 0.5 mm

shaft length -5.0 mm to 150 mm

lead diameter - 0.2 mm

contact diameter - center 0.2 mm, outer 0.5 mm

contact length - center lead protrudes from shaft 1.0 mm and is exposed 0.5 mm. The shaft (outer contact) is exposed 0.5 mm.

Instrument settings:

I. stimulator

a. electrical stimulation of brain

S_1 volts 5-10v

S_1 duration - 3.0 (x0.1)

S_1 frequency - 3.0 (x0.1)

S_1 delay - 3.5 (x0.1)

S_2 function - off

S_1, S_2 sync - S_1 del.

S_1 - pulses

b. when used to slave photostimulator

S_1 volts - 3.3 (x0.10)

S_1 duration - 15. (x0.10)

S_1 frequency - 3.0 (x0.1)

S_1 delay - 4.0 (x0.10)

II. Stimulus isolation unit

multiply input volts by - 0.1

coupling - direct

polarity - normal

III. A. C. preamplifier

input - 'use'

1/2 amplitude frequencies -0.1 to 100 H_Z

amplification - 1000X

IV. Oscilloscope

a. amplifier

input - negative terminal of channel 1

mode - Ch 1

trigger - Ch 1

volts/div - (.1-.5v)

b. time base

time/div - 50msec

slope - negative

coupling - AC fast

made - norm

V. Reflexor

data card brightness - 8 to 9

counter brightness - 7

push button

VI. Camera

film - 2 frames

film speed -0.25 (x1000) mm/sec

external control

Set I - Spontaneous Electrical Activity

Experiment 1 - Spontaneous Activity of Cortex and Tectum

After exposuring the brain, the NE-300 recording electrode was lowered on the surface of the cortex or tectum in the center of each

square millimeter and spontaneous activity was photographed and analyzed. The most characteristic responses are shown in Plate II, and the results present the range of responses.

Set II - Connections Between Cortex and Optic Tectum

Experiment II - Homotopic Recording of Cortex and Tectum

Using the NE-200 stimulating electrode, every square millimeter of one tectal lobe and cortical hemisphere was stimulated while recording from a homotopic point on the opposite lobe and hemisphere.

Experiment III - Heterotopic Recording of Cortex and Tectum

The same technique was used in this experiment as the previous one except every millimeter of the left hemisphere and lobe was recorded while each spot was stimulated on the right hemisphere.

Experiment IV - Stimulation of the Cortex While Recording From the Tectum

Every square millimeter on the cortex was stimulated using NE-100 stimulating electrodes and three sites on the right tectum were chosen for recording.

Experiment V - Stimulation of Tectum While Recording From the Cortex

The same technique was used here as in the previous experiment except four areas of the right tectum were stimulated while recordings were made from the cortex.

Set III - Visual Input to the Brain

Experiment VI - Bilateral Visual Stimulation While Recording From the Cortex and Right Tectum

After brain exposure, the photostimulator was located in a plane horizontal to and approximately 10 to 15 centimeters in front of the eyes of the turtle. As the eyes were stroboscopically stimulated, the cortical hemispheres and the right optic tectum potentials were recorded and analyzed at every square millimeter.

Experiment VII - Unilateral Visual Stimulation While Recording From the Cortex and Tectum

The same procedure was followed as in the previous experiment, except that a wet gauze and black paper patch were placed over the left eye and held in place by the orbit clamp of the sterotaxic apparatus, and the left tectum was recorded instead of the right. The reason only one tectal lobe was used in most experiments is that the dura had to be pushed aside for complete exposure of one lobe, and too much manipulation inevitably caused hemorrhaging.

Experiment VIII - Bilateral Visual Stimulation While Recording From the Cortical Hemispheres After Lesion of the Right Optic Tectum

Following exposure of the brain, the intact dura along the midline was retracted using weights, and the right optic tectum was lesioned using the blendtome. A number of preliminary experiments based on previous investigations provided evidence that the entire tectal cortex was lesioned.

These experiments were,

a) responses in the cortex after stimulation of the tectum were compared before and after the lesion

b) responses in the tectum after photostimulation of the eye were compared before and after the lesion.

After we were satisfied that the tectum was destroyed, the same experimental procedure was followed as that in Experiment VI except that recording was from only the cortex.

Experiment IX - Unilateral Visual Stimulation While Recording From the Cortical Hemisphere After Lesion of the Right Optic Tectum

Usint the same animal which was used in Experiment VIII, a wet gauze and black paper patch were placed over the right eye and held in place by the orbit clamp of the stereotaxic apparatus while the experimental procedure of Experiment VII was followed except that recording was from the cortex.

RESULTS

The results of the experimentation will be divided into the three sets mentioned in the Materials and Methods. Set I characterizes the spontaneous activity of the turtle brain; Set II connections within the cortical hemispheres and superior colliculi; and Set III, visual input to the brain.

Set I included one experiment demonstrating the frequency and amplitude of the spontaneous electrical activity in both the cortical hemispheres and the superior colliculi (Plate II). The cortical sites demonstrating the greatest amplitude of spontaneous activity were LC-8, 16-18, 26,27,36,37 and the homotopic sites on the opposite hemisphere. The amplitude reached a peak amplitude of 0.56 mv at LC-31 and the frequency averaged 55 cycles per sec. The tectal spontaneous electrical activity appeared homogeneous over the surface with amplitudes averaging 0.05 mv and frequencies similar to those in the cortex.

Set II included a group of four experiments involved with determining the presence of and characterizing the connections between the two major recipients of visual information, the cortical hemispheres and optic tecti. The first experiment was performed to examine possible intercortical or intertectal connections. An electrical stimulus was delivered over the surface of the hemisphere or lobe, while simultaneously recording over the surface of the opposite hemisphere or lobe. The second experiment was performed to determine connections leading from the cortex to the optic tectum and was done by applying an electrical stimulus over the cortical surface while simultaneously recording over the tectal roof. Finally, the third experiment was performed to verify

the presence of tecto-cortical connections by electrically stimulating the tectal surface while simultaneously recording from the cortical surface.

Application of an electrical stimulus to every site in one hemisphere elicited no evoked response in any site of the opposite hemisphere. Similarly, an evoked response could not be evoked in the tectal lobe by stimulation of every site in the opposite lobe.

The experimental procedure for determining cortical input to the optic tectum would obviously involve stimulation of all 66 cortical sites while simultaneously recording from all 28 tectal sites. From this data, at determination of the topographical organization of the cortex onto the tectum could be determined. A pilot study was performed in which three freely accessible sites RT-15, 21, 22 (Plate I) were recorded, while all 66 cortical sites were stimulated in order to verify the need for the extensive study mentioned above.

Input to RT-15 After Stimulation of the Cortex

Evoked responses were recorded at RT-15 from 11 of the 66 cortical sites stimulated; RC-4, 11, 12, 13, 23, 44, 50, 61, 62 and LC-2, 3, 56, 58 (Plates I, III, IV). At each site except LC-58, the initial waveform recorded was positive with a peak latency range of 21-76 msec and an amplitude range of 0.046 - 0.306 mv. Site LC-58 elicited an initial negative potential with a peak latency of 52 msec and and amplitude of 0.04 mv. The second waveform recorded at RT-15 from each cortical site stimulated, excluding LC-2 and LC-58, was a negative potential with peak latency ranges of 85-155 msec and amplitude ranges of 0.040 - 0.130 mv.

The positive potential evoked when stimulating LC-2 contained two peaks. The first having a peak latency of 25 msec and an amplitude of 0.160 mv; the second a peak latency of 68 msec and an amplitude of 0.130 mv. The third waveform was negative, corresponding in latency and direction to the above mentioned "second waveform" having an amplitude of 0.165 mv. Site LC-58 evoked a positive potential as its second waveform with a peak latency of 128 msec and an amplitude of 0.042 mv.

Input to RT-21 After Stimulation of the Cortex

Only two of the 66 cortical sites stimulated (LC-3 and RC-50) elicited a response. LC-3 elicited an initial positive response with a peak latency of 55 msec and an amplitude of 0.060 mv. The second waveform was negative with a peak latency of 133 msec and an amplitude of 0.050mv. RC-50 elicited an initial positive waveform, the peak latency of which was only 5 msec and the amplitude of which was 0.098 mv. It was followed by a negative potential with a peak latency of 50 msec and an amplitude of 0.120 mv and then by a positive potential having a peak latency of 94 msec and an amplitude of 0.040 mv.

Input ot RT-22 After Stimulation of the Cortex

Sites LC-3 and RC-4 elicited an initial positive response that had peak latencies of 36 and 41 msec and amplitudes of 0.130 and 0.264 mv. respectively. LC-3 elicited a second peak on the positive deflection with a peak latency of 68 msec and an amplitude of 0.144 mv. The peak latency of the third potential (133 msec) the direction (negative) and the amplitude (0.248 mv) was the same as the second potential recorded after stimulation of RC-4.

The final experiment in Set II involved characterizing the tectal input to the cortical hemispheres. To fully analyze the tectal input to the cortex, all 28 tectal sites should be stimulated while simultaneously recording from all 66 cortical sites. But as I only wished to find a connection, characterize the input, and look for topographical organization, a subexperiment of lesser magnitude was performed. Four widely separated tectal sites, RT-14, midway between 8 and 15, RT-20 and RT-23 (Plate I), were stimulated electrically while recording from all 66 cortical sites. Connections were found, and while characterizing the input, a repeating deflection latency pattern was noted. It was seen that the individual deflections comprising the response, either positive or negative, could be classified statistically according to their peak latencies: Between 16 and 35 msec, Class I; between 45 and 70 msec, Class II; and over 85 msec, Class III. This classification system was used in subsequent experiments, but the latencies mentioned above apply only to this particular set of experiments. The next step was to look for a topographical organization. As can be seen from Plates V, VI, VII, and VIII, the cortical sites eliciting the initial waveform with the greatest amplitude shifted from RC-32 in V, to RC-23 in VI, to RC-33 in VII, to RC-21 in VIII. This observation obviously intorduced the possibility that the optic tectum may in some way be spatially represented in the cortex. An informal follow-up study was performed in which widely separated tectal points were electrically stimulated while the cortex was recorded without the mm block limitation. The recording electrode was moved around while stimulating one tectal site, until the spot of

greatest amplitude was found. The stimulating electrode was then moved and again the cortical spot demonstrating the greatest amplitude was found. After four such moves, and combined with the previous results, a judgement was made as to the topographical organization of the tectum onto the cortex.

Input to the Cortex after Stimulation of RT-14

Evoked responses were found only in the ipsilateral hemisphere at RC-5, 6, 11-13, 21-23, 30-33, 40-43, 53 (Plates I, V). Of these, all but RC-53 responded with a Class I negative deflection as its initial waveform. The greatest amplitude of response was recorded at RC-32 (1.695 mv) and the amplitude decreased in all directions from this point; the rhinal sulcus being the lateral extent of the response. RC-53 elicited an initial low amplitude negative response having the characteristic latency of Class II.

All sites except RC-5, 6, 11, 12, 32, 33, 42, 43 exhibited a Class II negative deflection as a second waveform with RC-12 and 43 both exhibiting the greatest amplitude response (0.445 mv).

A Class III positive deflection was recorded at RC-13, 22, and 33, the greatest amplitude being 0.345 mv at RC-22.

Input to the Cortex After Stimulation of a Point Midway Between RT-8 and RT-15

Thirty-one cortical sites responded to stimulation of this tectal site. Ipsilateral potentials were recorded at RC-12-14, 20-24, 31-34, 41-44, 51-54, 60-62, and contralateral responses at LC-16-18, 35, 38, 46-48. (Plates I, VI). Within these responses, a pronounced Class I negative response was seen at RC-12-14, 22-24, 32-34 with the greatest amplitudes at RC-23 (1.150 mv); while RC-21, 31, 42-44, 52-54,

61 exhibited this negative Class I potential as a hump on the initial slope of the Class II response. Further, a low amplitude Class I positive waveform was seen in RC-41, 60, and 61.

RC-12-14, 20, 41, 51, 60 and LC-16-18, 35, 38, 46-48 exhibited a Class II negative deflection with the greatest amplitude recorded from LC-17 (0.44 mv); while RC-21-24, 31-34, 42-44, 53-54, 61, 62 exhibited Class II positive deflection. The area of greatest amplitude of this positive waveform included a large area. RC-53 and 54 both recorded amplitudes of 0.75 mv and RC-22-24, 32-34, 42-44, 52, all had amplitudes exceeding 150 mv.

A Class III negative waveform was recorded at RC-21-24, 31-34, 42-44, 52-54 with RC-24 exhibiting the greatest amplitude response (0.55 mv). RC-51 and 60 and LC-48 demonstrated a Class III positive deflection with RC-23 having the greatest amplitude (0.30 mv).

Input to the Cortex After Stimulation of RT-20

Responses were evoked at RC-11-13, 20-23, 30-33, 40-43. (Plates I, VII). All of these evoked responses contained a Class I negative deflection as the initial waveform. The greatest amplitude deflection was recorded at RC-21 (1.45 mv), with RC-21, 22, 31, 32 all exhibiting amplitudes exceeding 1.0 mv.

A Class II deflection was also negative and was pronounced at RC-11, 12, 20, 22, 30 while recorded only as a hump on the descending arm of the Class I negative potential at RC 13, 21, 22, 31, 32. The greatest amplitude of this Class II deflection was recorded at RC-12, (0.35 mv), with the remainder (vis., RC-11, 20, 22, 30) being 0.20 mv or over.

RC-42 exhibited a Class III negative waveform having an amplitude of 0.20 mv while RC-13, 21, 22, 32 exhibited a Class III positive deflection. The greatest amplitude positive waveform was recorded at RC-21 and 22 (0.25 mv).

Input to the Cortex After Stimulation of RT-22

Evoked potentials were elicited in RC-6, 11-14, 21-23, 31-33, 42-44, 53, 54, 61, 62. (Plates I, VIII). RC-6, 11-14, 22, 23, 31-33, 43, 44 exhibited a Class I negative deflection initial waveform with RC-33 having the greatest amplitude response (1.0 mv). RC-53, 61 and 62 demonstrated a response containing a Class I positive waveform with RC-61 exhibiting the greatest amplitude deflection (0.10 mv).

Class II waveforms were recorded as negative deflections as RC-11-13, 21, 22, 31-33, 42, 43, 53, 54. The amplitude of the deflection reached a peak at RC-32 (0.70 mv).

RC-22 and 33 both exhibited a Class III positive waveform having an amplitude of about 0.20 mv.

Visual Input

Set III involved a set of 4 experiments which were performed to demonstrate and characterize the visual input to the brain. The stimulus in all cases was a stroboscoptic stimulus. The stimulus was delivered bilaterally to observe evoked potentials in the cortical hemispheres and optic tectum, and compared with the evoked potentials elicited by unilateral photic stimulation. To determine interaction between the retino-tectal system and retino-thalmo-cortical system, the tectal roof was electrolytically lesioned and the visual evoked response was again observed in the cortex.

Visual Input to the Cortical Hemispheres - Bilateral: Animal A

Bilateral photic stimulation elicited responses in LC-1, 7-9, 15-18, 25, 26, 28, 36, 38, 46-48 and RC-6, 13, 14, 22-24, 33, 34, 41, 51-53 (Plates I, IX). After characterizing waveforms comprising the response, a definite demarcation could be seen in rostral and caudal potentials, Rostrally, the responses were of a higher amplitude and could be classed according to peak latencies. The classification involves Class I deflection with peak latencies from 78 to 105 msec, Class II from 110 to 160 msec, and Class III from 190 to 290 msec.

Rostrally, there was a Class I negative waveform recorded at all sites, i.e. LC-1, 7-9, 15-18, 25 (with a mean peak latency of 85 msec), and RC-6, 13, 14, 22-24, 33, 34 (with a mean peak latency of 93 msec). The greatest amplitude Class I deflection was recorded from LC-7 (0.83 mv) in the left hemispheres and from RC-23 (0.98 mv) in the right.

A Class II negative deflection was recorded from LC-1, 7-9, 15, 16, 18 and RC-6, 13, 14, 22, 23, 33, 34 with amplitudes recorded as high as 0.45 mv (RC-14).

A Class III deflection was common to LC-7, 16 and RC-13, 14, 21, 23. In all but RC-23, this waveform was positive.

Caudally, LC-26 exhibited six low amplitude deflections having peak latencies ranging from 17 to 250 msec. LC-28, 38, 46-48 and RC-41, 51-53 all responded with the same potential; that being a low amplitude, (0.1 mv or below) response, with four deflections having peak latencies ranging from 45-105 msec. LC-36 exhibited an evoked response containing an initial 0.1 mv positive deflection, and a second 0.15 mv negative deflection with peak latencies of 100 and 165 msec respectively.

Visual Input to the Optic Tectum - Bilateral: Animal A

Bilateral photic stimulation elicited a response in all eleven exposed sites recorded on the right tectum, i.e. RT-7, 8, 13-15, 20-22, 26-28 (Plates I, IX). The remaining sites, RT-2, 6, and 16, were inaccessible. The evoked responses were of relatively low amplitude and seemed to fall into two categories; those in the anterio-medial sites which displayed an initial negative waveform, and those in the posterio-lateral sites which displayed an initial positive waveform.

RT-7, 13, 14, 20, 26 exhibited an initial negative deflection with amplitudes ranging from 0.08 to 0.17 mv and peak latencies from 45 to 60 msec. The waveforms following this peak were low in amplitude (0.15 mv or below) and contained peak latencies from 65 to 180 msec.

RT-8, 15, 21, 22, 27, 28 exhibited initial positive waveforms with peak latencies ranging from 25 to 85 msec and amplitudes from 0.05 to 0.24 mv. Waveforms following this positive peak were again low in amplitude (below 0.15 mv) with latencies extending up to 223 msec.

Visual Input to the Cortical Hemispheres - Unilateral: Animal B

Unilateral photic stimulation elicited responses only in the contralateral sites LC-1, 7-9, 16-18, 26-28, 36, 37 (Plates I, X). As in the Bilateral: Animal A experiment, the rostral sites displayed evoked potentials which fit the classification set up, whereas the caudal sites did not.

The rostral sites included LC-1, 7-9, 16-18, 26, 27 and in all cases except LC-26, the initial waveform was a Class I negative deflection. In LC-26, the Class I negative deflection was preceeded by a prepotential. The greatest amplitude of this Class I waveform was

recorded at LC-16 (1.2 mv) with LC-8, 17, 26, 27 all recording over 0.70 mv. This Class I deflection exhibited a double peak at LC-8, 16-18, 26, 27 (Plate X). The peak analyzed in these cases was the initial one.

A Class II negative response was seen at LC-8, 17, 18, 27 with the greatest amplitude being recorded at LC-18 (0.5 mv).

Caudally, LC-28 exhibited a long duration negative deflection with about five peaks, reaching 0.725 mv at the highest point. LC-36 exhibited a 0.43 mv negative and a low amplitude positive deflection with peak latencies of 115 and 245 msec. Finally, the evoked response at LC-37 consisted of a single 0.50 mv negative potential with an initial peak latency of 130 msec.

Visual Input to the Optic Tectum - Unilateral: Animal B

Unilateral photic stimulation elicited responses in all recorded contralateral tectal sites; LT-3-5, 10-12, 17-19, 24, 25. The only sites inaccessible to recording were LT-1, 9, 23 and RT-2, 16, 28. As in the Bilateral: Animal A experiment, the anterio-medial response could be distinguished from posterio-lateral responses according to the direction of the initial waveform.

Lt-3-5, 11, 12, 19, 25 exhibited an initial negative response ranging in amplitude from 0.10 to 0.45 mv and in initial peak latency from 22 to 60 msec. The waveforms following this peak included peaks both positive and negative with amplitudes ranging from 0.10 to 0.25 mv and latencies up to 270 msec.

LT-10 17, 18, 24 exhibited an initial positive waveform with

amplitudes ranging from 0.25 to 0.30 mv with initial peak latencies from 45 to 55 msec. The deflections following this peak included both positive and negative potentials having amplitudes up to 0.35 mv and peak latencies up to 240 msec.

Visual Input to the Cortical Hemispheres - Bilateral: Animal C

To further characterize the major input to the cortex, the experiments performed on Animal C present only evoked potentials containing waveforms exceeding an amplitude of 0.15 mv. As can be seen in comparing the plates from Animals A and B (Plates IX, X), and Animal C (Plates XI, XII), the caudal cortical responses present in Animals A and B are not presented here.

Bilateral photic stimulation elicited a response in LC-1, 2, 7-9, 16-18, 25, 26 and RC-5, 6, 12-14, 21-23, 31-34. The waveforms present in the responses fit a classification of peak latencies, but the limits had to be re-arranged from the Animal A experiments. Class I deflections are those waveforms with peak latencies ranging from 110 to 125 msec, Class II from 150 to 190 msec, and Class III from 240 to 300 msec.

All of the above mentioned sites exhibiting a response except LC-2, 17 and RC-21 demonstrated a Class I negative deflection as their initial waveform. The latter three sites exhibited this Class I negative response preceeded by low amplitude prepotentials. The greatest amplitude of this Class I response was seen at LC-16 (0.90 mv) in the left hemisphere and RC-23 (0.60 mv) in the right.

A Class II negative response was demonstrated in LC-7, 8, 9, 16-18, and RC-13, 21-23, 31-33; in all cases except LC-8, this was the deflection immediately following the Class I.

A Class III response was seen in LC-1, 7-9, 16, 17, 26, and RC-23, 32. In all cases except RC-23, the deflection was positive.

Visual Input to Cortical Hemispheres - Unilateral: Animal C

Unilateral photic stimulation elicited a response in the contralateral sites LC-1, 7-9, 16-18, 25-28 (Plates I, XII). The deflection of the evoked response was classified according to the peak latency characteristics set up in the Bilateral: Animal C experiment.

A Class I negative deflection was the initial waveform recorded in each of the above mentioned sites except LC-17, where a low amplitude positive-going prepotential preceeded it. The greatest amplitude recording was from LC-16, where this Class I deflection was recorded as a 0.74 mv potential.

A Class II negative potential was recorded from LC-7, 16, 17, 27, 28 where it was the waveform immediately following the Class I deflection.

Finally, a Class III positive deflection was recorded from LC-7, 17, 26, 27 which was low in amplitude and of a long duration.

Visual Input to Cortical Hemispheres After Lesion of the Right Optic Tectum - Bilateral

Bilateral photic stimulation elicited responses in RC-5, 6, 12-14, 21-23, 31-33, 42-44, 52-54, 60-62, and 66 (Plates I, XIII). Only RC-4, 11, 14 were inaccessible. The waveform pattern could again be classified according to peak latencies. Class I deflections were those with peak latencies ranging from 98-108 msec, Class II, from 128 to 165 msec, and Class III from 185 to 310 msec.

A Class I negative deflection was recorded as the initial waveform at RC-13, 14, 22, 23, 32, 42-44 with a mean initial peak latency of

100 msec. The greatest magnitude Class I response was recorded from RC-23 (1.185 mv).

A Class II deflection was recorded as the negative deflection reading on the descending slope of the Class I waveform in all the previously mentioned sites and as the first negative waveform in RC-5, 6, 12, 21, 31, 52-54, 60-62, 66. The greatest amplitude Class II response was recorded from RC-23.

A Class III negative response was recorded at RC-33, 52, and a Class III positive waveform at RC-60.

Visual Input to the Cortical Hemispheres After Lesion of the Right Optic Tectum - Unilateral

Unilateral photic stimulation elicited evoked potentials in RC-5, 6, 12-14, 21-24, 31-34, 42-44, 52-54, 60-62, 65, 66, when using the same animal used in the bilateral experiment (Plates I, XIV). The waveform pattern conformed to classification based on the peak latency ranges set up in the previous experiment.

RC-22, 23, 32-34, 43, 44 exhibited a Class negative deflection with a mean initial peak latency of 102 msec. The amplitude ranged from 0.25 to 1.25 mv decreasing in all directions. The sites exhibited a Class II negative deflection as their second waveform, while RC-5, 6, 12, 21, 24, 31, 42, 52-54, 60-62, 65, 66 exhibited this deflection as their first. The Class II waveform was of greatest magnitude at RC-44 (0.77 mv) decreasing in all directions with the least decrease occuring medio-anteriorly.

The Class II deflection was negative in direction and was exhibited as the second waveform in RC-5, 6, 12-14, 52, 53, 62 and the third at

RC-22, 33, 34, 43.

To insure control for this experiment, even when data was only recorded from the right cortex, the left hemisphere was tested at selected sites to assure a continuing lack of visual input.

PLATE I

Dividing the cortical hemispheres and tectal lobes of the turtle into squares 1 mm by 1 mm gave 66 cortical and 28 tectal squares. The squares were numbered according to the scheme in the adjacent plate and are referred to as RC-42 (right cortical hemisphere - square 42) or LT-11 (left tectal lobe - square 11) throughout the paper. The lines presented in this plate extending from LC-8 through LC-16 and ending in LC-25 and in homotopic squares on the opposite hemisphere represents the orientation of the rhinal sulcus.

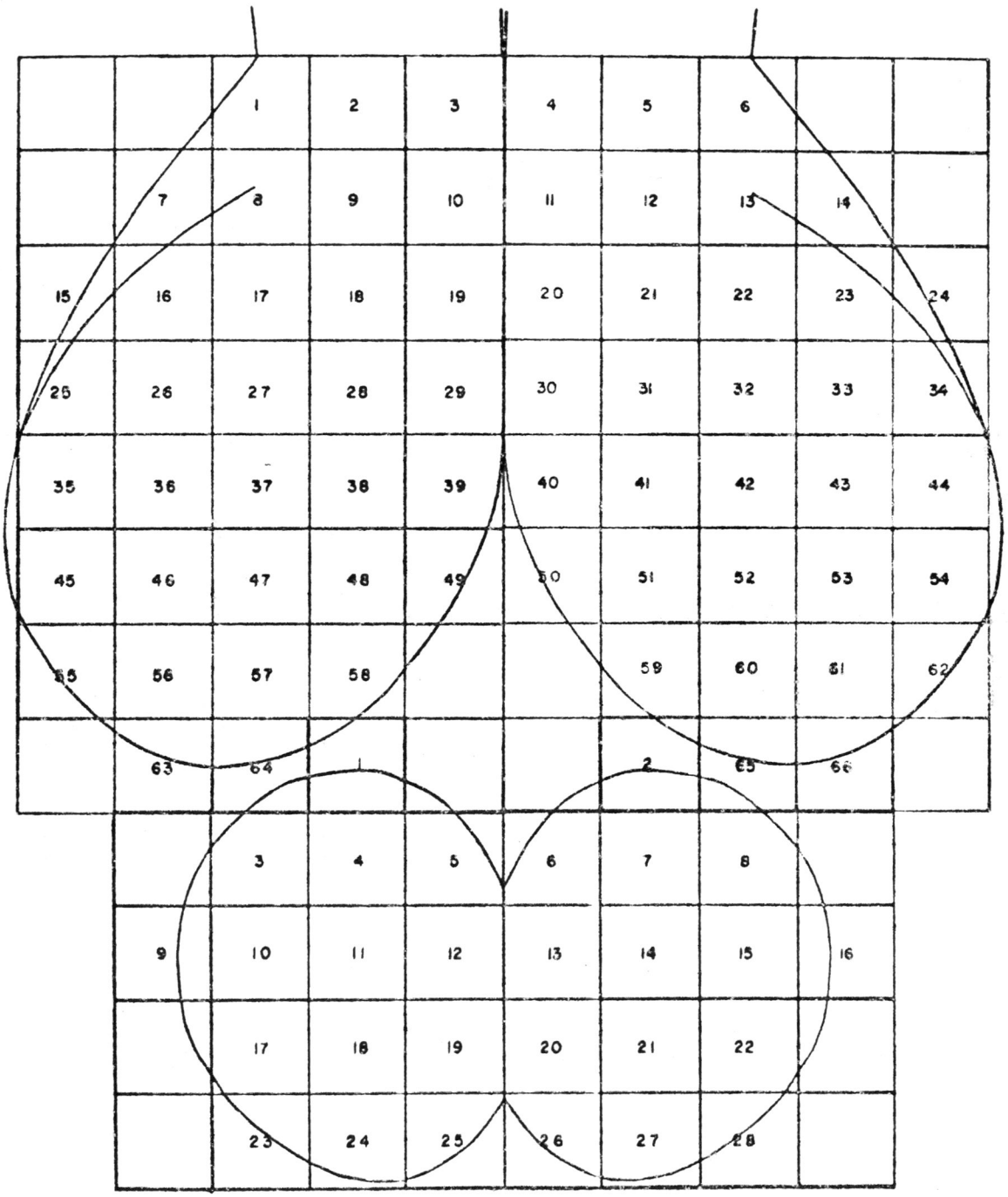

1 2 3 4 5 6
7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24
25 26 27 28 29 30 31 32 33 34
35 36 37 38 39 40 41 42 43 44
45 46 47 48 49 50 51 52 53 54
55 56 57 58 59 60 61 62
63 64 1 2 65 66
3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22
23 24 25 26 27 28

PLATE II

Spontaneous activity of the cortex and optic tectum recorded from the center of each accessible cortical and tectal square.

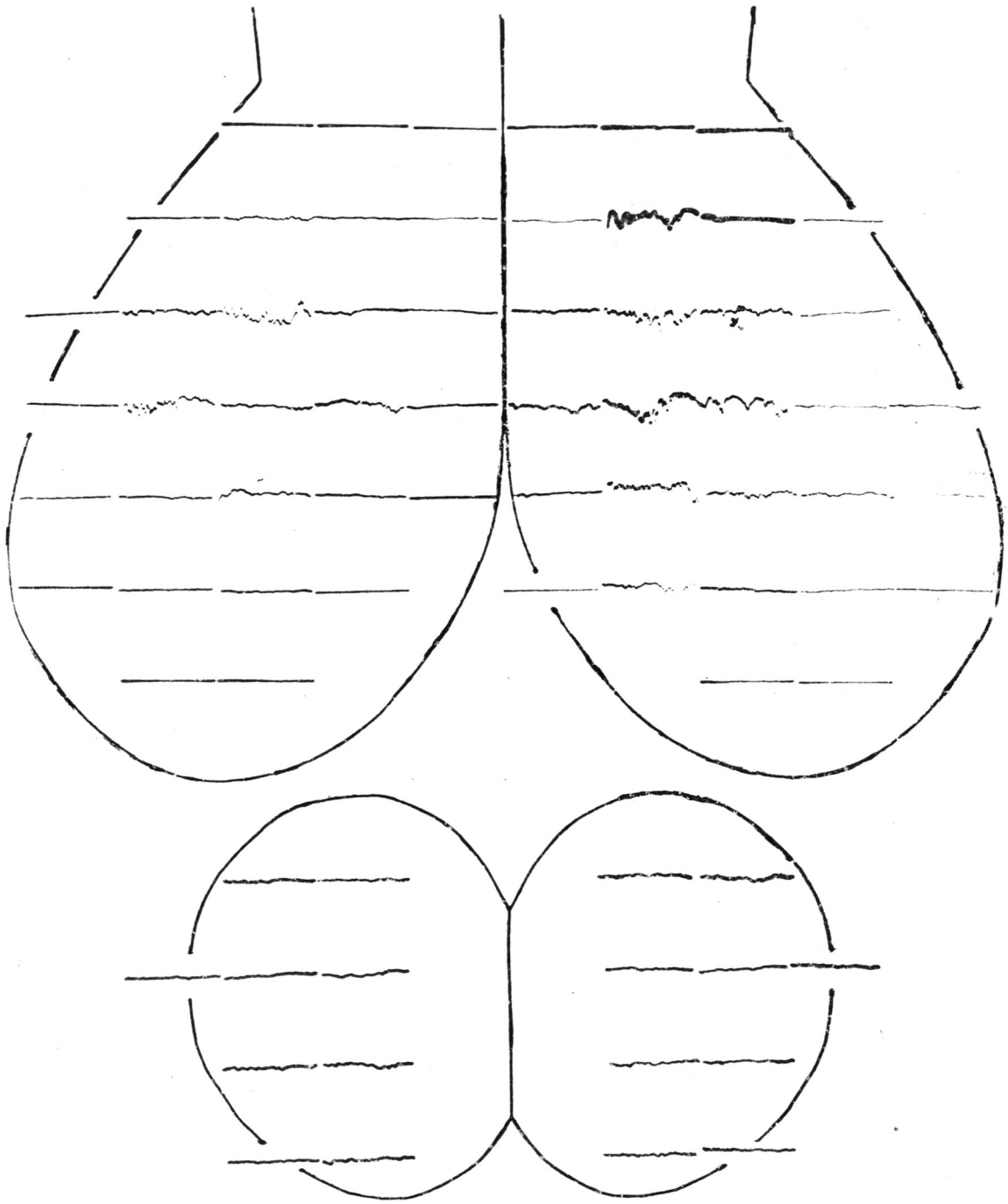
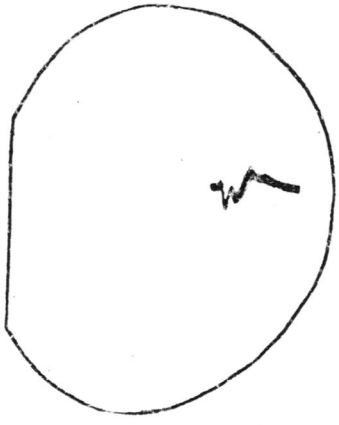
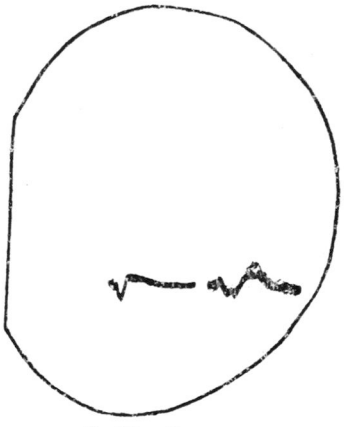

1.0 v
0.5 sec

PLATE III

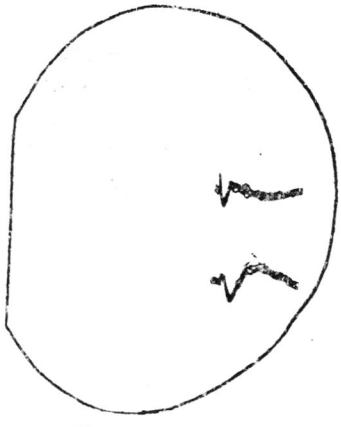
In determining the cortical input to the tectum, each of the 66 cortical squares were stimulated electrically while 3 selected tectal sites were recorded. (RT-15, 21 and 22). This plate presents responses recorded at the above mentioned sites after stimulation of cortical squares LC-2, 3 and RC-4, 11, 12, 13 (Plate I). If no response was recorded after stimulation of the cortex, the tectal site is represented as a blank.



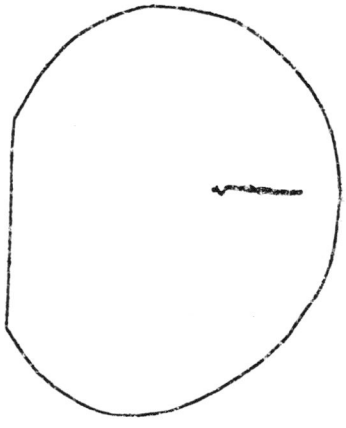
LC 2



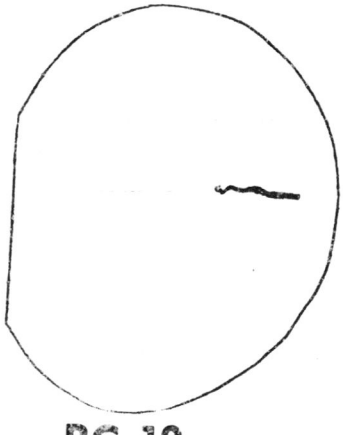
LC 3



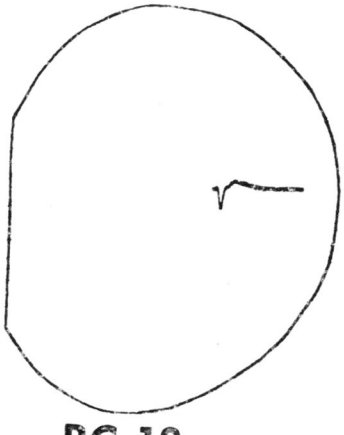
RC 4



RC 11



RC 12



RC 13

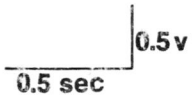
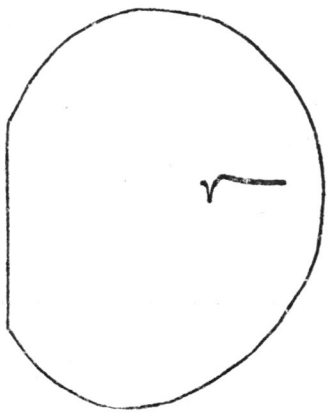
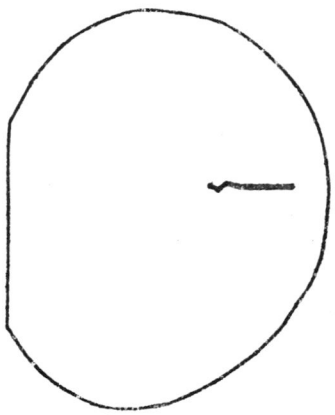


PLATE IV

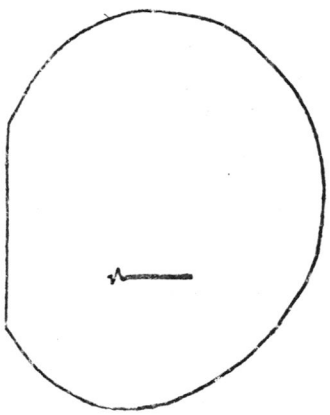
Responses recorded at RT-12, 21 and 22 after stimulation of cortical squared LC-56, 57 and RC-23, 44, 50, 61, 62. (Plate I) As in Plate III, lack of a response in one of the three tectal sites is represented as a blank.



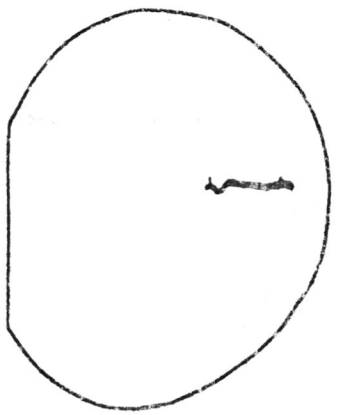
RC 23



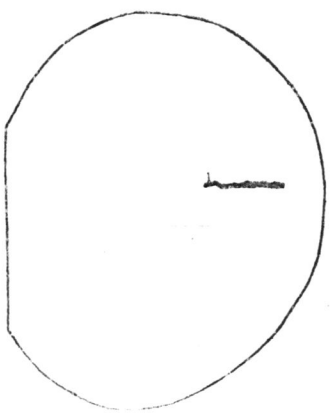
RC 44



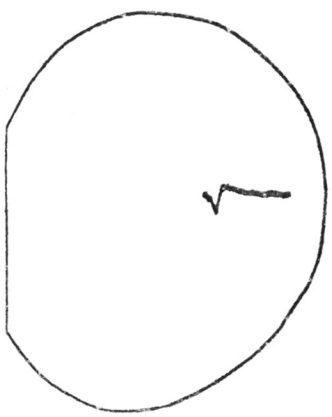
RC 50



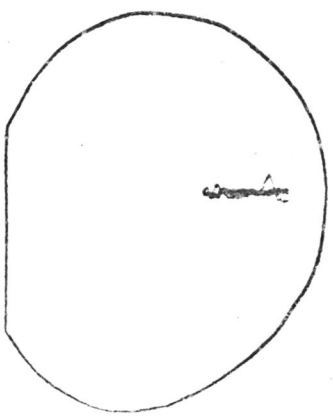
LC 56



LC 58



RC 61



RC 62

0.5v

0.5 sec

PLATE V

Responses elicited in the cortex after stimulation of RT-14 (represented by black spot on tectum). Plate I

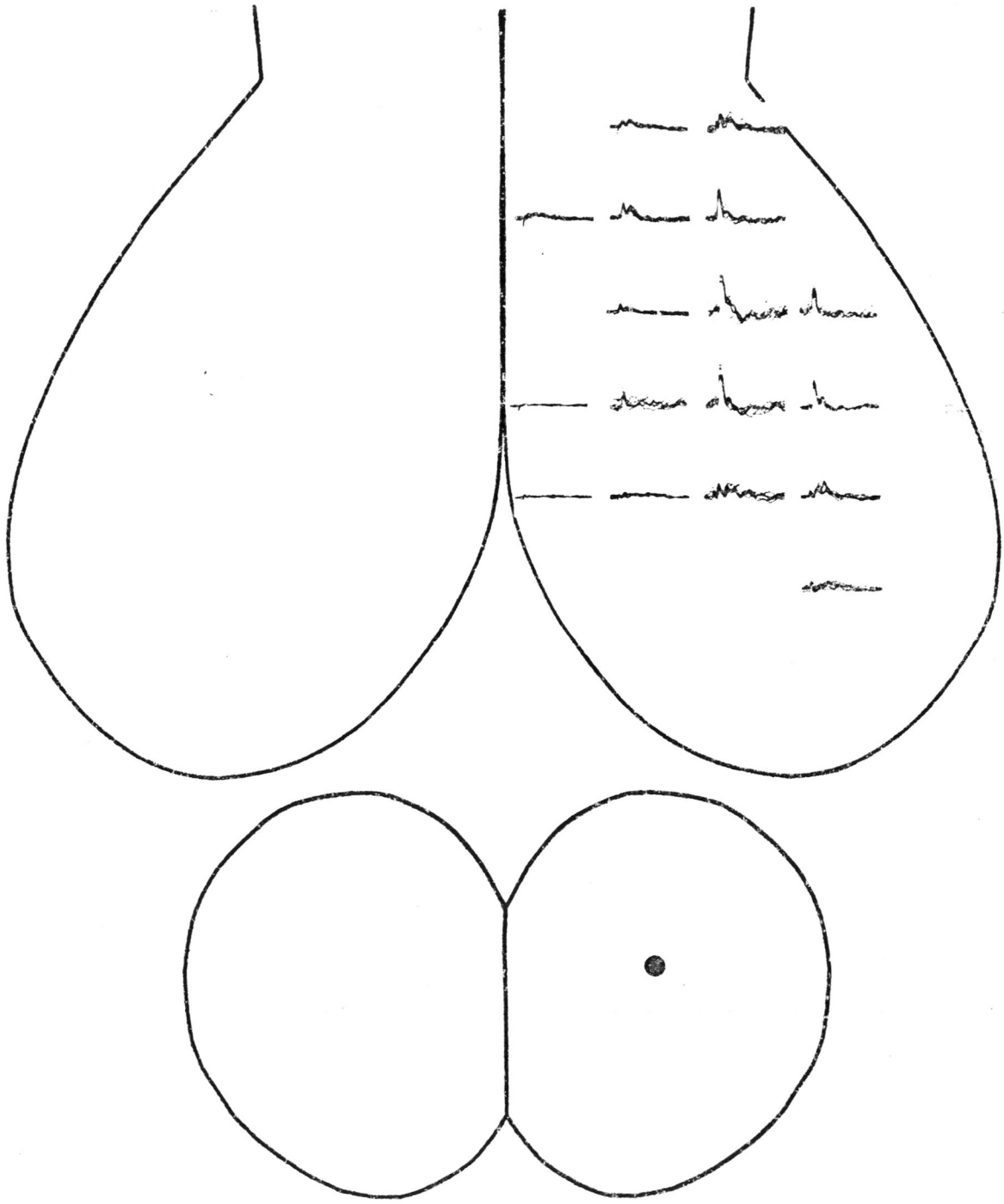

0.5 v
0.5 sec

PLATE VI

Responses elicited in the cortex after stimulation of a site located between RT-8 and RT-15 (represented by black spot on tectum). Plate I

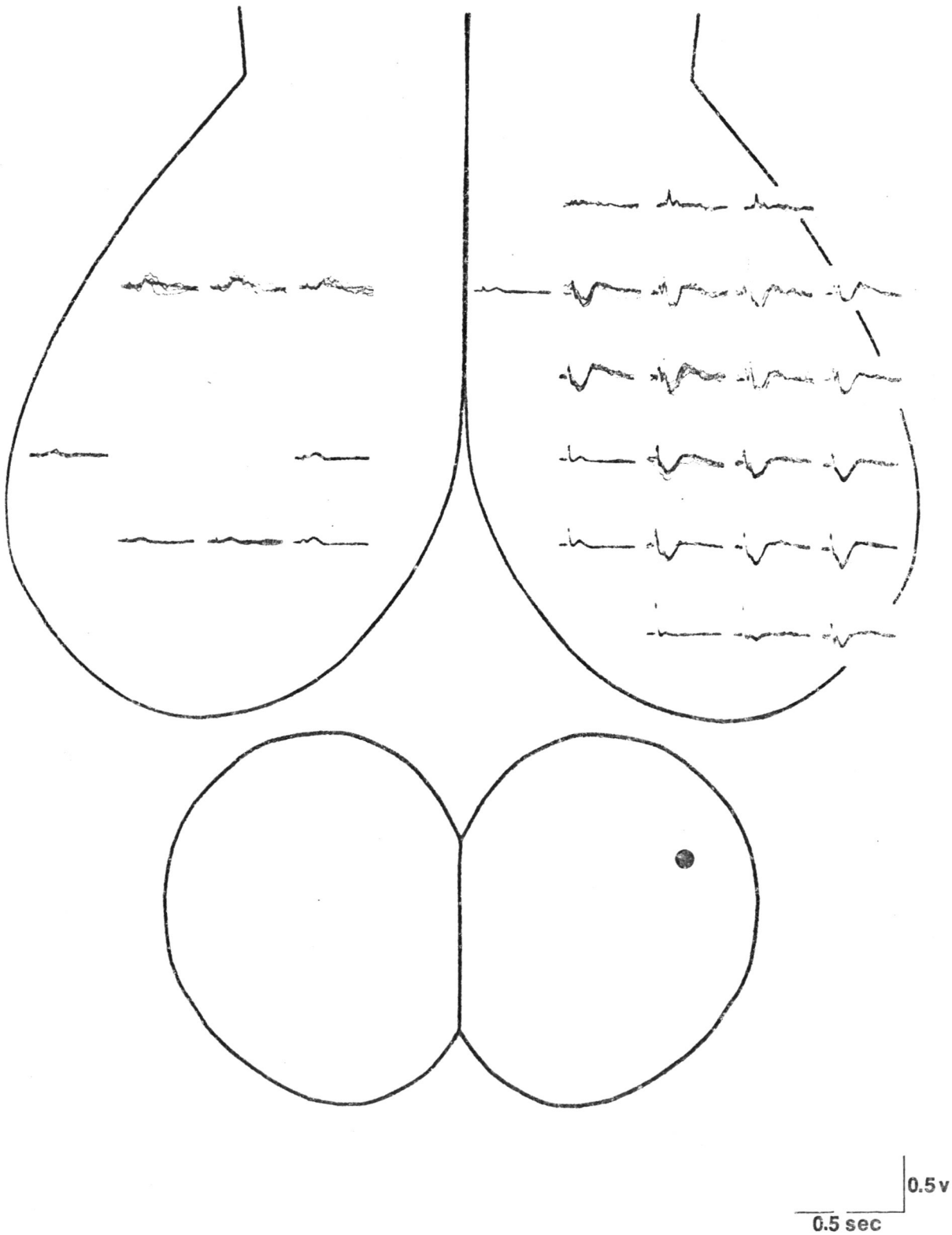

0.5 v
0.5 sec

PLATE VII

Responses elicited in the cortex after stimulation of RT-20 (represented by a black spot on tectum). Plate I

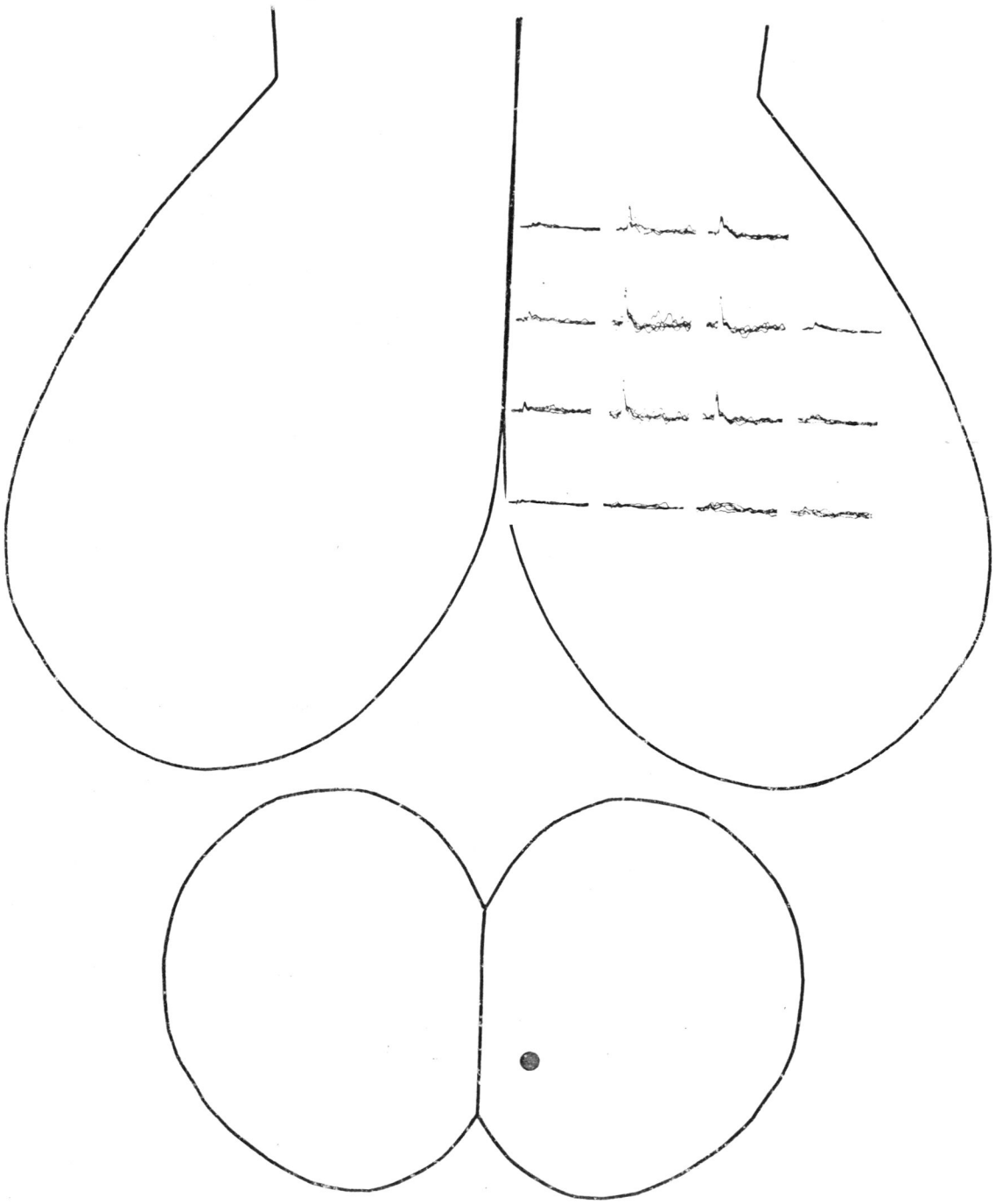

0.5 v
0.5 sec

PLATE VIII

Responses elicited in the cortex after stimulation of RT-22 (represented by a black spot on tectum). Plate I

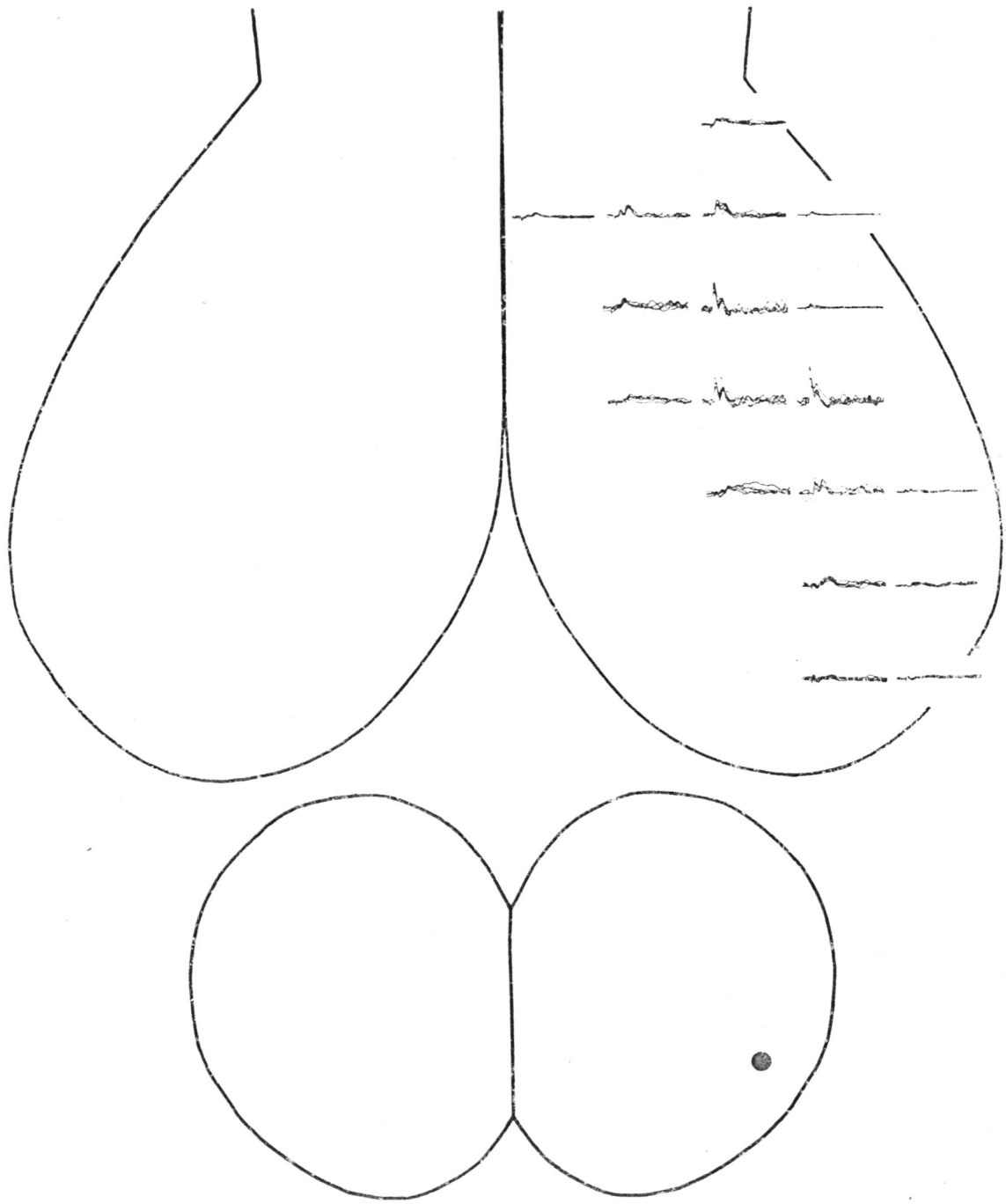

0.5 v
0.5 sec

PLATE IX

Responses elicited in the cortical hemispheres and right optic tectum after bilateral stroboscopic stimulation.

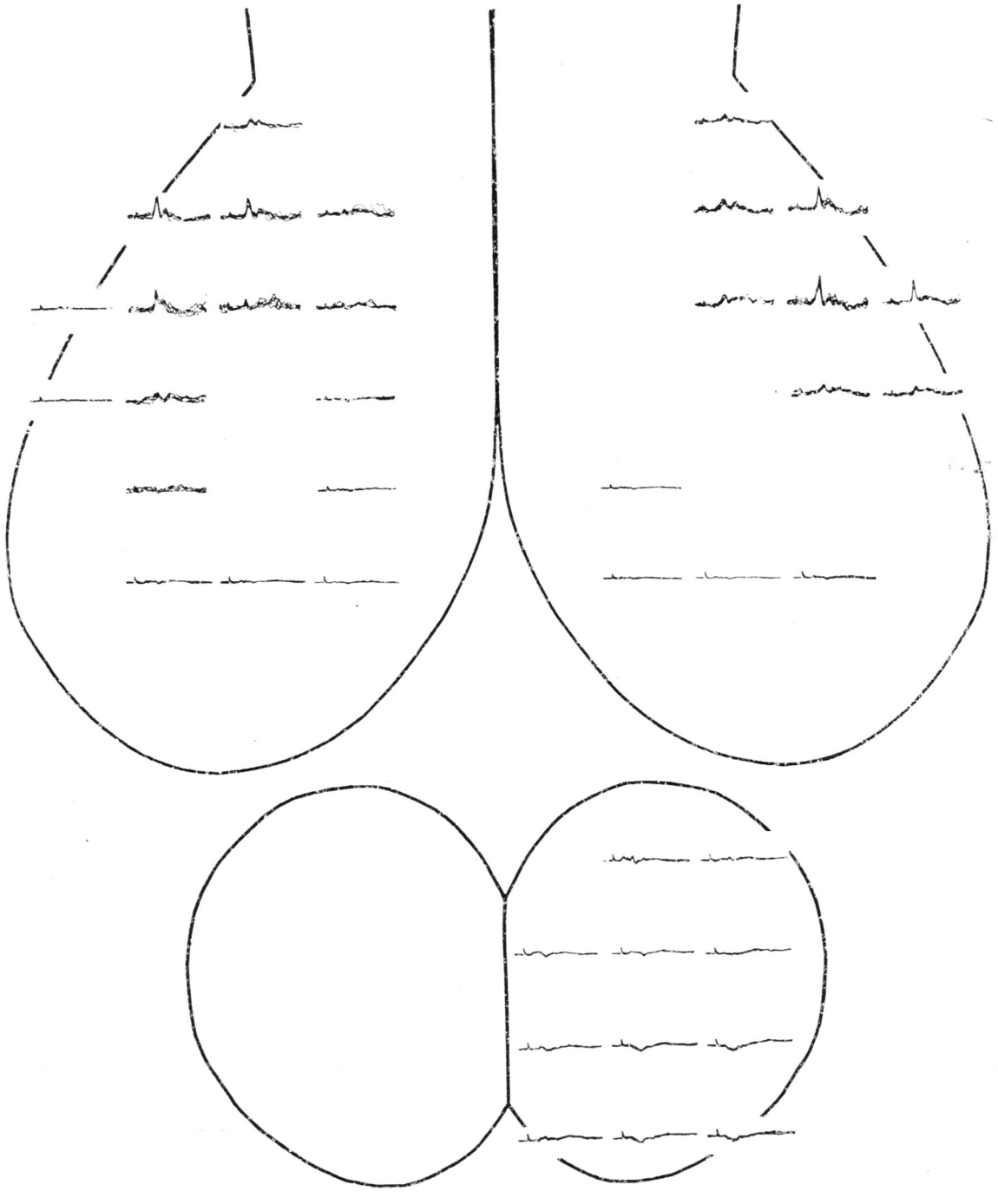

0.5 v
0.5 sec

PLATE X

Responses elicited in the cortical hemispheres and optic tectum after unilateral stroboscopic stimulation.

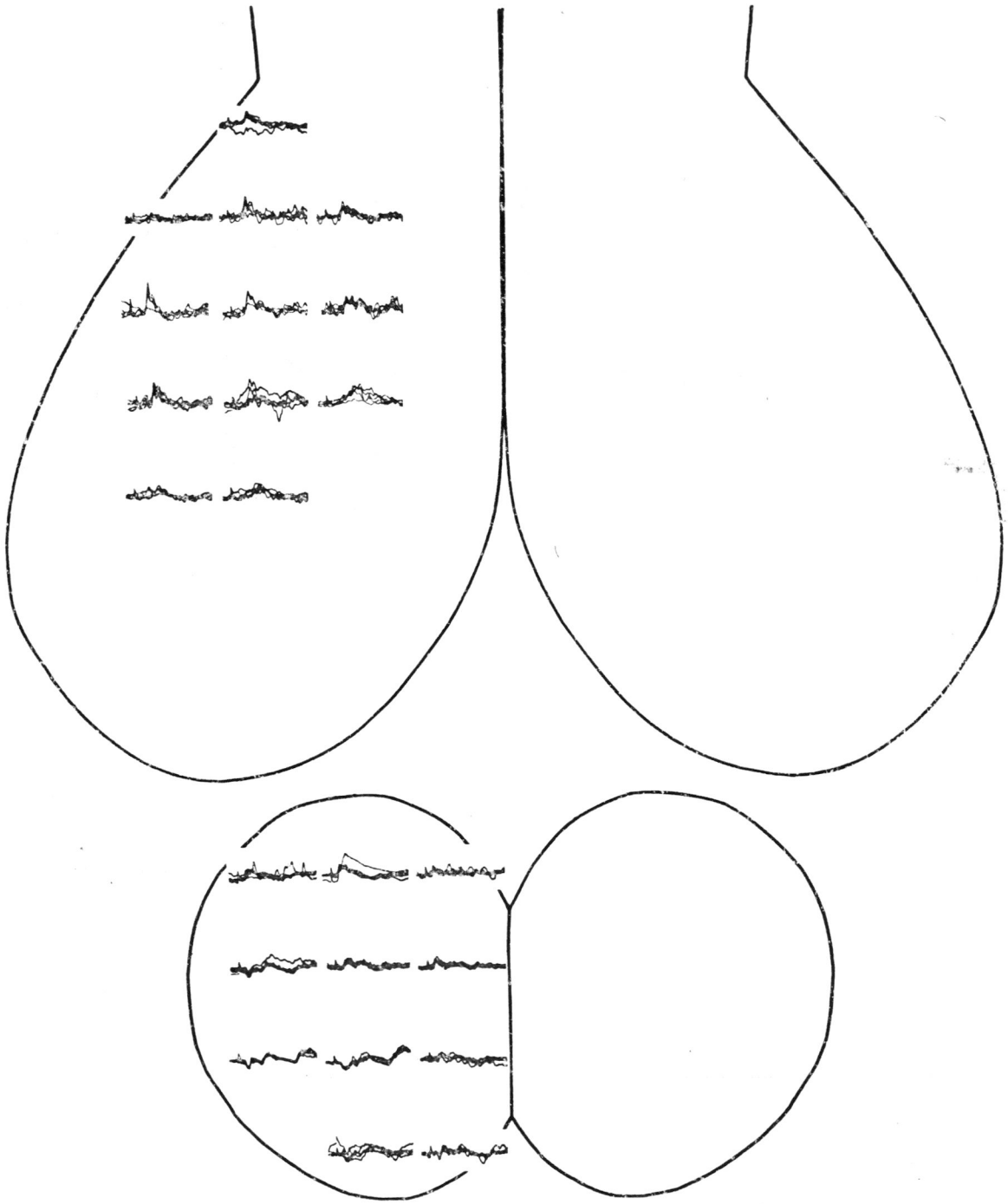

0.5 v
0.5 sec

PLATE XI

Responses elicited in cortical hemispheres after bilateral stroboscopic stimulation.

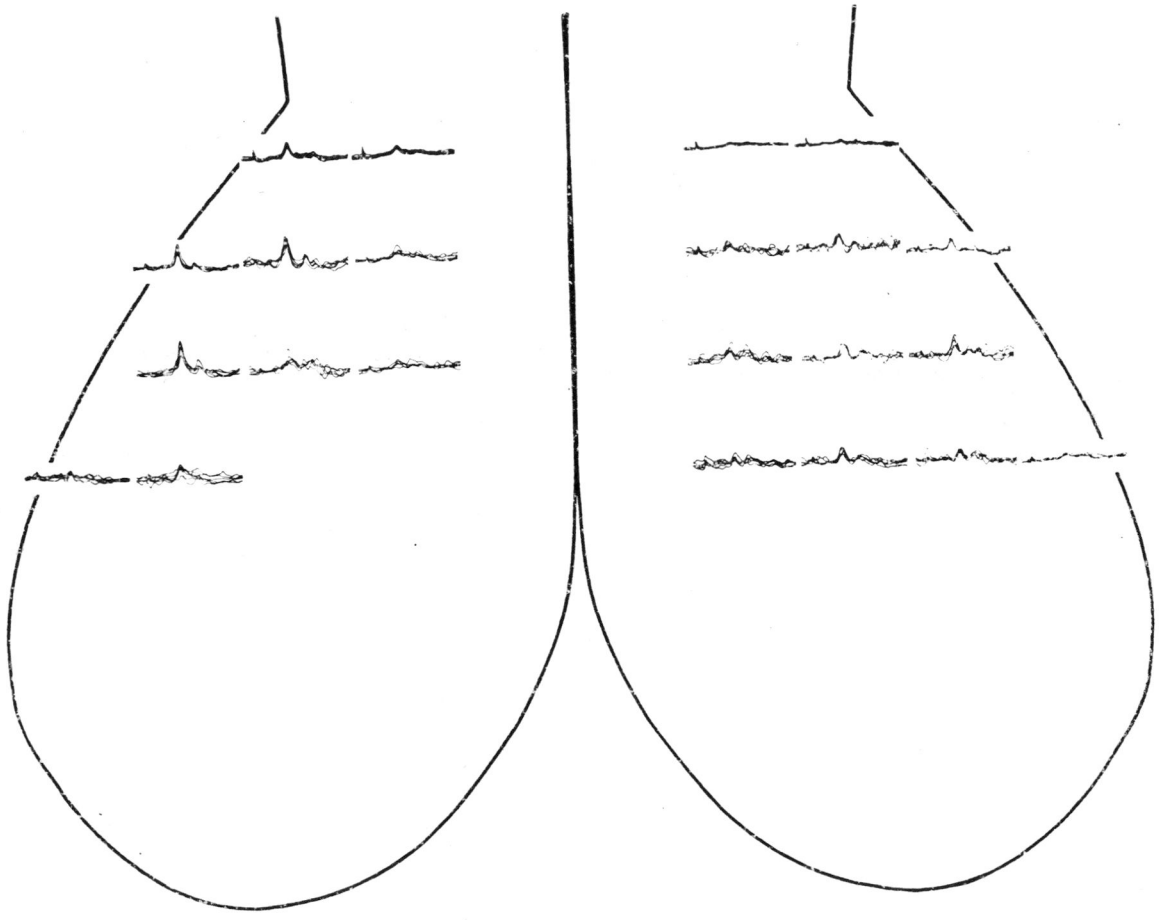

0.5v
0.5 sec

PLATE XII

Responses elicited in the cortical hemispheres after unilateral stroboscopic stimulation.

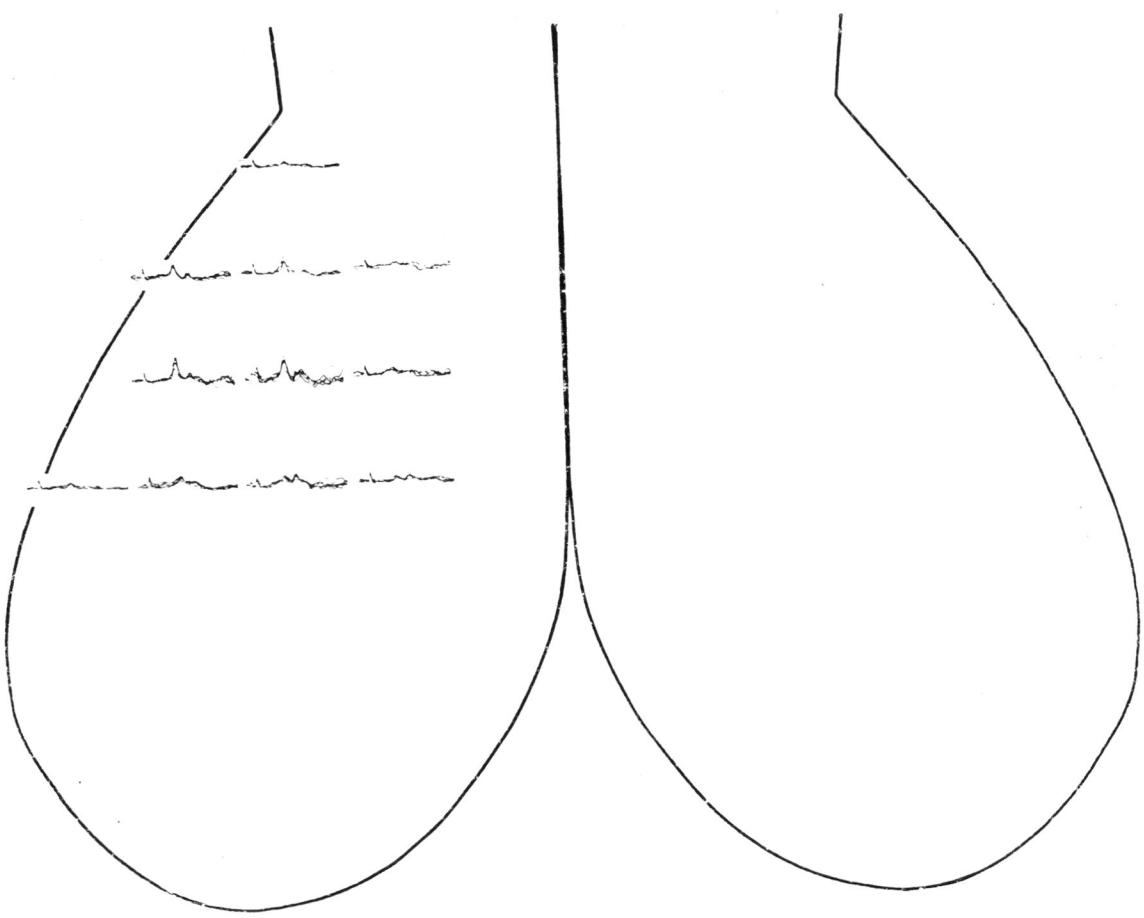

0.5v
0.5 sec

PLATE XIII

Responses elicited in the right cortical hemisphere after lesion of the right optic tectum when stimulated with bilateral light flashes.

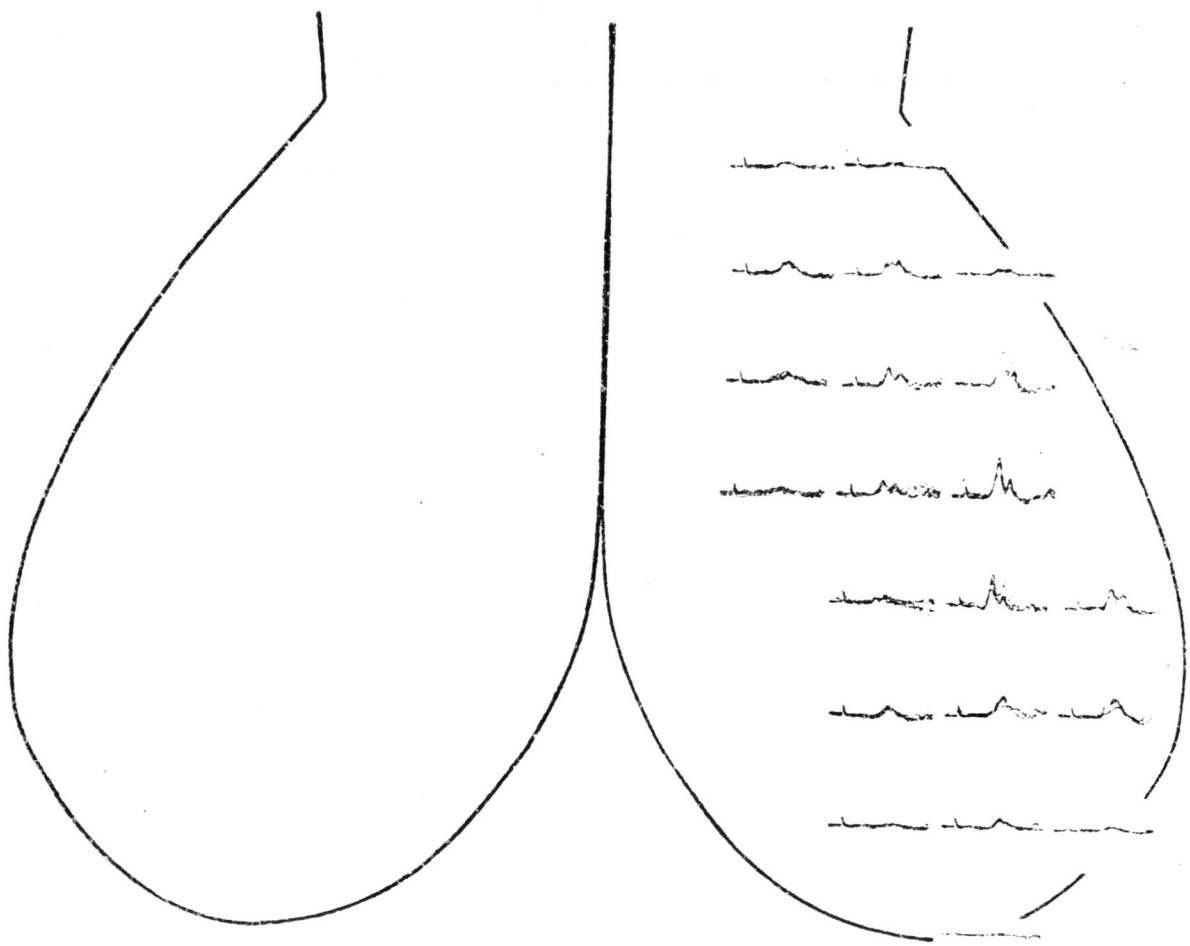
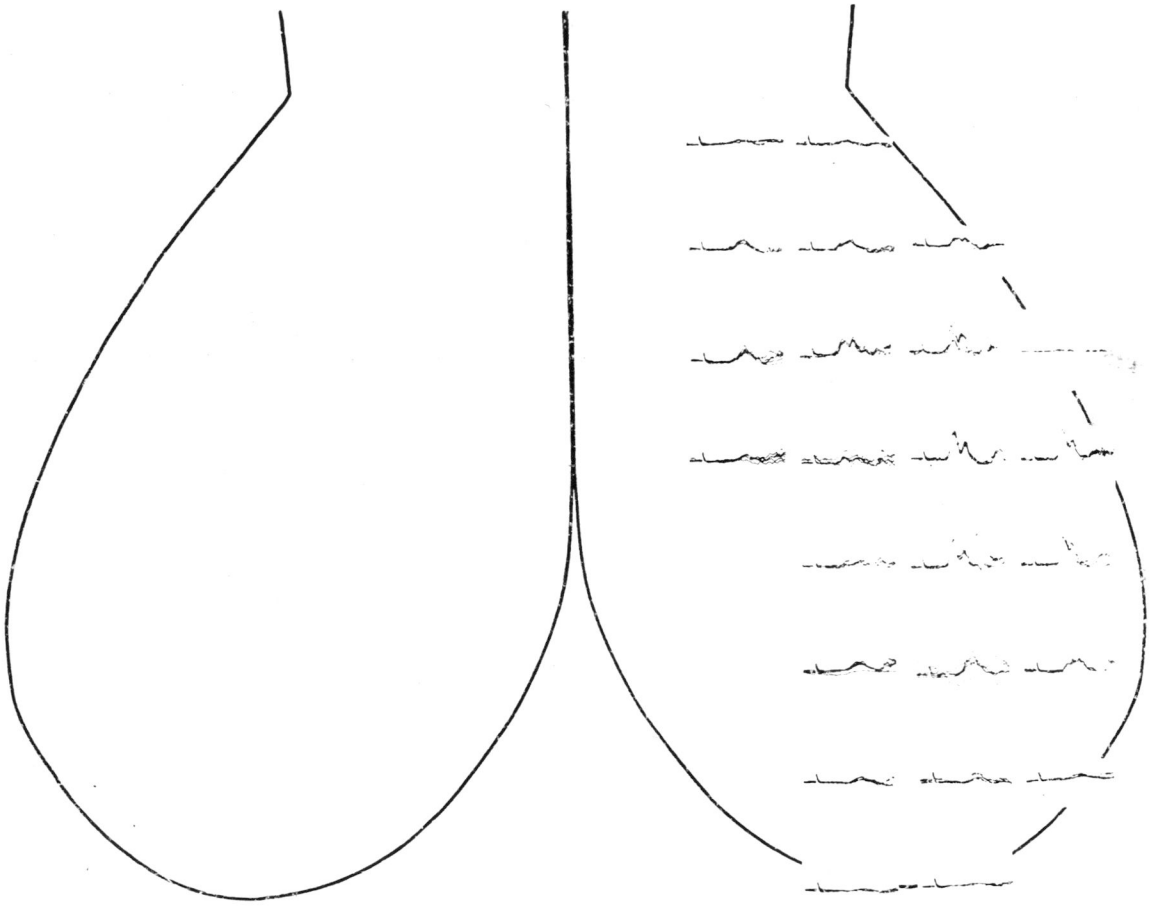

0.5v
0.5 sec

PLATE XIV

Responses elicited in the right cortical hemisphere after lesion of the right optic tectum when stimulated with unilateral stroboscopic light flashes.



0.5 v

0.5 sec

DISCUSSION

Set I involved recording the spontaneous electrical activity over the entire surface of the cortices and superior colliculi with the realization that the electrocorticogram is poorly understood. The brain is a mass of electrical circuits, each firing randomly with the corresponding signals adding or subtracting from the output, or that which was recorded. A hypothesis is that the areas of the cortex demonstrating the greatest spontaneous activity have the densest neuron population. This area in the turtle includes LC-8, 16-18, 26-28, 37 (Plate I) and the homotopic sites on the right hemisphere (Plate II); and corresponds to the area found by Kruger & Berkowitz (1960) and Orrego (1961) to have the greatest amplitude of spontaneous electrical activity. The optic tectum presents a homogenious amplitude response over the entire surface with the average amplitude being less than that found in the cortex.

Set II was performed to determine and characterize any connections existing between the cortical hemispheres, between the superior colliculi or between cortical hemispheres and superior colliculi. Recording from one half of the brain while simultaneously electrically stimulating the opposite is one method used to determine whether any connections exist between the two and if any, the characteristics of the pathway. My negative results agree with the findings of Mazurskaya and Smirnov (1966) using Emys orbicularis and S. J. Putnam (personal communication) using Pseudemys scripta. Further, Armstrong (1950) found no anatomical evidence for tectal cross-connections in the lizard. But in a similar preparation with Emys orbicularis, Orrego (1961) found evidence of

multisynaptic cross connections in the caudal hemispheres, which can possibly be attributed to the volume conducting properties often seen in the electrophysiological studies of the brain.

The cortical sites eliciting a response in the three tectal sites have no apparent spatical correlation (Plates I, III, IV). No pharmachological studies, such as those by Mazurskaya and Smirnov (1966), were performed to rule out the possibility of antidromic impulses arising from the established tecto-cortical pathway. If there is cortical input to the superior colliculus, it can be generally characterized as having a deep synaptic relationship (initial positive deflection) a being of low amplitude, and with a peak latency of 50-60 msec, which indicates a multisynaptic relationship.

A review of the literature indicates that cortical potentials have been evoked by electrical and chemical stimulation of the optic tectum of the turtle (Mazarskaya and Smirnov, 1966). The presence of electrically evoked potential's were confirmed and characterized in my work. In general, the input is to the ipsilateral hemisphere with the initial waveform negative, which indicates a dendritic synaptic relationship. The peak latency of this initial deflection ranged from 16-35 msec. Amplitudes reached 1.5 mv in the area of LC-17 and 27 and RC-22 and 32, and decreased in all directions from them. The cortical site eliciting a response having the greatest amplitude seemed to be dependent on which tectal site was stimulated (Set I). In determining whether an area is represented topographically on another area in the brain, a punctate stimulus is delivered and the area of greatest amplitude is picked out. Moving the stimulus location should move the area of greatest

amplitude correspondingly. As the evidence from the first experiment indicated that the tectum may be topographically represented on the cortex, a follow up experiment was performed in which 4 widely separated points on the right optic tectum were electrically stimulated while simultaneously searching for the site on the right cortex presenting the greatest amplitude response. Rostro-caudal movements of the stimulating electrode could not be detected with recording electrode as they all produced their greatest amplitude responses in the same point in the cortex. Medio-lateral movements of the stimulating electrode over the 3.0 mm tectal surface moved the site of greatest amplitude potential in the corresponding direction a distance of 0.5 mm. In other words, the entire tectal surface appears to be represented in a block of cortex as wide as the electrode and 0.5 mm long extending in a medio-lateral direction 2.5 mm caudal to the rostral extent of the cortex. As mentioned previously, Brown (1970) demonstrated a linear area contralis in the all-cone retina of the turtle, P. scripta elegans. In humans, the area centralis is disc-shaped and the cortical representation of the area centralis is similarly disc shaped. Heric & Kruger (1965) demonstrated a topographical organization of the retina onto the superior colliculus, but a generalized whole field retinal flash elicits a homogenous tectal response. Therefore, even though there is a precise point to point relationship between the retina and the tectum, there appears to be a magnification of the linear area centralis so that fibers spread uniformly over the surface of the tectum. If a generalized stimulation could be delivered simultaneously over the entire surface of the optic tectum and

and a recording taken from the entire surface of the cortex, we would not get a homogenous amplitude response, but would find the bar previously mentioned eliciting the greatest amplitude response, and a decreased amplitude potential for the surrounding areas. The presence of this bar seems to indicate that the cortex may be more than a "general pallium," and that the "non-specific nature of the reptilian cortex" should be reevaluated.

Set III was designed to demonstrate characteristics of the visual input to the brain. Unilateral and bilateral stroboscopic stimulation was delivered while simultaneously recording from the cortices and tecti. This should characterize the retino-thalmo-cortical system and the retino-tectal system. A subsequent experiment was performed to look for any interactions between the two systems by observing the cortical visual evoked response after electrolytic extirpation of the optic tectum. To demonstrate the individual variability in the normal cortical response to photic stimulation, the results of two animals (A and B) are presented (Plates IX, XI). These animals presented visual evoked responses in the contralateral hemisphere only as was seen by Kruger & Berkowitz (1960) using Alligator mississippiensis and Karamian and Vesselkin (1966) using Emys orbicularis. This disagrees with the findings by Mazurskaya & Smirnov (1966) in which they found a delayed input to the ipsilateral hemisphere of Emys orbicularis. The visual input appears to occupy an area including LC-7-9, 16-18, 25-28, 35-38 and the homotopic sites on the right hemisphere. This corresponds approximately to the "visual cortex" of Orrego (1961). The input is to the surface dendritic layer (negative deflection) with peak latencies from 78-105 msec in one animal

and 110-125 msec in the other. The amplitudes reached 1.2 mv in the rostro-lateral areas and decreased in all directions. There appeared to be slight variations in the input to the contralateral hemisphere when the stimulation was bilateral and when it was unilateral. As there is no cross connection between the hemisphere, the interaction obviously occurs before entry into the cortical surface.

As the retino system is considered the primary visual projection system in the reptile, the visual evoked response recorded from the optic tectum is important. There seems to be an evoked response of homogeneous amplitude over the contralateral tectal roof. Heric and Kruger (1965) found a precise topographical organization of the retina onto the tectum, indicating that if the entire retina was responding to photic stimulation and the response over the tectal roof centralis must spread over the surface as mentioned in the previous set. The polarity of the initial waveform introduces an interesting correlation with anatomical work of Armstrong (1950). Plates X and XI present the evoked responses in the optic tectum demonstrating a surface dendritic synaptic relationship (negative deflection) in the anterio-medial areas, and a deeper synaptic relationship (positive deflection) in the posterio-lateral areas. Armstrong (1950) found that the rostral optic tract of the lizard projects to the anterio-medial areas while the caudal optic tract projects to the posterio-lateral areas. The retino-tectal system is considered to be a monosynaptic system from the optic nerve to the tectal roof. But the peak latency of the initial deflection after photic stimulation ranges from 20 msec to as much as 60 msec. This delay is explained first when considering the multisynaptic pathway through the

retina, and second after observing the nerve fiber diameter and degree of myelination.

We have thus far demonstrated the characteristics of the retino-thalamo-cortical input and the retino-tectal input. But only by observing one of the systems independent of the other can the results be verified. The optic tectal roof was extirpated from Emys orbicularis in an experiment by Mazurskaya and Smirnov (1966). They demonstrated the persistence of the cortical visual evoked responses but did not characterize them. If the cortical representation of visual input is not altered after extirpation, then is follows that the two pathways are not interrelated. As can be seen when comparing Plates X, XI, XII and XIII with XIV and XV, extirpation of the tectal roof in the turtle acts as a releasing mechanism allowing a much larger area to respond to the photic stimulus. When considering the depth of synapse and response latency and amplitudes over the cortex, there seems to be no real variation. The second apparent change is the location of the greatest amplitude response. This site appears to shift caudally after the tectal destruction. The input seen in Set II may be the factor which normally alters the retino-thalamo-cortical input.

IMPLICATED RESEARCH

The electrophysiological studies done here introduce two major possibilities for further investigation.

a) There is a centro-rostral concentration of greatest amplitude cortical response when considering either spontaneous electrical activity, visual input, or tectal input. This concentration is indicative of a topographical organization of the visual field, lending more credence to the theory that this general pallium in the reptile is the precursor to the mammalian 7 layered neopallium.

b) There appears to be a definite interaction between the retino-thalamo-cortical system and the retino-tectal system. Interruption of the latter pathway by extirpation of the tectal roof has a "releasing" effect on the cortical response to photic stimulation, i.e. more of the cortical surface responds than before extirpation.

REFERENCES CITED

Armstrong, J. A. 1950 An experimental study of the visual pathways in a reptile (Lacerta vivipara). J. Anat. (Lond.) 84: 146-167.

Brown, K. T. 1969 A linear area centralis extending across the turtle retina and stabilized to the horizon by non-visual cues. Vis. Res. 9(9): 1053-1062.

Curwen, Alice D. and Ruth N. Miller 1939 The pretectal region of the turtle, Pseudemys scripta troostii. Jour. Comp. Neur. 40: 97-227.

Dawson, W. R., George Bartholomew 1958 Metabolic and cardiac responses to temperature in the lizard, Dipsosauris dorsalis. Phys. Zool. 31 : 100-111.

Forbes, A., S. Burleigh and Negland 1955 Electrical responses to color shift in frog and turtle retina. Jour. Neurophysiol. 18(6): 517-535.

Forbes, A. and Helene Deane 1958 Electrical studies of color vision in the turtle. Exptl. Cell Res. Supp. 5: 440-450.

Graf, V. 1967 A spectral sensitivity curve and wavelength discrimination for turtle Chrysemys picta picta. Vision Res. 7(11): 915-928.

Granda, A. M. 1962 Electrical response of the light and dark adapted turtle eye. Vis. Res. 2(11): 343-356.

Granda, A. M., and Charles Stirling 1966 The spectral sensitivity of the turtle's eye to very dim lights. Vis. Res. 6(3/4): 143-152.

Hall, W. C., and Ford F. Ebner 1970 Thalamotelencephalic projection in the turtle (Psuedemys scripta). J. Comp. Neurol. 140: 101-122.

Heric, T. M., and L. Kruger 1965 Organization of the visual projection upon the optic tectum of a reptile (Alligator mississippiensis). J. Comp. Neur. 124: 101-111.

Huber, G. Carl, and Elizabeth Carolina Crosby 1926 On thalamic and tectal nuclei and fiber paths in the brain of the American Alligator. J. Comp. Neur. 40: 97-227.

Karamian, A. I., N. P.Vesselkin, M. G. Belekhove and T. M. Zagorulka 1966 Electrophysiological characteristics of tectal and thalamo-cortical division of the visual system in lower vertebrates.

Kosareva, A. A. 1967 Projection of optic fibers to visual centers on turtle (Emys orbicularis). J. Comp. Neur. 130: 263-276.

Kruger, Lawrence and Ellis C. Berkowitz 1960 The main afferent connections of the reptilian telencephalon as determined by degeneration and electro-physiological methods. J. Comp. Neur. 115: 125-140.

Mazurskaya, P. Z. and G. D. Smirnov 1966 Some characteristics of optic projections of the telencephalon in the turtle. Dokl. Akad. Nauk. SSSR 167(6): 1414-1417.

Orrego, F. 1961 The reptilian forebrain. I. The olfactory pathways and cortical areas in the turtle. Arch. Ital. Biol. 99: 425-445.

Orrego, F. and D. Lisenby 1962 The reptilian forebrain. IV. Electrical activity in the turtle cortex. Arch. Ital. Biol. 100: 17-30.

Sokol, S. and W. R. A. Muntz 1966 The spectral sensitivity of the turtle. Vis. Res. 6(5/6): 285-292.