THE UNIVERSITY OF MANITOBA

MESENCEPHALIC RAPHE NUCLEI REGULATION OF AFFERENT INPUT TO THE LATERAL GENICULATE: DEPENDENCE UPON ENDOGENOUS SEROTONIN AND ANTAGONISM BY LYSERGIC ACID DIETHYLAMIDE

by

David R. Humpherys

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
DOCTOR OF PHILOSOPHY

FACULTY OF MEDICINE
DEPARTMENT OF PHYSIOLOGY

WINNIPEG, MANITOBA
APRIL, 1974

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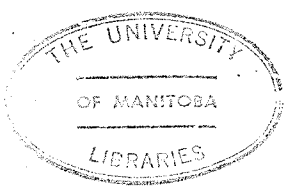

ACKNOWLEDGEMENTS

In the course of planning the experiment, carrying out the research and writing up the results which lead to a finished doctoral thesis one undergoes a tremendous learning experience. It is usually the case that the help and adive of other persons contributes a great deal to the shaping of the final product. Such is the case here. I am very grateful for the help, advice and time others have given to make this endeavor possible.

To the University of Winnipeg Psychology Department I owe thanks for providing the laboratory which has been put under my supervision and in which this research was done. Thanks go to John Menzies and Sharon Alward who assisted me diligently in my experiments, and to Dana Orhiel for preparations of tissues for histological study. Thanks go out to Earnie Samulaites and Ken Krebbs of the University of Winnipeg Electronics and Technical Services Department for their valuable help in building apparatus and trouble-shooting faulty equipment.

Special thanks go to Hugh Spencer for invaluable technical advice and to Dr. Larry Jordan who has given me very useful instruction and who has served as an example of a good scientist.

A very special thanks goes out to Dr. Ken Hughes, my advisor, who for the past three years has been a constant source of inspiration. He has never been too busy to lend a helping hand and has been the source of much appreciated advice. His grant from the Department of National Health and Welfare has provided financial support for this research. I am very grateful to you Ken, for your interest in my work, for teaching me to think and communicate, and for being a good friend.

Finally, I am greatly indebted to my lovely wife, Julie, whose love and encouragement have served as assurance that I was doing the right thing in pursuing a scientific career. Her patience and understanding during the disruption of family activities caused by my work has been a source of great inspiration to me; for

that reason I dedicate this thesis to her. Julie, you are indeed an angel.

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ABSTRACT

Unit activity from the lateral geniculate nucleus was studied in unanesthetized immobilized cats. In the first experiment the dorsal raphe nucleus was stimulated electrically to test the hypothesis that the raphe nucleus of the mesencephalon exerts control over neuronal discharge in the lateral geniculate. In an effort to determine if any observed effects were specific to various anatomical areas of the midbrain, points in the lateral tegmentum were also stimulated. We found that stimulation of the raphe nucleus resulted in a selective depression of photically driven neuronal activity in the lateral geniculate but had minimal effect on spontaneous discharge in the absence of light. Stimulation of various loci lateral to the raphe nucleus caused a mixture of non-specific facilitory and depressive effects.

Next we pretreated subjects with either p-chlorphenylalanine (PCPA), a specific depletor of serotonin, or alpha-methyl-p-tyrosine (AMT), a depletor of catecholamines in an effort to determine if the stimulation effects were dependent upon functional concentrations of endogenous amines in the brain. The typical depression of geniculate activity following raphe stimulation was abolished in PCPA treated subjects, but was restored by subsequent administration of 5-HTP, the immediate precursor of serotonin. Stimulation of the raphe nucleus in AMT treated animals had the same selective depressive effects on geniculate activity as in control subjects.

In the next series of experiments the effects of raphe stimulation on geniculate activity was studied before and after the administration of saline, lysergic acid diethylamide (LSD), or its non-hallucinogenic congener 2 Brom-Lysergic Acid (BOL). These studies provided a test of whether raphe stimulation effects could be selectively potentiated or antagonized by LSD.

We observed that LSD, not BOL or saline, antagonized the depressive effects of raphe stimulation on photically driven activity in the lateral geniculate. Both spontaneous and light evoked activity in the lateral geniculate were considerably heightened after LSD.

The results suggested that the raphe nucleus could regulate the transfer of afferent information from the optic nerve to the lateral geniculate by the synaptic release of serotonin and that this regulatory function was selectively antagonized by LSD.

Since Hofmann's (59) discovery of the psychotomimetic properties of lysergic acid diethylamide (LSD), there has been considerable interest in the effects of this and other so-called hallucinogenic agents on sensory and perceptual processes. Clinical observations on humans (17, 67, 68, 123) showing the apparent predilection of LSD for the visual system, have prompted numerous investigators to study the effects of this substance on electrophysiological activity in visual pathways of animals. Although intensive research has so far failed to provide an adequate neurophysiological model of a drug-induced hallucinoid state, several studies have succeeded in identifying various anatomical loci and physiological and pharmacological variables thought to be involved in LSD action in the CNS.

While early evoked potential studies on the actions of LSD suggested cortical synapses as a site of action, (88, 99, 100,101) recent attention has been focused on subcortical visual areas, particularly the lateral geniculate nucleus (LGN). Evarts and his co-workers (42) found that small intra-arterial doses of LSD (30 ug/kg) had a profound depressant effect on geniculate field potentials evoked by optic nerve stimulation. Doses many times greater than that required to depress LGN potentials had no effect on cortical potentials evoked by stimulation of the optic radiation. Subsequent experiments from Bishop's laboratory (20) have confirmed Evart's earlier observations on the LGN. Studies on the iontophoretic application of LSD in the lateral geniculate (36, 97, 126) have shown that with low ejecting currents, LSD depresses focal potentials evoked by optic nerve stimulation without affecting antidromic activation or glutamate excitation of LGN neurons. Higher ejecting currents with LSD cause depression of all LGN responses including glutamate excitation and intidromic invasion. Recent studies on the effects of LSD on the receptive field properties of LGN neurons (60, 91) have shown that this

drug does not uniformally depress all lateral geniculate neurons. With low intravenous doses, some cells responded with acceleration of spontaneous and light-evoked activity, other cells showed a depression of both spontaneous and light evoked activity. With higher doses (200 ug), inhibition of cellular activity was predominant.

It seems apparent from these studies that LSD exerts a dose-dependent selective action in the lateral genicualte nucleus. The mechanism responsible for this action remains unkown; however, a number of hypotheses have been put forth. Curtis and Davis (36) proposed that LSD acts by preventing the release of excitatory transmitter from optic nerve terminals while Phillis et al (97) have suggested that a direct postsynaptic action on the LGN is involved. A number of recent investigations (3, 4, 21, 22, 23, 43) have found that LSD exerts a selective action on certain neurons in the brainstem, a finding giving rise to speculation that LSD effects in the LGN may not be direct but mediated by brainstem pathways synapsing upon the lateral geniculate (126). Among the important questions to resolve in evaluating this hypothesis are : 1) do brainstem pathways exert any physiological control over lateral geniculate activity, 2) is there anatomical evidence for synaptic connections between the brainstem and lateral geniculate, and 3) if the above are found to occur, does LSD exert a selective action on these particular pathways.

Numerous electrophysiological experiments (16, 40, 125, 130) have shown that LGN activity can be altered by electrically stimulating various regions of the upper pons and mesencephalon, giving support to the idea that certain brainstem structures normally play a role in regulating transmission through the LGN. Recent flourescence histochemical studies (6, 49) of the lateral geniculate in the rat have demonstrated the presence of 5-hydroxytryptamine (5-HT) containing terminals originating from cell bodies in the mesencephalon.

These observations have resulted in the proposal that 5-HT may be a synaptic transmitter involved in brainstem regulation of LGN excitability (30, 60, 126). This notion is particularly interesting in relation to the pharmacological mechanism of LSD action. Gaddum (51) first observed LSD antagonism of 5-HT action on smooth muscle preparations and suggested that the central actions of LSD might be due to antagonism of 5-HT in the brain. Several investigators have shown that iontophoretically applied LSD antagonizes the excitant effect of iontophoretically applied 5-HT on some (21, 22, 23, 103) while mimicking the depressant effects of 5-HT on other neurons. Biochemical studies have repeatedly demonstrated that LSD causes an increase in endogenous 5-HT in the brain accompanied by decreased metabolic turnover of this amine (44, 45, 46, 111).

Some authors have reported that various behavioral reactions to LSD in animals can be altered by interfering with synthesis or storage of brain 5-HT. Aghajanian and his co-workers (3, 4, 43, 55) have shown that neurons in the raphe nuclei, the primary source of 5-HT-containing cell bodies in the brain, are sensitive to the depressant effects of intravenous or iontophoretically administered LSD.

From these studies it seems apparent that LSD exerts a rather complex action on 5-HT containing pathways in the brain. A hypothesis that LSD selectively alters lateral geniculate activity by specifically interfering with 5-HT transmission in pathways roriginating in the brainstem would gain considerable support if it could be demonstrated that: 1) 5-HT pathways exert a physiological effect on LGN activity and 2) this activity is selectively altered by LSD without affecting LGN responses to non-5-HT pathways.

Previous studies reporting altered LGN activity in response to brainstem stimulation (16, 40, 135) have indicated numerous loci effective in facilitating of depressing lateral geniculate discharge; however, no attempt

has been made to determine whether effects were due to current spread through the brain, nor has the pharmacological identity of possible mediating pathways been elucidated. Although histochemical studies have demonstrated that 5-HT terminals, presumably from the raphe nuclei, are present in the LGN, the physiological and functional significance of these connections is not known. That 5-HT-containing raphe neurons play a role in regulating activity in sensory pathways is an idea supported by some very recent observations on the somatosensory system. Samamin et al (108) found that raphe stimulation caused a reduction in somatosensory evoked potentials in the cortex of the rat. This effect was markedly facilitated after administration of LSD but depressed by strychnine. Other reports have shown that responses of lamina 5 cells in the spinal cord to nocioceptive stimuli are depressed by raphe stimulation (84). These investigators found that the stimulation effects were abclished after treatment with p-chlorphenylalanine (PCPA), a potent 5-HT synthesis inhibitor. (74) LSD blocked the response of lamina 5 cells to raphe stimulation but not to stimulation of adjacent mesencephalic structures. Concurrent behavioral studies (5, 89) indicated that raphe stimulation produced marked analgesia in both rats and cats. These observations of raphe influence on somatosensory pathways demonstrate an apparent functional relationship which appears to be selectively altered by LSD. In what appears to be the only study on the effects of raphe stimulation on the auditory system, Sheard and Aghajanian (118) found that stimulation produced behavioral dishabituation to auditory stimuli, a condition also observed under LSD but blocked with PCPA.

Whether the raphe nuclei exert similar control on the visual system, possibly through the lateral geniculate, and whether interference with such a functioanl connection is responsible for LSD effects on the LGN, is not known. Information regarding this possibility would provide valuable insight into

mechanisms underlying LSD effects on the visual system and its relationship to 5-HT neurotransmission in the CNS.

The present study was initiated to investigate the following: 1) the effects of electrical stimulation of the raphe nuclei and surrounding mesencephalic loci on the spontaneous and lightevoked discharge in lateral geniculate neurons, 2) a comparison of the effects of selective depletion of endogenous pools of brain 5-HT and catecholamines on geniculate responses to electrical stimulation, and 3) the effects of intravenous LSD on lateral geniculate responses to raphe and mesencephalic stimulation.

In an attempt to determine whether our observations on LSD were unique to its hallucinogenic properties, we will compare the effects of this substance with 2-bromo-lysergic acid, a potent peripheral 5-HT antagonist, but devoid of hallucinogenic effects (67).

EXPERIMENT I

METHODS

Subjects: A total of fifty-five adult cats weighing between two and five kilograms were used in this study. All animals were judged to be in good health before any experimentation proceeded.

Surgical Preparation: All animals were surgically prepared for recording and stimulation one week prior to the day of experimentation. Cats were anesthetized with pentobarbital sodium (Nembutal), 25 mg/kg intravenously. All surgery was performed under aseptic conditions. A unilateral craniotmy was performed in the region overlying the lateral geniculate nucleus at coordinates, Anterior-Posterior, +3.0 - +8.0; Lateral, 7.0 - 12.0. A stainless steel cylinder was cemented to the skull covering the exposed brain subsequently filled with mineral oil, and a polyethylene cap was screwed into the cylinder providing a seal. A polyvinyl pedestal was cemented to the anterior portion of the skull according to a modified version of the techniques described by Humpherys et al (65). This procedure allows attachment of the animal's head to the sterotaxic frame during experimentation without having to use traumatizing ear bars.

Two stainless steel concentric bipolar electrodes with tip separation of 1 mm. were stereotaxically implanted in the midbrain. One electrode was positioned according to the coordinates given by Berman for the dorsal mesencephalic raphe nucleus (A-P, -1.5; L, 0.0; D-V, -2.0 -4.0). The second electrode was placed in the lateral tegmental area of the midbrain according to the coordinates A-P, +1.0; L, 2.0 - 5.0; D-V, -2.0 -4.0. In order to hit the dorsal raphe nucleus in the cat from a vertical approach it is necessary to drill a hole through the boney tentorium which separates the occipital lobe from the underlying midbrain. This was accomplished by

passing a hollow metal guide between the cerebral hemispheres after resection of the dura. The guide is lowered until it touches the tentorium. The bit of a drill is lowered inside the guide, a hole in the tentorium is drilled and both drill and metal guide are removed and the electrode is lowered into place according to predetermined coordinates.

Both electrodes are then cemented in place and fitted with two-prong connectors for later hook-up to electrical stimulators. After completion of surgery animals were given an antibiotic and allowed to recover for a minimum of one week prior to experimentation.

Experimental Procedure: On the day of experimentation the cat was anesthetized with halothane 70% N_2O 30% O_2 mixture. A polyethylene catheter was inserted in the femoral artery for continous recording of arterial blood pressure. The femoral vein was also cannulated for administration of drugs during the experiment. The thigh wound was then infiltrated with xylocaine and sutured closed.

A small 1/4 inch incision was made at the back of the neck for insertion of a ground electrode and the wound was then infiltrated with xylocaine and sutured closed. The subject was then clamped into the stereotaxic frame by means of the pedestal previously cemented to the skull.

The polyethylene cap was removed from the stainless steel cylinder and the dura overlying the suprasylvian gyrus where the microelectrode would be inserted was resected. The animal was then fitted with an endotracheal tube, anesthesia was withdrawn and tubocurarine (1 mg/kg) was administered intravenously. Throughout the duration of the experiment additional amounts of tubocurarine were given (at the rate of 3 mg/hour) to maintain immobility. Since we were not equiped to monitor expired CO_2 a number of procedures were employed to minimize the probability of hypercapnia during the experi-

ment. The animal was ventillated with a Harvard Dual Phase respirator with stroke volume and rate selected according to the weight of the animal and in accordance with parameters shown by others (81,122) to maintain an adequate degree of ventillation over long periods of time. Apparatus and tracheal tube dead space were held at a minimum of 11 cc. The ratio of inspiration time/expiration time was set to 40-45% thus facilitating pulmonary emptying and increasing venous return to the heart. To prevent slowly developing anoxia due to atelectasis, a Harvard Hyperinflation Valve was employed in the respiratory circuit which caused the lungs to overinflate to a pressure of 30 cm. of H_2O every 15 minutes.

Recording Procedures: A glass microelectrode (tip diamenter, 1-3 microns) filled with 2M NaCl was lowered into the cylinder and placed on the surface of the brain with the aid of a dissecting microscope. The cylinder was then filled with a 4% agar-Ringer solution which hardened quickly to form a closure over the cranial cavity, bringing brain pulsations to a minimum. Prior to lowering the electrode, a reading on a Kopf micromanipulator was taken and used as a reference point to determine the depth, in microns of all cells recorded from during the experiment. Cellular spike activity was fed into a high impedance FET probe connected to a Grass P-16 microelectrode preamplifier with filters set to attenuate all DC slow potential and EEG activity. Output of the preamplifier was fed into an oscilloscope audio monitor, and Schmitt trigger interface of a PDP-8 computer (Digital Equipment Corporation) for on-line monitoring and analysis of data. The microelectrode was lowered into the brain according to the coordinates previously determined for the lateral geniculate nucleus. Units in the lateral geniculate and optic radiation were recorded. While the electrode was being lowered, a light in front of the animals eyes was flashed on and off in order to determine whether the units encountered were light responsive. Cell body

spikes (62,63) responsive to light were found at a depth of between 12 and 15 millimeters below the cortical surface while fiber spikes (62,63) responsive to light were usually encountered between 5 and 11 millimeters below the cortical surface. These light responsive fibers were assumed to be axons of geniculate cells running through the optic radiation. No attempt was made to distinguish between the various subpopulations of geniculate cell types.

Electrical Stimulation Procedure: Electrical stimulation was delivered via Grass S-4 Square Wave Stimulators and SIU5 Isolation Transformers with stimulation parameters of 100 Hz, 100 microseconds duration and 9 volts. Rough calculations of tissue impedance indicated approximate current valve to be 100 - 200 microamperes. Similar stimulus parameters have been employed by others studying the raphe nucleus (3, 4).

Visual Stimulation: The animals eyes were dilated with atropine and nictitating membranes were retracted by applying neosynephrine. The visual stimulus was a stationary 6 watt bulb behind a transluecent screen 1 meter away from the animals eyes. Illumination of the lamp gave rise to a circular shaped stimulus approximately 1 inch in diameter resulting in diffuse stimulation of the retina. All experiments were performed in darkness with the only other source of light being the oscilloscope screens and indicator bulbs on the equipment in the shielded room.

Testing Procedure: When a unit was isolated and found to be light responsive, a carefully controlled testing procedure was employed to determine whether its response was altered by electrical stimulation of raphe and tegmental loci. It was felt desirable to test the effects of stimulation on three different kinds of lateral geniculate activity; spontanious activity preceeding the onset of the visual stimulus, spike patterns during the visual

stimulus, and after discharge immediately following the termination of the visual stimulus.

Since this experiment called for repeated sampling of activity from the same cell in order to test for selective effects of raphe and tegmental stimulation, it was important to know to what extent various factors influencing cellular excitability contributed toward the variability in successive records. Such variables as habituation to the visual stimulus changing physiological state, drift of the microelectrode, and changes in arousal are among many of the factors potentially capable of disguising the true effects of experimental manipulations when tested in sequence.

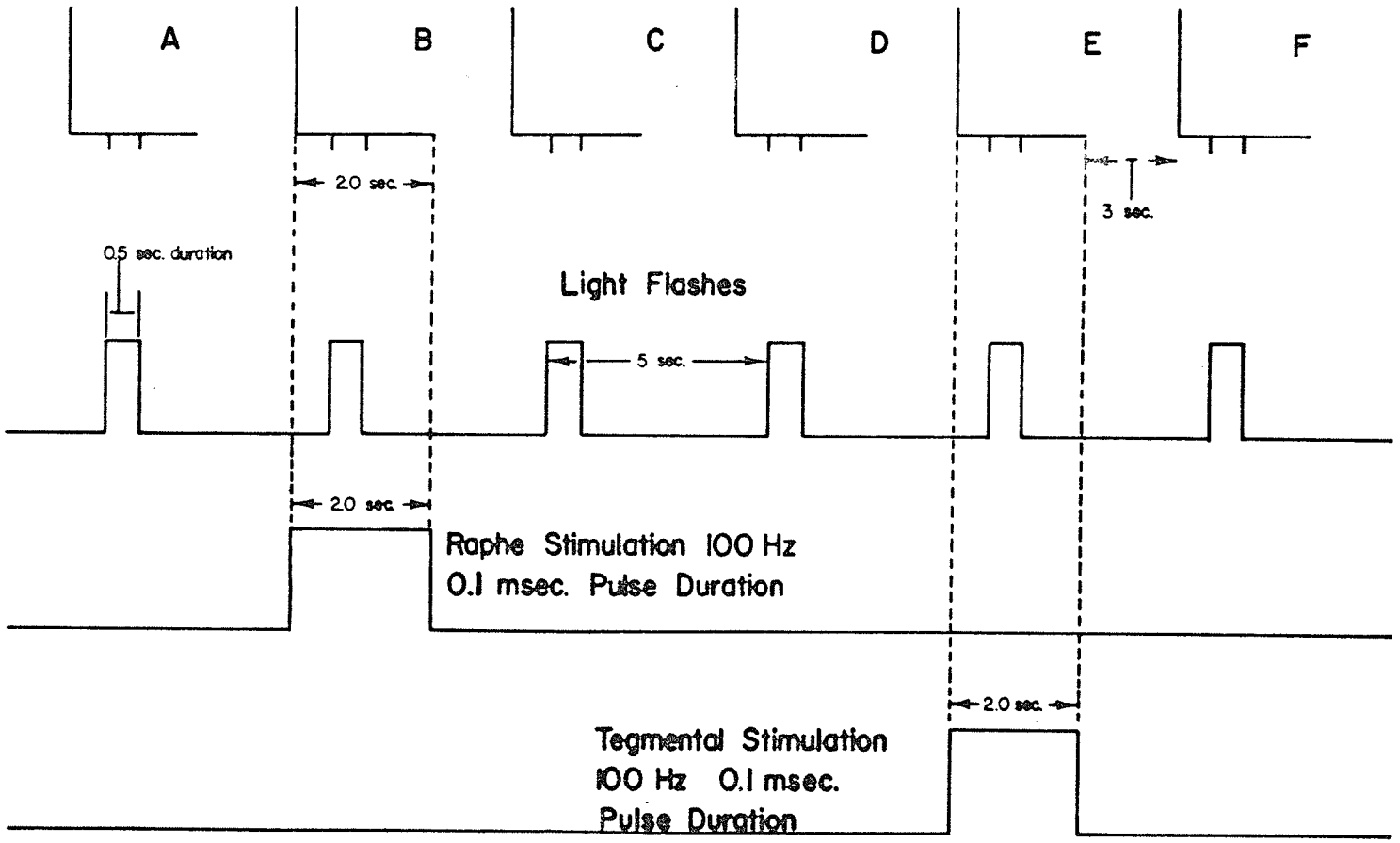
In an effort to minimize interpretational difficulties we developed a multiple frequency histogram computer program, designed to digitize spike trains from successive samples and store them in separate memory fields on a recycling basis.

Figure 1 illustrates how this program was interfaced with the procedure of sequential presentation of the visual stimulus and electrical stimulation. We use the term sequence to mean six presentations of the visual stimulus (500 msec duration), the onset of each consecutive stimulus being separated by 5 seconds. Presentation of the visual stimulus, electrical stimulation, and triggering computer sampling of spike trains was controlled by programmable logic circuts (Lehigh Valley Electronics). The logic circuits trigger the computer to begin sampling spike activity 500 msec before the onset of each visual stimulus, continuing during the visual stimulus and 1 second after its termination resulting in a total of a 2 second sampling period. The initiation of a sampling period is triggered 500 msec. prior to the onset of each visual stimulus in the sequence. The term trial will refer to any single 2-second sampling period; consequently each trial contains a sample of activity prior to, during, and after the visual stimulus. The six trials

FIGURE 1

stration of computerized testing sequence. One sequence is composed of six presentations ne light flash. The computer is triggered to sample geniculate activity 500 msec. before, nsec. during and 1 sec. after the onset of the visual stimulus, the entire sampling per- being termed a trial. Separate trial histograms were computed and summed over 50 repe- ons of the experimental sequence. Raphe stimulation occured during Trial B while teg- al stimulation was presented in Trial E.

Sequential Computerized Pulse Frequency Histograms

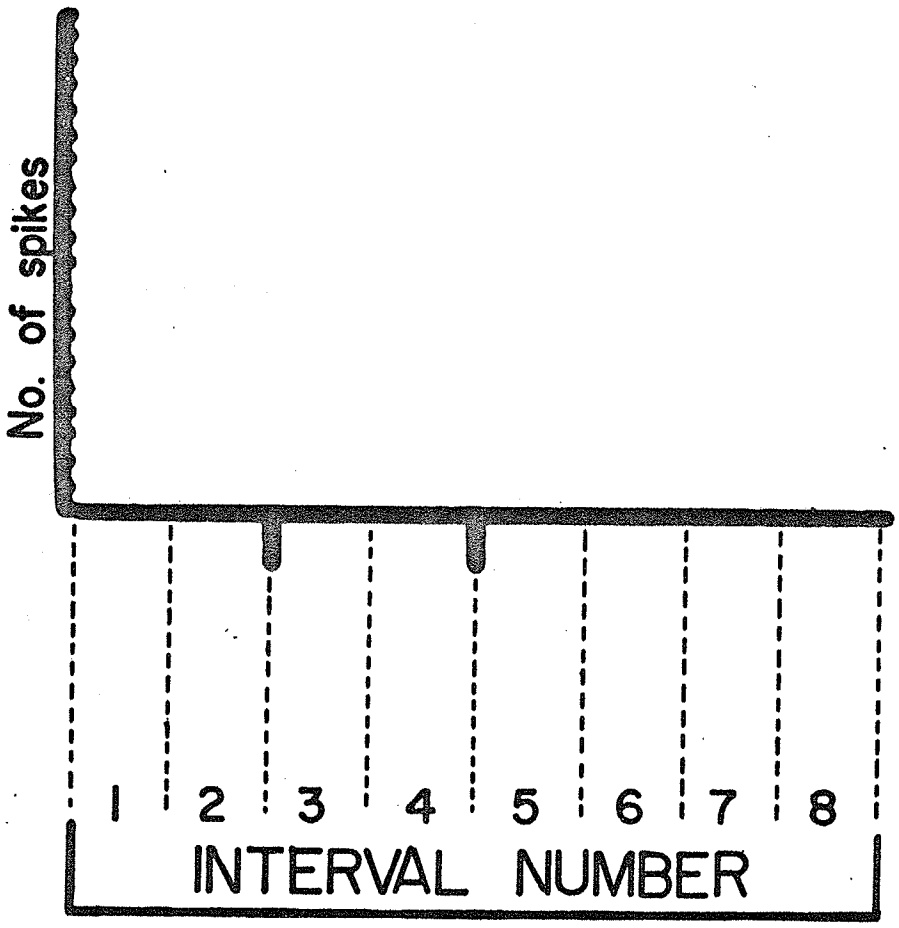


in a sequence (Figure 1) are denoted by the leters A through F. During trial B, the second in the sequence of six, electrical stimulation through the Raphe electrode was turned on for the entire 2 seconds (see Figure 1). The following two trials, C and D occur in the absence of electrical stimulation allowing an assessment of possible after effects and recovery from the electrical stimulation in trial B. Electrical stimulation through the tegmental electrode is turned on during trial E. Trial F occurs without stimulation as in A, C and D. After the termination of trial F there is a 5 second delay period after which the sequence is repeated starting again with trial A. Each trial in a sequence is sampled and stored in separate memory fields by the computer; as the sequence is repeated the spike trains during each trial are stored, summed with previous samples of the appropriate trial, and displayed as pulse frequence histograms. In these experiments the sequence was repeated 50 times so that the events unique to each trial were sampled 50 times. By using this method of data sampling were confident that any change in the excitability or responsiveness of the cell as a function of time would be equally represented in all trial histograms; therefore, significant differences between different trial histograms (i.e., comparing A vs B) are likely to be attributable to the electrical stimulation.

Data Analysis: All data analysis was based on the summed contents of the individual frequency histograms. A 2-second trial, represented on the X-axis of the histogram, was divided into 400 discrete time intervals or bins. Each bin represented a 5-millisecond time interval (refer to Figure 2). The Y-axis of the histogram represents the number of spikes for a given bin over 50 summed trials. A subroutine in the computer program divided the 400 bins into eight analysis intervals of 50 bins each. Referring to Figure 2, it can be seen that analysis intervals 1 and 2 correspond to the 500 msec. of

FIGURE 2

Breakdown of completed trial histogram into intervals for statistical analysis.



X Axis divided into 8 equal 250 msec. intervals (50 bins)

For each interval: Computation of

1. Total spikes
2. Mean spikes/bin
3. Variance

pre-stimulus spontaneous activity. Intervals 3 and 4 correspond to the 500 msec. during the visual stimulus as indicated on the histogram by two vertical cursors extending below the X-axis. Intervals 5, 6, 7, and 8 correspond to the 1 second period of time following the termination of the visual stimulus. For each of the eight analysis intervals the total number of spikes, mean number of spikes per bin, and variance were computed and printed out. This procedure was followed for each of the six individual trial histograms resulting from 50 repetitions of the sequence. These analysis intervals formed the basis of all statistical comparisons used in all experiments.

In evaluating the effects of raphe and tegmental stimulation on geniculate discharge certain assumptions formed the basic rationale of the statistical comparisons made. First it was assumed that 50 repetitions of the experimental sequence with the electrical stimulators turned off would yield six summed trial histograms showing discharge patterns which would not differ significantly from each other. Any differences between the six histograms accumulated over 50 repetitions of the sequence with identical treatment in all trials should be attributable to normal error variance. This assumption was substantiated in initial pilot experiments. Second, if there were no after effects resulting from raphe stimulation in trial B or tegmental stimulation in trial E, then histograms A, C, D, and F should not differ from one another significantly (refer to Figure 1). These four histograms could be considered independent control samples of geniculate activity upon which to assess the effects of stimulation in either histogram B or E. Significant differences in comparisons between histograms A, C, D, or F might possibly reveal an ordered effect related to relative position in the sequence or the presence of after effects of the electrical stimulation or both effects combined.

In determining specific effects of electrical stimulation comparisons between histograms A and B were made for raphe stimulation; comparisons between histograms A and E were made for tegmental stimulation (refer to Figure 1). In making statistical comparisons between any two histograms, the eight consecutive analysis intervals (see Figure 2) for each respective histogram were paired (i.e., interval 1, histogram A with interval 1, histogram B; interval 2, histogram A with interval 2, histogram B, etc.) and a Student's t-Statistic for difference between means was computed for each pair of analysis intervals giving rise to eight separate t-tests (two-tailed) for each pair of histograms being compared.

Histology: At the end of each experiment the animal was given a lethal dose of Nembutal, the brain was removed quickly and fixed in a 10% formalin solution. Later the brains were cut into blocks, embedded in paraffin and sectioned on a microtome. Sections 10u. thick showing the location of the electrode path were stained with cresyl violet and photographed.

RESULTS

The effects of electrical stimulation of raphe nuclei and lateral tegmental region were studied in 176 cells in the lateral geniculate and optic radiation. Figure 3a - 3b shows typical histological sections with arrows indicating the postion of the tip of the stimulating electrodes in the raphe and tegmental areas of the midbrain. In all animals studied there were no significant changes in heart rate or blood pressure during stimulation of lateral midbrain regions. With raphe stimulation there were no cardiovascular changes resulting from stimulation within the midbrain; however, in three different animals the electrode was located in the raphe area of the pons resulting in a substantial fall in blood pressure during stimulation with quick recovery and no apparent change in heart rate. Data from these animals was excluded from the sample.

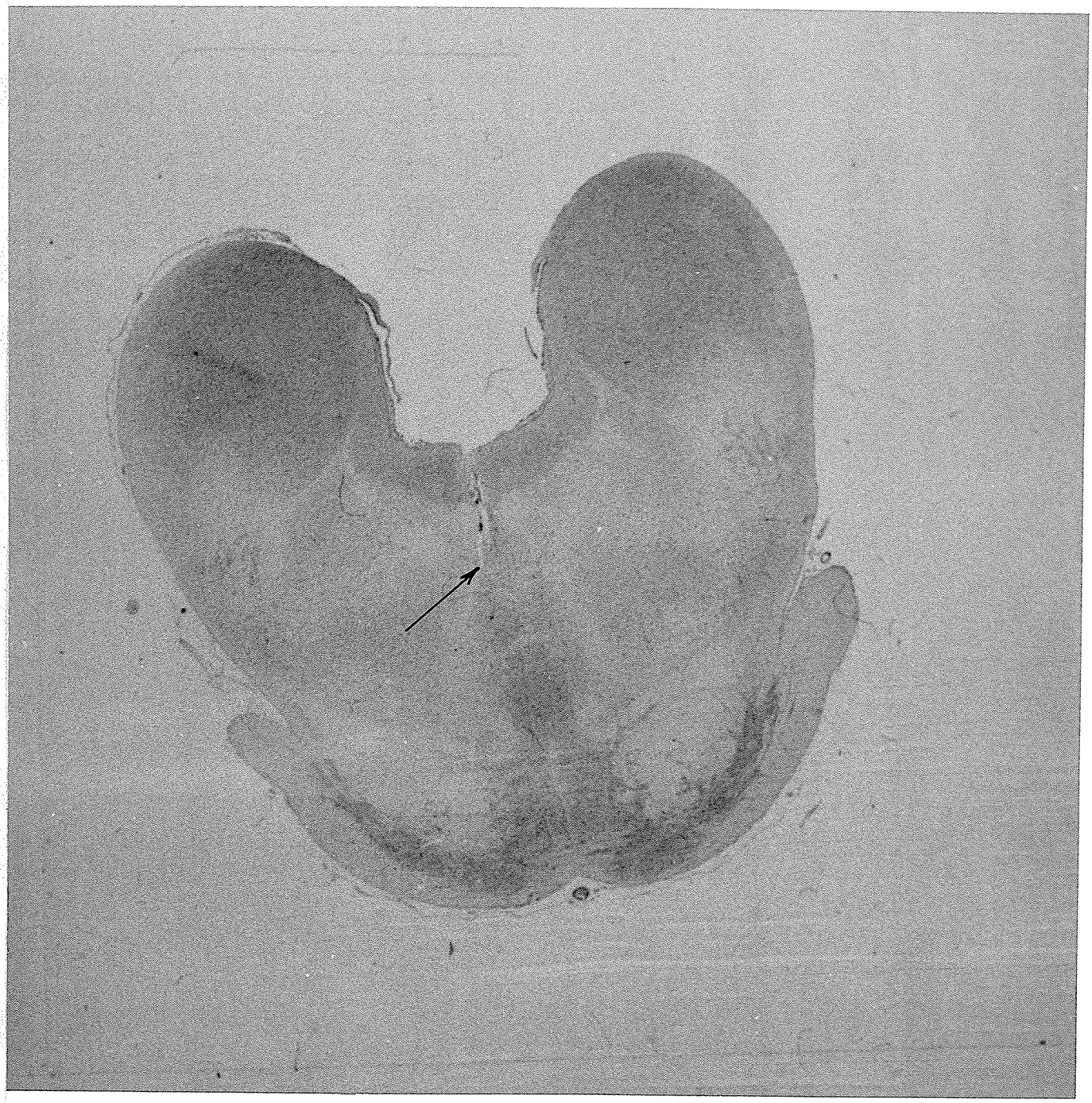
Effects of Raphe and Tegmental Stimulation on Geniculate Discharge:

Figure 4 illustrates the effects of raphe and tegmental stimulation on a geniculate unit. Figure 4a shows a control histogram obtained from sequence trial A depicting the characteristic prestimulus spontaneous activity, light-evoked discharge (ON response) and post-stimulus activity (OFF response). When compared to the histogram in Figure 4b it is found that raphe stimulation

FIGURE 3

Typical histological section showing tip of electrode path in the dorsal raphe nucleus. (A) and lateral tegmental area (B) arrows point to the location of the electrode tip.

A



B

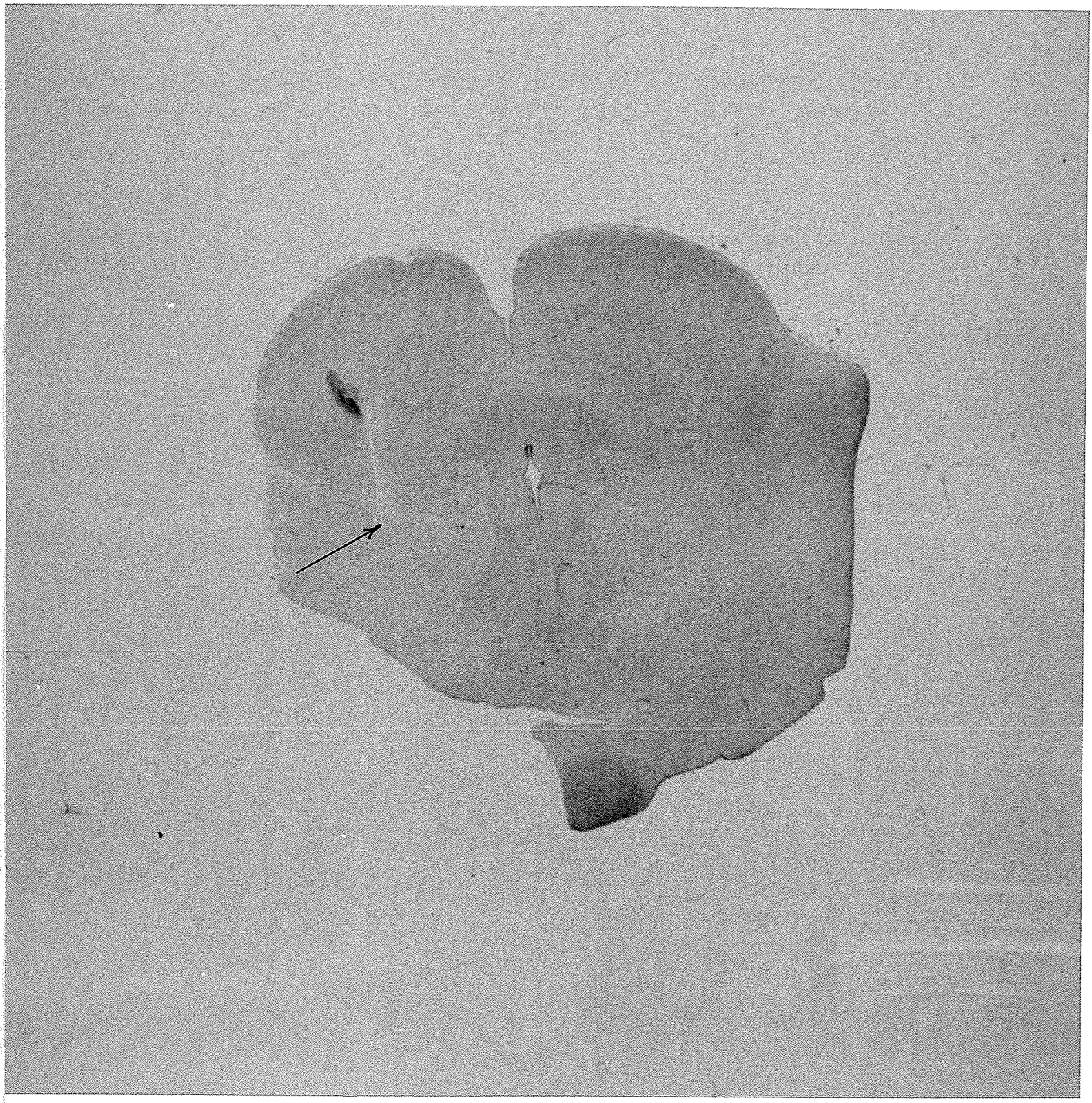
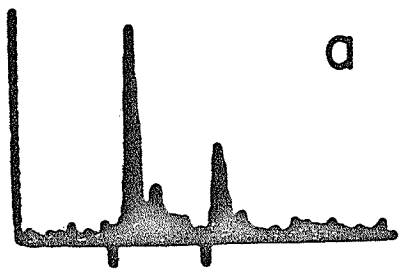


FIGURE 4

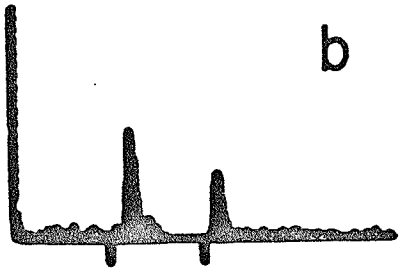
Effects of raphe and tegmental stimulation on lateral geniculate activity; a and c, control trials with no stimulation; b, raphe stimulation; d, tegmental stimlation.

CONTROL



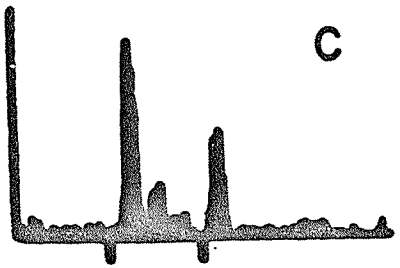
a

RAPHE STIMULATION



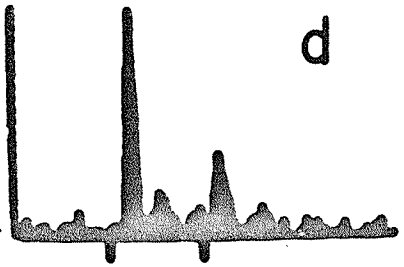
b

CONTROL



c

TEGMENTAL STIMULATION



d

caused a signific ($p < 0.01$, $t=3.11$, $DF=98$) depression of the ON response (corresponding to analysis interval 3) with no significant difference in pre-stimulus spontaneous activity (Intervals 1 and 2). There was a significant depression of activity in Intervals 4 and 5 ($p < 0.05$) with no significant differences in Intervals 6, 7, and 8. Comparison between the histogram in Figure 4a and 4c showed no significant differences. Tegmental stimulation in this case (Figure 4d) had a slight but non-significant facilitatory effect on the ON response.

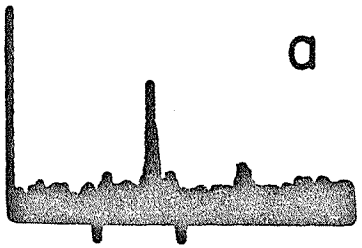
Figure 5 shows the reversability of stimulation effects typical of that observed in 70% of our sample (120 cells). There were no significant differences found in comparing the histograms in Figures 5a, 5c, 5d, and 5f. In this cell, stimulation of the raphe nucleus caused a significant ($p < 0.01$) depression of the ON response, and a slight but insignificant depression of Post-stimulus discharge. Tegmental stimulation in this cell showed a significant ($p < 0.05$) acceleration of discharge throughout all intervals although visual inspection of Figure 5e indicates that the peak of the ON response seems smaller than control values.

In serveral of the cells encountered, raphe and tegmental stimulation had mixed effects which sometimes resulted in non-significant t-ratios even in the

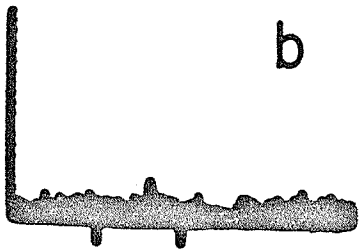
FIGURE 5

Six sequential trial histograms (a-f) showing reversability of stimulation effects on geniculate activity; b, raphe stimulation; e, tegmentum stimulation; acdf, control.

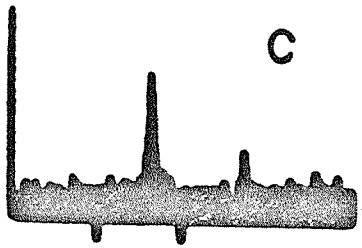
CONTROL



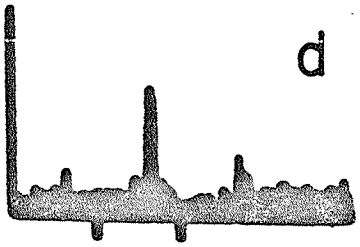
RAPHE STIMULATION



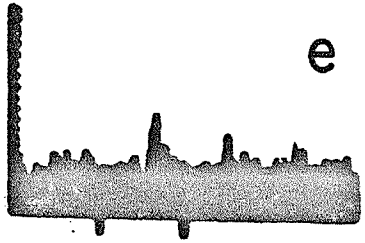
CONTROL



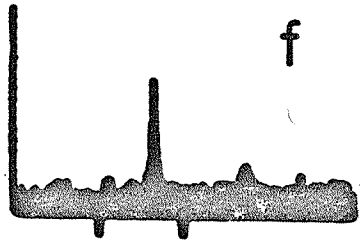
CONTROL



TEGMENTAL STIMULATION



CONTROL



face of obvious changes upon visual inspection. Figure 6 illustrates this effect.

Comparing the histograms in Figures 6a and 6b, it can be seen that the first peak of the ON response is substantially reduced in 6b as compared to 6a; nevertheless a non-significant t-ratio resulted presumably because of the considerable enhancement of the second ON peak (Observe arrows). There was a significant ($p < 0.01$) reduction in Interval 5 of the histogram in 6b corresponding to the peak of the OFF response. It is interesting to note that tegmental stimulation (Figure 6e) caused a significant enhancement ($p < 0.05$) of pre-stimulus spontaneous activity, no significant change in post-stimulus after discharge, and a similar profile during the stimulus (ON response) as found with raphe stimulation. In the second cell shown in Figure 6a - 6b, raphe stimulation (Figure 6b) had a significant ($p < 0.05$) depressant effect in all analysis intervals except 1 and 2 corresponding to pre-stimulus spontaneous activity. Tegmental stimulation (Figure 6e) showed a significant acceleration of the OFF response in Interval 5 ($p < 0.05$) while exhibiting virtually identical depressant effects on the ON response found with raphe stimulation (see arrows).

Figure 7 shows another example of mixed effects with raphe and tegmental stimulation. Raphe stimulation resulted in a significant ($p < 0.05$) enhance-

FIGURE 6

Illustration of mixed facilitatory and depressant effects of raphe (b,B) and tegmental (e,E) stimulation on two separate cells. Arrows point to peaks common to both kinds of stimulation in each cell.

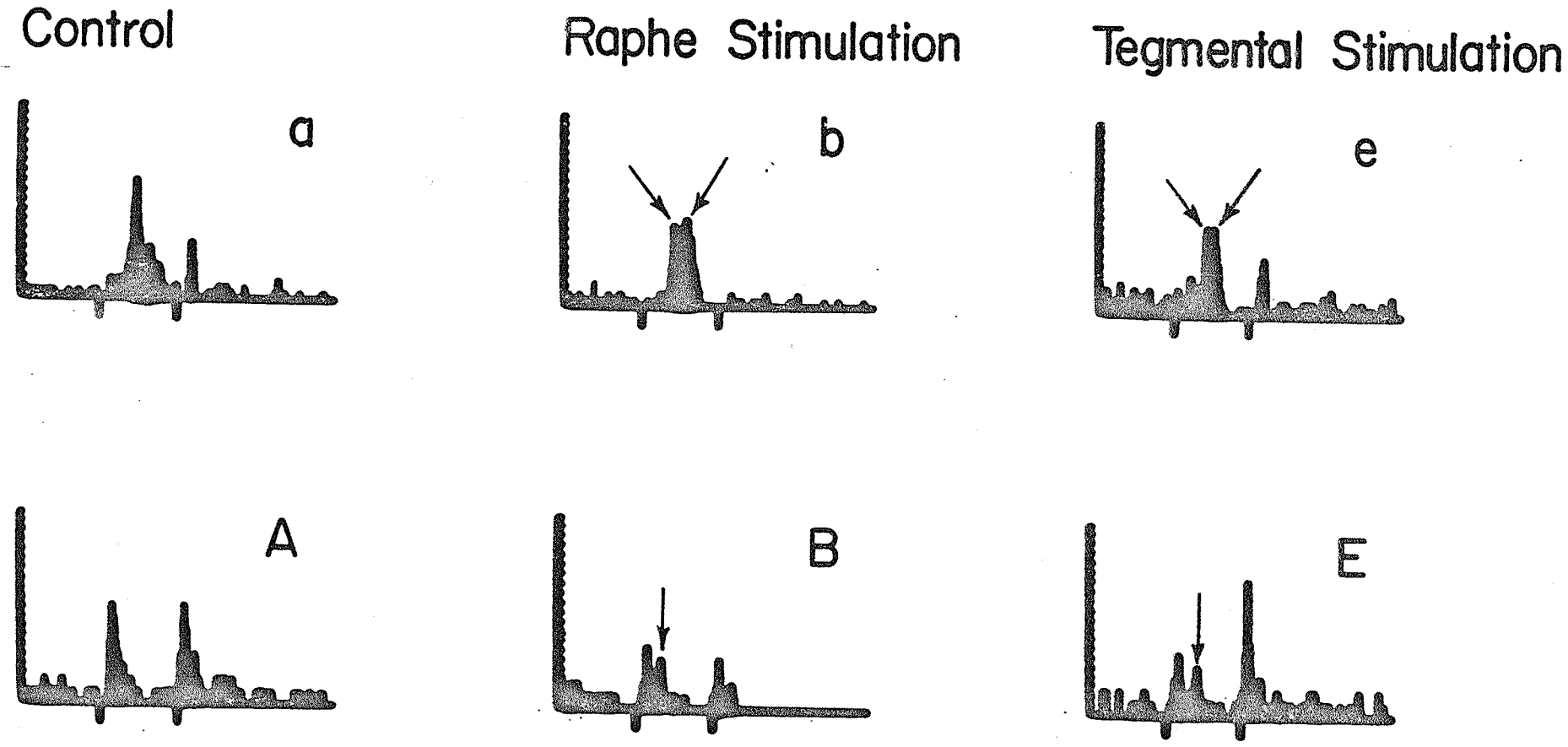
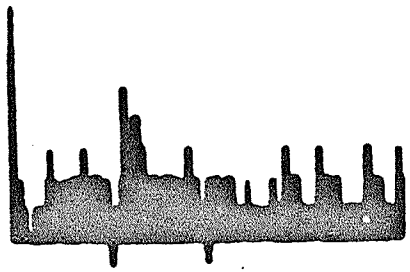

Control
Raphe Stimulation
Tegmental Stimulation
a
b
e
A
B
E

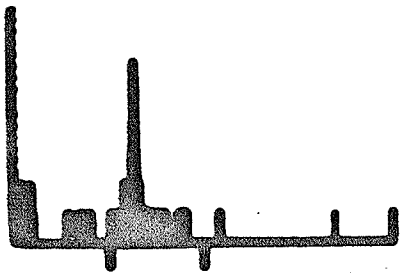
FIGURE 7

Mixed facilitatory and depressant effects with raphe stimulation. Tegmental stimulation is purely facilitatory.

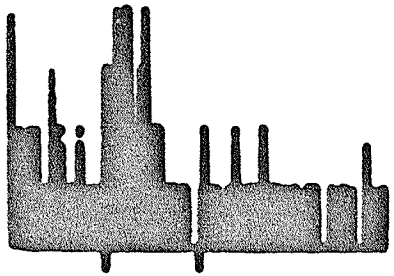
CONTROL



RAPHE STIM.



TEGMENTAL STIM.



ment of the peak of the ON response accompanied by profound depression of all other activity. Tegmental stimulation, on the other hand (Figure 7e), showed a significant facilitation ($p < 0.01$) of the ON response accompanied by significant ($p < 0.05$) facilitation of all other activity in the cell.

Analysis of Mixed Stimulation Effects:

Since several of the cells we encountered in our sample showed a mixture of depressant and facilitatory responses to raphe and tegmental stimulation, we made a detailed comparison of stimulation effects in all cells. This comparison was made for any given cell by comparing the effects of raphe stimulation with control (the eight analysis intervals in trial histogram B vs. the eight analysis intervals in trial histogram A), and the effects of tegmental stimulation with control (the eight analysis intervals in trial histogram E vs. the eight analysis intervals in histogram A). In each individual comparison between paired analysis intervals, if a significant t-ratio ($p < .05$) was obtained, it was recorded appropriately as a facilitatory of depressant response for that particular interval. If a non-significant t-ratio was obtained it was recorded as no effect. The total number of cells responding to raphe and tegmental stimulation with facilitation, depression, or no effect in the eight analysis intervals was recorded and expressed as

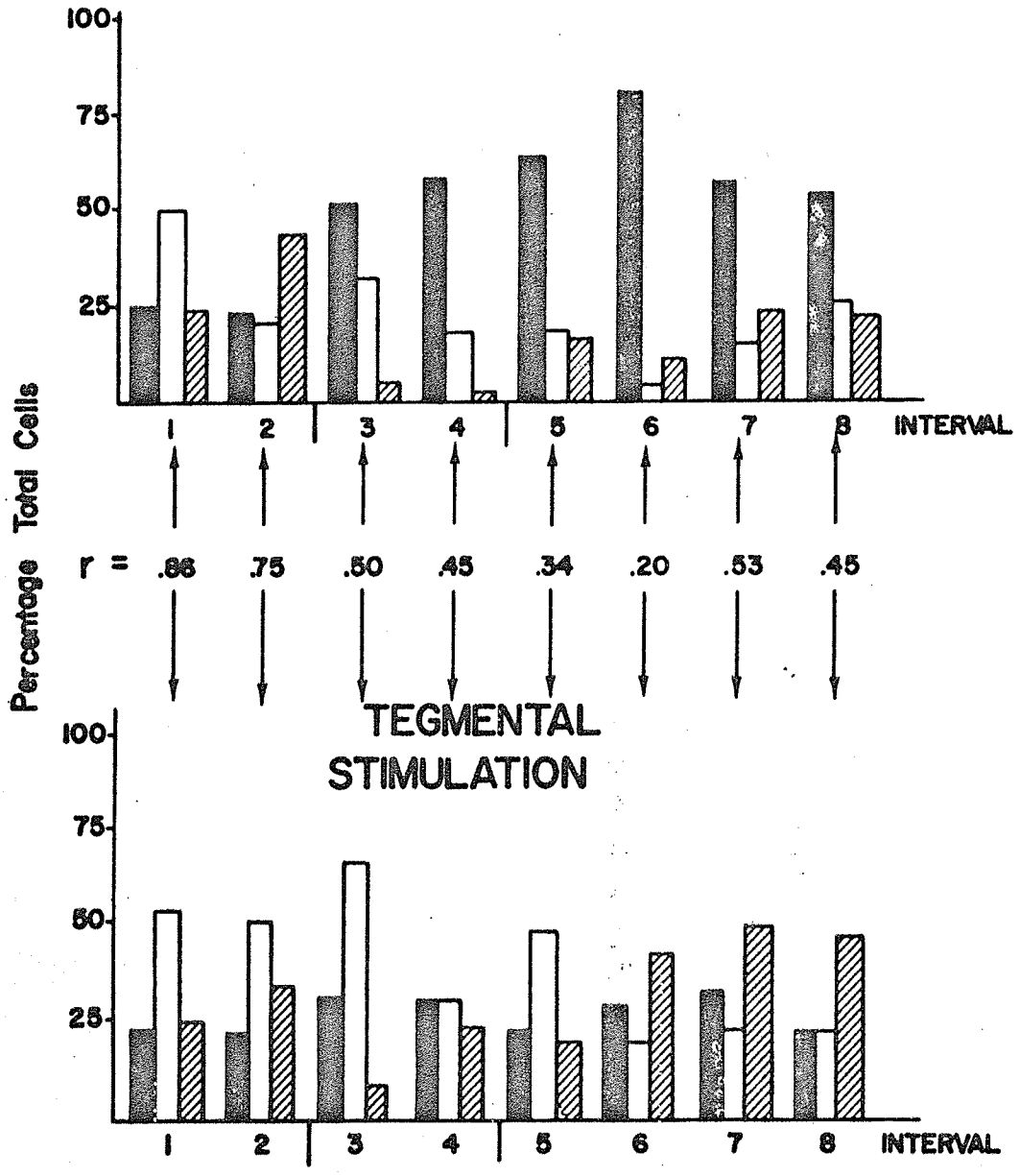
a percentage of the total sample of cells. In addition, stimulation data from all cells were pooled and a Pearson Correlation Coefficient for each pair of Raphe-Tegmental Stimulation Analysis intervals was computed.

Figure 8 summarizes the results of these comparisons. The number of cells (expressed as a percentage) showing inhibitory, excitatory, or no response in each analysis interval to raphe stimulation (top graphe) and tegmental stimulation (bottom graph) is shown. Between the two graphs is the correlation coefficient for each analysis interval covering the entire sample of 140 cells. This number does not include 36 cells of the original 176 where stimulation effects were tested. Eighteen of these 36 cells were non-responsive to the visual stimulus and the other 18 came from three animals where raphe stimulation caused obvious cardiovascular changes.

It can be seen from Figure 8 that depression was the predominant effect of raphe stimulation between Intervals 3 and 8, with the peak depression (70% and 80% of all cells) in Intervals 5 and 6 corresponding to the usual peak in the OFF response. It is interesting to note that only 25% of all cells showed predominant depression during pre-stimulus activity (Intervals 1 and 2) while over 55% of the cells showed depression of light-evoked activity (Intervals 3 and 4). These results show a greater tendency for geniculate cells

FIGURE 8

Comparison of raphe and tegmental effects on geniculate activity in the eight analysis intervals of the trial histogram for all cells in sample. Black bars, significant ($p < 0.05$) depression; white bars, significant facilitation; hatched bars, no significant effects. Correlation coefficient computed for each pair of analysis intervlas. Ordinate expressed as a percentage of total sample of cells. Intervals 1 and 2, pre-stimulus spontaneous activity; intervals 3 and 4, light on; intervals 5-8, light off.


RAPHE STIMULATION
100
75
50
25
Percentage Total Cells
1
2
3
4
5
6
7
8
INTERVAL
r =
.66
.75
.50
.45
.34
.20
.53
.45
TEGMENTAL STIMULATION
100
75
50
25
1
2
3
4
5
6
7
8
INTERVAL

to become depressed by raphe stimulation when photically driven and during photic-related afterdischarge than during spontaneous activity in the absence of a visual stimulus.

Electrical stimulation of the tegmental area, on the other hand, shows much less specificity for photically driven discharge in geniculate cells. Over 50% of the cells showed a facilitation of pre-stimulus spontaneous activity with tegmental stimulation: 66% respond with increased activity during Interval 3, corresponding to the initial peak of the ON response, although Interval 4, corresponding to the second half of the light-on period was characterized by any predominant effects. Interval 5, corresponding to the initial peak of the OFF response, showed facilitation in 55% of the cells while the remaining post-stimulus Intervals (6, 7, and 8) showed a mixture of facilitatory, depressant, and no effects.

These results indicate that tegmental stimulation has a tendency to exert a general facilitatory action on the spontaneous activity and the initial peak of the ON and OFF response of geniculate activity. At the same time there appears to be mixed effects on longer latency light-evoked and post-stimulus discharge.

The Pearson Correlation Coefficients show that during pre-stimulus

activity there is considerable predictability between the two stimulation effects (Interval 1 and 2). By squaring the correlation coefficients (r^2) we find that in Interval 1 and 2, 73% and 56% respectively of the variability of one stimulation effect can be accounted for by specifying the value of the other. Predictability is considerably reduced in the remaining analysis intervals reaching the lowest level in Interval 6 ($r = .20$, $r^2 = .04$). These results indicate that there is a high correlation between raphe and tegmental effects on pre-stimulus spontaneous activity but low correlation on photically related (ON and OFF) activity.

Anatomical Specificity of Mixed Effects on Tegmental and Raphe Stimulation:

Since the previous analysis was performed without consideration of anatomical placement of stimulating electrodes, we felt it desirable to determine if position of the stimulating electrodes influenced the proportion of cells responding primarily with facilitation or depression to raphe and tegmental stimulation.

Using the results of the comparisons previously mentioned, all cells from a given animal were compared to see if they responded to raphe and tegmental stimulation similarly in each analysis interval. Following this the results of histological examination of electrode pathways were noted for that

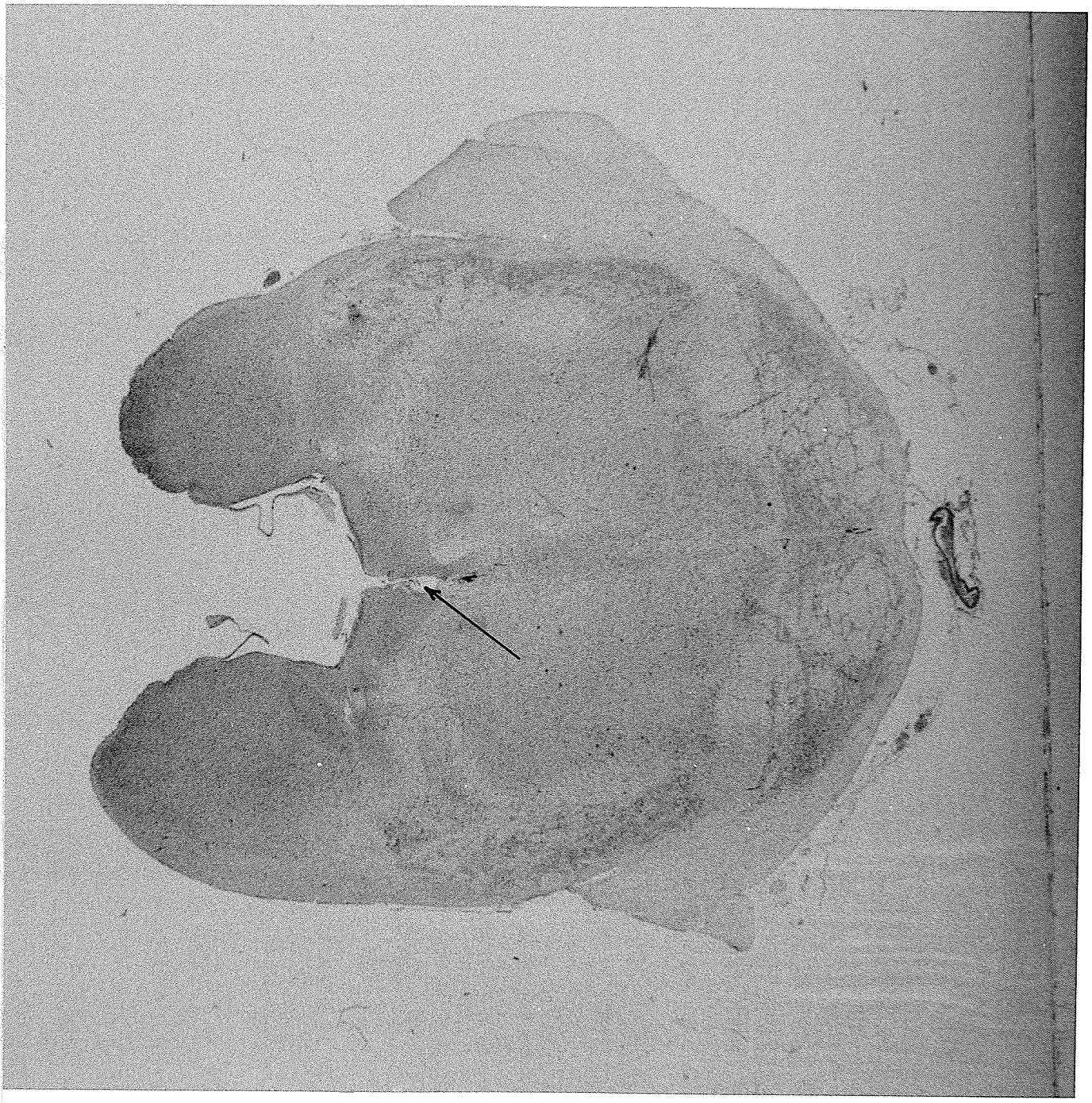
animal. An example of these comparisons is summarized in Table 1. Referring to Table 1, it can be seen that in animal #R-7, all four cells sampled responded very similarly to both kinds of stimulation. All cells responded to raphe stimulation in analysis Interval 1 with significant ($p < 0.05$) acceleration of firing. Three out of four cells in this animal responded in Interval 2 with no significant change in firing. Response of all cells in the remaining analysis Intervals was predominantly depression. The response to tegmental stimulation in these cells was primarily depressant or no effect with only few cases of facilitation. Figure 9 shows the placement of the raphe and tegmental electrodes in this animal. It can be seen that the raphe electrode was directly in the dorsal raphe nucleus of the midbrain while the tegmental electrode was on the lateral boundary of the periaquaductal gray matter. In other animals where the tegmental electrode had been placed in or near the periaqueductal gray matter the response to stimulation was primarily depressant or without effect with very few facilitatory responses.

Referring back to Table 1, it can be seen that in animal R-22 there was a mixture of facilitatory and depressant effects of raphe stimulation in a

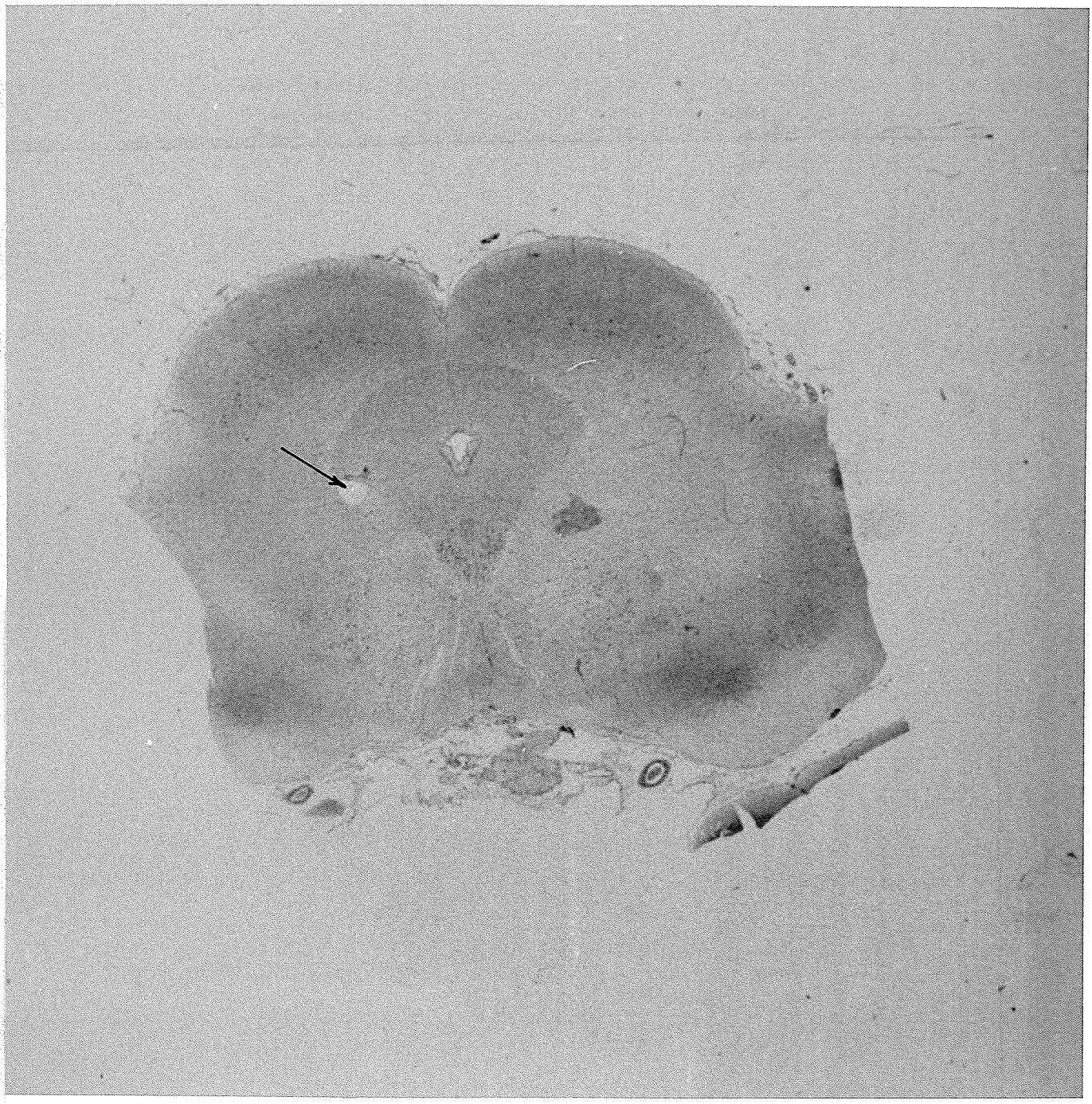
FIGURE 9

Histological sections from animal R-7 showing electrode tip in dorsal raphe nucleus, (A) and on the edge of periaquaductal gray (B).

A



B



number of intervals. The responses in these cells to tegmental stimulation was primarily of a facilitatory nature with the exception of the last three analysis intervals. Figure 10 shows the location of electrode tips in this animal. It can be seen that the position of the raphe electrode is slightly lateral to the midline but very close to the dorsal raphe nucleus.

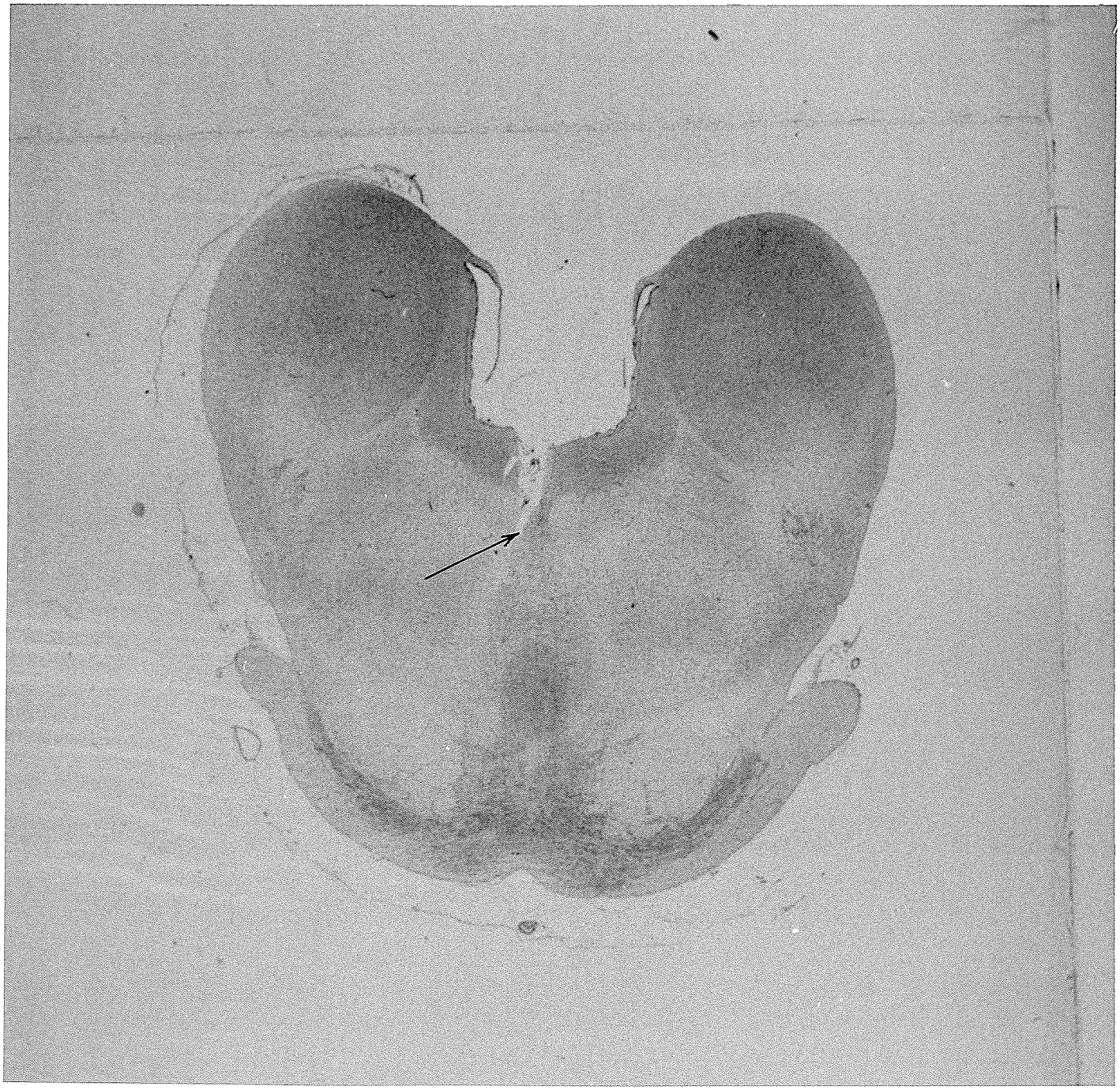
The location of the tegmental electrode is below the superior colliculus but slightly dorsal to the central tegmental field. Animals with similar placements or placements more ventral in the central and lateral tegmental areas showed primarily facilitatory effects on geniculate activity in most analysis intervals.

Referring again to Table 1, animal R-31 showed primarily depressant responses to raphe stimulation. Tegmental stimulation caused facilitory responses in Intervals 3, 4, and 5 but a mixture of effects in all other intervals. Figure 11 shows the histological results from this animal. The raphe electrode is again located in the dorsal raphe nucleus while the tegmental electrode is located lateral to the periaquaductal gray matter in the ventral superior colliculus. Placements in this area in other animals resulted in a mixture of facilitatory and depressant responses to stimulation via the tegmental electrode.

FIGURE 10

Histological sections from animal R-22. Tip of raphe electrode slightly lateral to midline (A). Second electrode tip below superior colliculus, lateral to PAG and superior to central tegmental field (B).

A



B

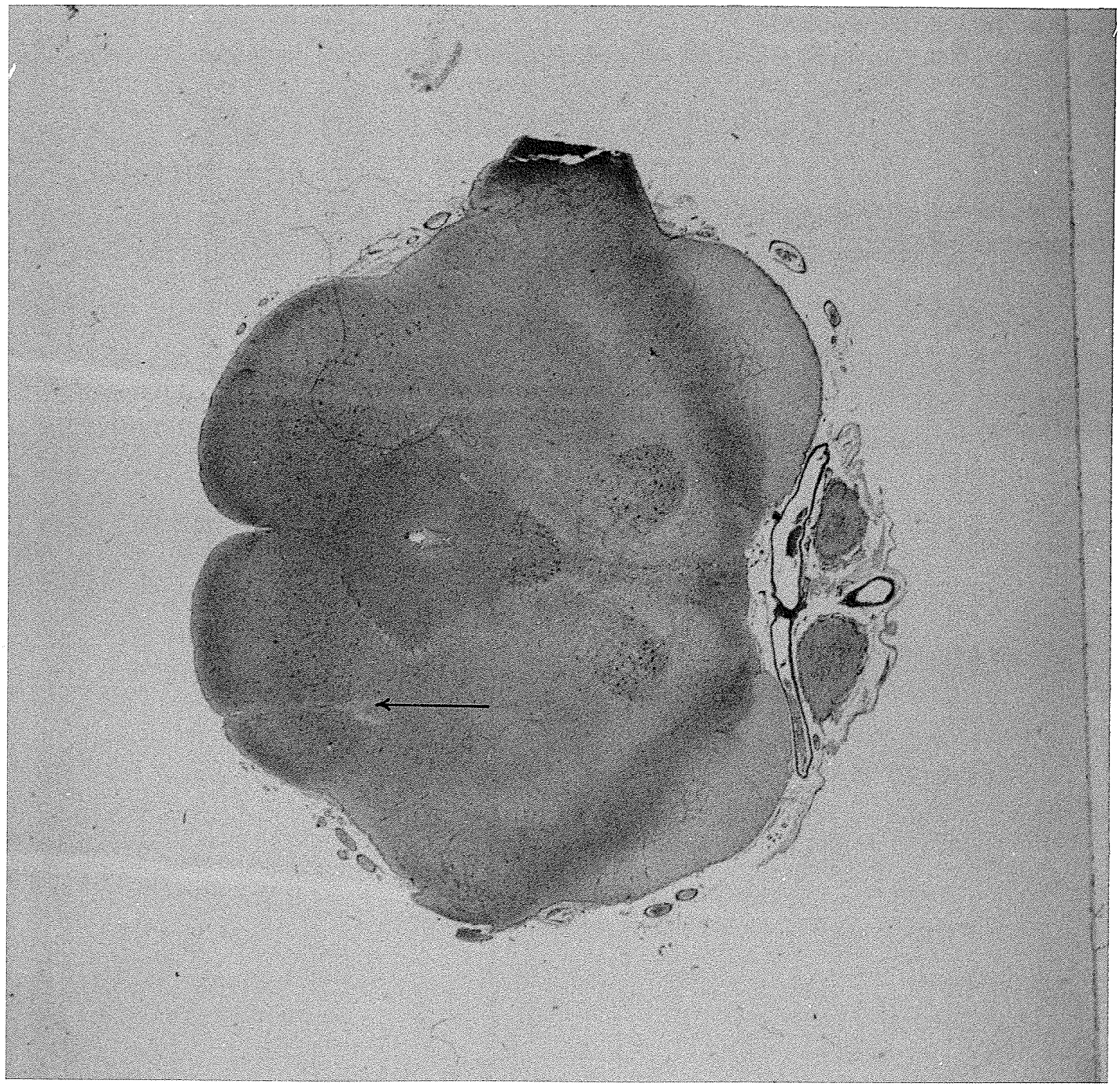
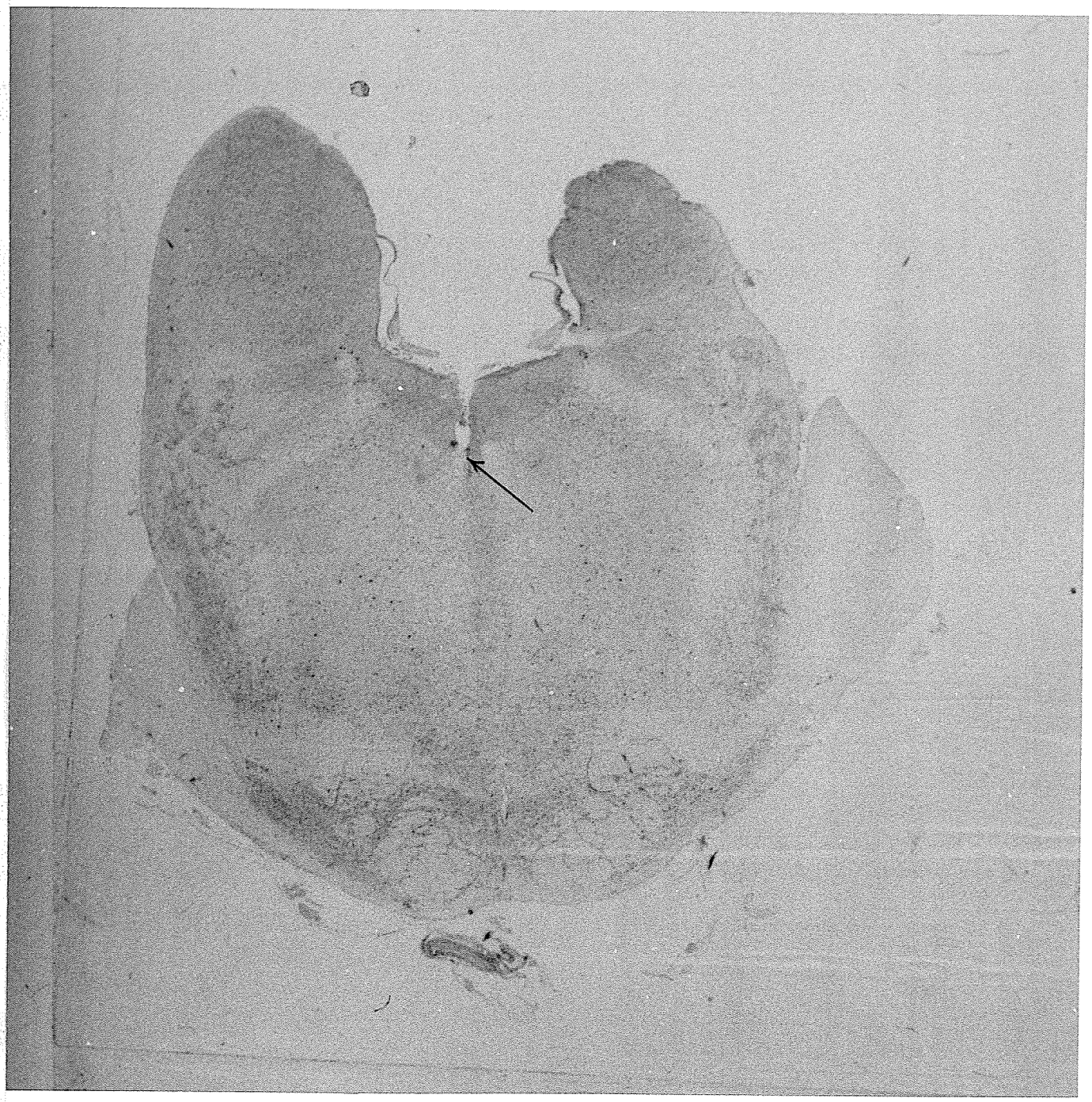


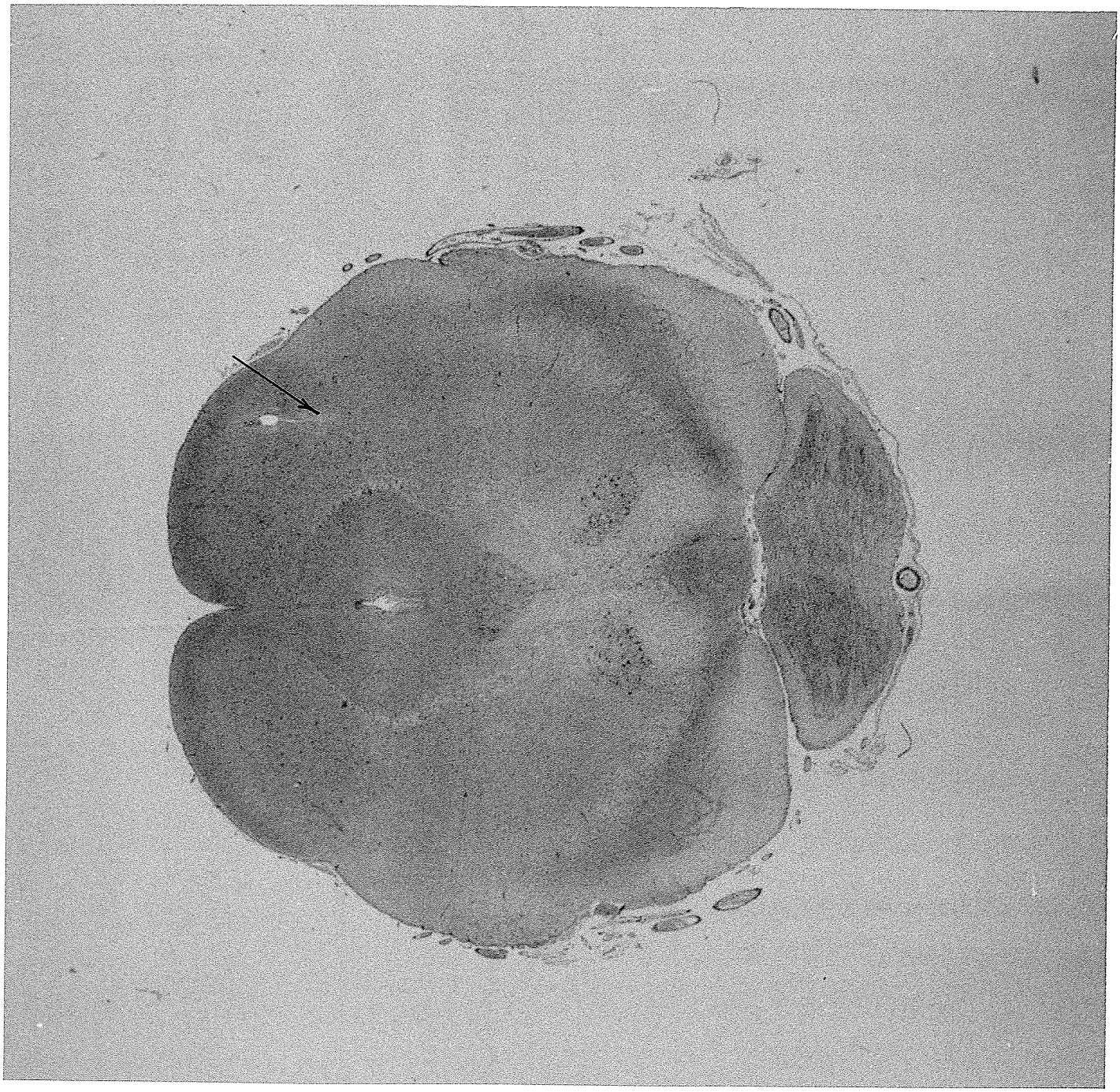
FIGURE 11

Histological sections from animal R-31. Electrode tips located in dorsal raphe nucleus (A), and slightly ventral to superior colliculus (B).

A



B



These results indicate that movement of the raphe electrode slightly lateral from the midline increases the likelihood of excitatory responses in some analysis intervals during stimulation. The position of the tegmental electrode seems crucial in determining what kinds of effects are elicited in geniculate cells during stimulation. Placements in and near the central and lateral tegmental field result in primarily facilatatory responses while positions in and near the periaquaductal gray matter produce depressant responses. Placements between these two areas usually result in a mixture of effects during stimulation.

Specificity of Stimulation Effects to Visual Cells:

In an effort to determine if stimulation of raphe and tegmental areas had the same general effects on non-visual cells, we sampled a total of 18 cells from 8 cats which were not responsive to the visual stimulus. In each of these 8 cats visually responsive cells were also encountered which showed typical responses to raphe and tegmental stimulation.

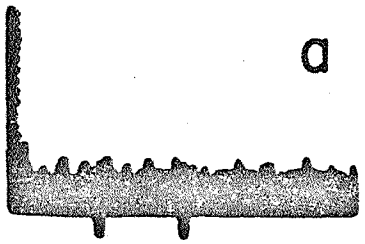
Figure 12 shows two such cells. These cells were found in two different animals at a depth of between 16 - 18 mm. below the cortical surface. In both cases there were no significant effects of raphe or tegmental stimulation on the level of maintained discharge in these neurons.

FIGURE 12

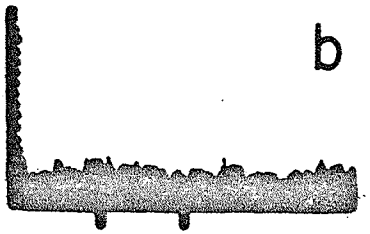
Effects of raphe (b) and tegmental (e) stimulation on two cells non-responsive to visual stimulus.

CELL NO. R-5-3-3

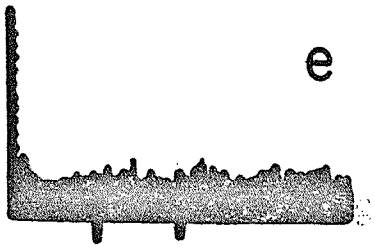
CONTROL



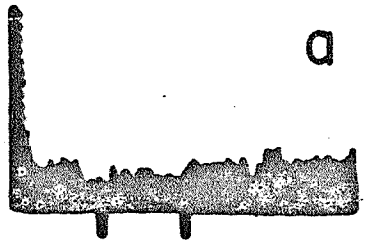
RAPHE STIM.

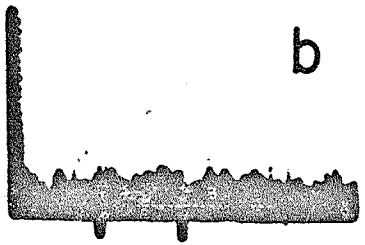


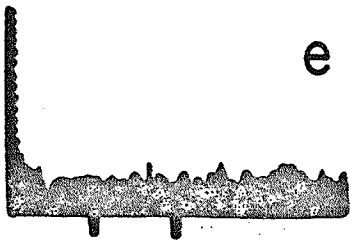
TEGMENTAL STIM.



CELL NO. R-9-1-4







This finding was characteristic of all 18 non-light responsive cells in our sample.

DISCUSSION

We have shown that lateral geniculate cells respond to stimulation of the raphe nucleus with a depression in spike activity. Stimulation of various locations lateral and superiolateral to the raphe nucleus result in a mixture of excitatory and inhibitory responses of geniculate cells. Most cells in our sample showed immediate recovery or return to prestimulation patterns of firing following the termination of either raphe or tegmental stimulation.

The stimulation effects observed appear to be specific to cells which are light responsive since none of the cells that did not respond to light showed stimulation effects. This finding confirms the notion that specific pathways in the brainstem selectively modulate lateral geniculate discharge activity.

One of the most interesting findings in this study was that the depressive effects of raphe stimulation were most apparent when the cell was being photically driven or shortly after the termination of the visual stimulus corresponding to the OFF discharge. In contrast, the excitatory effects of tegmental stimulation appeared to be most prominant during the pre-stimulus spontaneous activity and during the initial peak of the ON and OFF response to photic stimulation. The implications of these findings regarding the possible form of synaptic connections between the lateral geniculate and various brainstem pathways, is of interest. It has been suggested that 5-HT, presumably from raphe pathways, may act to prevent the presynaptic release of excitatory transmitter from the optic nerve (126) or preventing their access to receptor sites thus reducing the geniculate response to light. If this were true, one would expect that activation of raphe pathways would have a depressive effect on geniculate response to light without significantly affecting the rate of spontaneous activity in the absence of light. One difficulty with this idea is the finding that optic nerve fibers are themselves spontaneously

active in the absence of light and undoubtedly contribute to some extent to the spontaneous discharge of lateral geniculate cells (81, 113). Nevertheless, numerous studies have shown that spontaneous discharge can be substantially altered in geniculate cells by lesioning or stimulating various areas of the brainstem, indicating that at least in part the spontaneous discharge in geniculate cells is influenced by brainstem pathways (16, 40, 125, 130). Thus, one might expect, according to the above theory, stimulation of the raphe nucleus would result in a substantial depression of photically related activity in the geniculate (ON and OFF) originating from the optic nerve with a less prominent effect on the spontaneous discharge of these neurons. The extent to which the spontaneous activity of a given geniculate cell is influenced by raphe stimulation would then be determined by the extent to which the optic input contributed to the overall spontaneous rate of firing. Our data supports this notion. If there were direct synaptic connection between the raphe fibers and the geniculate cells it is difficult to see how the depressive effects of raphe stimulation is minimal during spontaneous activity but maximal when the geniculate is driven by light - presumably a time when there is an increase barage of excitatory input. It would seem more likely in light of these results that as previously suggested the influence of raphe fibers on the lateral geniculate cells is brought about by influence on optic nerve terminals.

One alternative that must be considered is the possibility that during stimulation of the raphe nucleus there is a build up of released transmitter substance. If this were to occur one might expect that there would be greater depression in consecutive analysis intervals in the geniculate cells. The observation that the peak depression occurs in interval six with subsequent reduction in intervals 7 and 8 is not consistent with such a hypothesis.

One might argue that if intervals 1 and 2 are compared with intervals 7 and 8 the activity in the latter is much more depressed than the former and therefore indicates that raphe depression is quite prominant during spontaneous activity after the light goes off. Contrary to this view are several studies which show that afterdischarge in geniculate cells is often considerably extended in time (32, 63, 90, 96) so that is probably improper to consider the activity in intervals 7 nand 8 as being a return of normal spontaneous activity independent of photic experience. Thus, although the possibility of transmitter buildup cannot be ruled out at this time it is consistent with our data to suggest that this is not responsible for the apparent selectivity of raphe induced depression of photic related activity.

An important question to consider here is whether the occasionally observed excitatory effects of raphe stimulation are direct (i.e., synaptic) or whether they occur as a result of current spread from the electrode tip to surrounding midbrain pathways having an excitatory influence on the geniculate.

As the result of some iontophoretic studies (21, 22, 23, 103) some authors have suggested that 5-HT may be excitatory at some synapses and inhibitory at others. The possibility arises that 5-HT-containing raphe fibers influence lateral geniculate cells not only by virtue of presynaptic influence of optic nerve activity but by a direct postsynaptic connections some of which may be excitatory. The results of this experiment cannot directly be applied to this question; however, we observed that if the raphe electrode deviated from the midline it increased the probability of excitatory effects. In addition when excitatory effects were observed they were correlated with the excitatory effects of tegmental stimulation (See Table One). These findings support the notion that the excitatory effects

sometimes observed during raphe stimulation might be due to current spread from the stimulating electrode.

In our study, stimulation of the midbrain tegmentum had excitatory and inhibitory influence on the discharge of LGN cells. The relative predominance of these effects seemed to be related to the position of the electrode within a large region of the lateral midbrain. These findings are consistent with other studyings on the effects of reticular formation stimulation on LGN cells (16, 40, 130). The profile of tegmental stimulation effect on LGN cells is considerably different from the effects of raphe stimulation. Since there was greater variation in tegmental electrode placements than raphe electrode placements it is difficult to obtain a unified picture of the effects of tegmental stimulation, on the LGN. This fact however, was advantageous in that it served as a good control is assessing the anatomical specificity of raphe stimulation effects. Over 55% of the cells in our sample responded to tegmental stimulation with a general facilitation of both spontaneous and light-evoked activity. Electrode placements in these animals were in the central and lateral tegmental field of the rostral mesencephalon.

These results are most easily accounted for by proposing that tegmental pathways in the mesencephalic reticular formation have a direct modulating effect on lateral geniculate cells, regulating their general excitability.

In considering the functional significance of the findings on raphe stimulation it is important to take note of some of the work from other laboratories. Sheard and Aghajanian found that electrical stimulation of the raphe caused dishabituation in rats previously habituated to an auditory stimulus (118). Samanin and his co-workers have found a reduction of soma-

tosensory evoked potentials with stimulation of the raphe nucleus in rats (108). Recent studies on the effects of raphe stimulation on sensation of pain have shown that behavioral analgesia in rats and cats is obtained by raphe stimulation (5, 84, 89). These workers have also shown that raphe stimulation reduces activity in lamina 5 cells to nocioceptive stimuli in cats. All these studies suggest that the raphe pathways play some role in regulating the excitability of various sensory relay nuclei to afferent input from sensory receptors. Our results indicate a similar role in the visual modality. It seems plausible that the raphe nucleus is part of a network functioning as a sensory gating mechanism and perhaps mediating such phenomenon as habituation, and attention.

EXPERIMENT II

METHODS

Subjects: A total of 35 cats were used in this experiment.

Procedure: All surgical and preliminary experimental procedures in this experiment were identical to those previously described.

Testing Procedure and Data Analysis:

In this experiment we utilized two kinds of testing and data analysis. We felt it desirable to sample as many cells in each PCPA and AMT treated subject as possible and compare the results in the sample to those obtained in the previous experiment. Consequently for all cells except the last one sampled in each subject, the procedure was identical to that reported in the previous experiment. With each animal after several hours of testing an arbitrary decision was made that the next cell sample would be the last one. These cells were first tested in a similar manner as before; however, after the testing sequence had been repeated 50 times instead of going on to another cell the animal was given an injection of 5-HTP in the case of PCPA pretreatment or L-DOPA in the case of AMT pretreatment. Twenty to thirty minutes after the injection the cell was again re-tested with 50 repetitions of the testing sequence. Using this procedure it was possible to observe whether the effects of stimulation in the pretreated animal were altered after administration of the precursor. It has been reported that within a half-hour after administration of 5-HTP in PCPA treated animals that serotonin levels in the brain begin to increase (74). In the case of catecholamines, a complex series of events occurred after treatment of AMT treated animals with L-DOPA, dopamine levels in the brain increase significantly during the first hour with noradrenaline levels remaining low; during the second hour and afterwards the noradrenaline levels increase significantly (33).

In the experiments with the precursors, 5-HT and L-DOPA, a slightly different technique was employed in analyzing the data. For each cell in which the precursors were tested, there were five basic histogram comparisons. Before the administration of the precursor the effects of raphe and tegmental stimulation were assessed by comparisons between trial histogram A and B for raphe effects and A and E for tegmental effects as in the previous experiment. To determine the effect of the precursor on general cellular response in the absence of stimulation, trial histogram A before administration was compared with trial histogram A after the administration (see Figure 1). To test the effects of stimulation after administration, the post-drug histogram A was compared to the post-drug histograms B and E (Figure 1). As in all previous work comparisons were made by pairing analysis intervals in the respective histograms and the t-statistic was employed.

Drug Administration:

The ten animals used in the PCPA experiments were treated with daily doses of 300 mg/kg of p-chlorphenylalanine (Sigma) dissolved in a phosphate buffer injected subcutaneously for three days prior to the experiment. It has been reported that this treatment regimen produces a 95% depletion of endogenous 5-HT in the brain (37). 5-Hydroxytryptophan (100 mg/kg) was injected very slowly intravenously 20-30 minutes prior to re-testing of the cell.

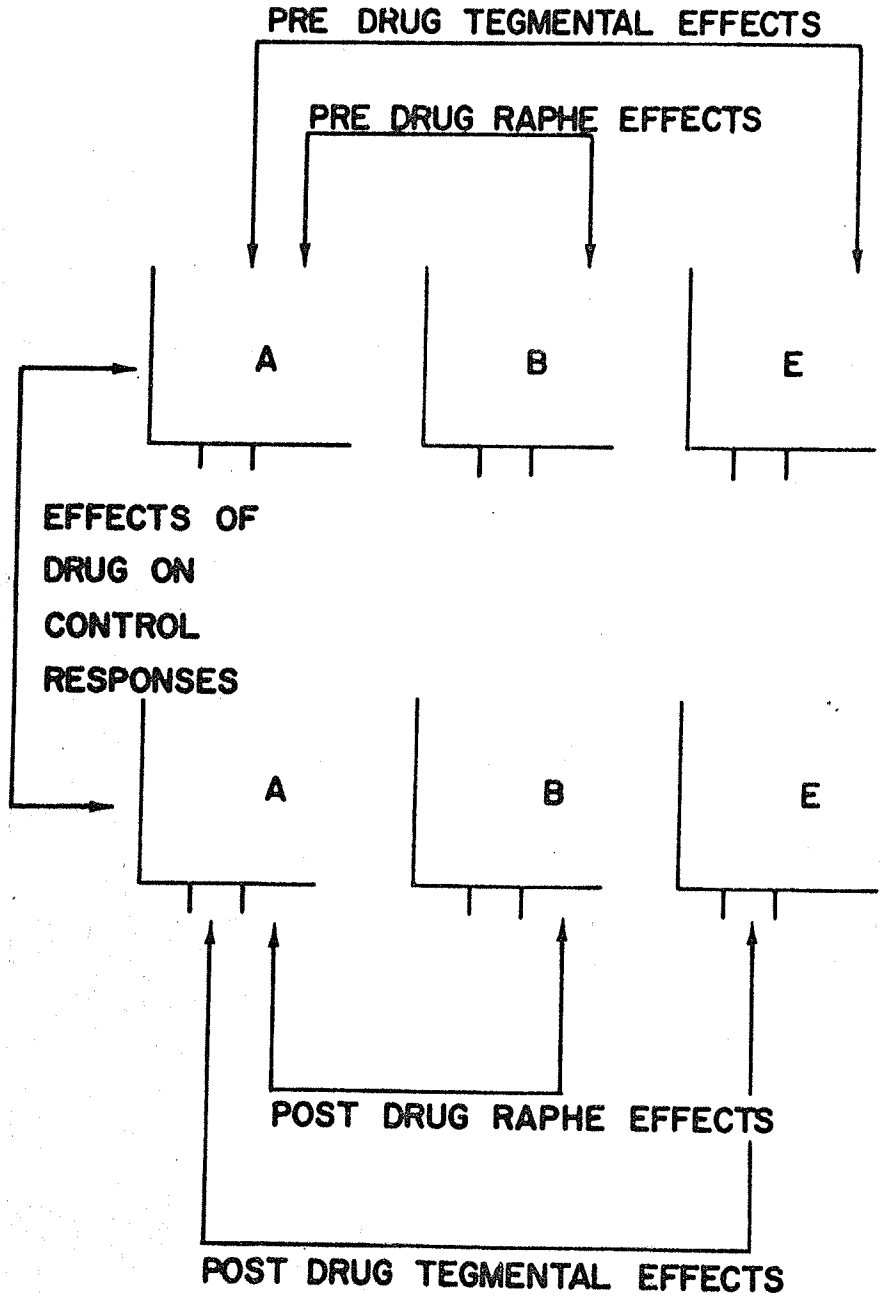
The five animals used in the AMT experiment were injected subcutaneously with 275 mg/kg of AMT dissolved in saline 10-12 hours prior to testing. It has been shown that this treatment schedule produces an 80% depletion of catecholamines.

L-DOPA was given 100 mg/kg with a slow intravenous infusion prior to retesting the last cell in each AMT treated subject.

Ten animals were tested with 100 micrograms LSD, five were tested with

FIGURE 1

Diagram indicating method of assessing effects of drug treatment on geniculate responses to raphe and tegmental stimulation. Top row of histograms sampled prior to drug injection (pre-drug histograms); bottom row sampled after injection (post-drug histograms). Arrows indicate which two histograms were paired for a given comparison. A, control - no stimulation; B, raphe stimulation; C, tegmental stimulation.


PRE DRUG TEGMENTAL EFFECTS
PRE DRUG RAPHE EFFECTS
A
B
E
EFFECTS OF DRUG ON CONTROL RESPONSES
A
B
E
POST DRUG RAPHE EFFECTS
POST DRUG TEGMENTAL EFFECTS

50 micrograms LSD, five were tested with 100 micrograms BOL and ten were tested with 2cc. of physiological saline. In some of the animals the effects of LSD or BOL were tested more than once on different cells. When this occurred the second injection was always given at least three hours after the first. It has been shown that LSD disappear from the brain within the first 1 - 2 hours after administration (45).

RESULTS

Effects of Raphe and Tegmental Stimulation in PCPA treated Cats:

A total of 48 cells were studied in 10 cats treated with PCPA. Figure 2 illustrates typical effects of raphe and tegmental stimulation on geniculate cells from three different PCPA treated animals. In cell P-1-3-1, raphe stimulation had a significant facilitatory effect ($p < 0.05$) in intervals 1 and 4. There were no significant depressant effects in any intervals. In cell P-4-1-2, raphe stimulation produced no significant differences in any analysis intervals compared to the control. In cell P-7-1-5, raphe stimulation produces a slight but insignificant facilitation in intervals 2, 3, and 5. In cell P-1-3-1, tegmental stimulation had no significant effects except a facilitory effect in interval 4, the same effect observed with raphe stimulation in that cell. In cell P-4-1-2, tegmental stimulation showed a significant facilitatory effect in interval 1, a significant depressant effect in interval 3 (corresponding to the peak of the ON response ($p < .05$) and a slight but non-significant depressant effect in intervals 6, 7, and 8. In cell P-7-1-5, there was a slight but non-significant facilitatory effect in interval 5.

These results indicate that in PCPA-treated cats geniculate responses to raphe stimulation are very atypical when compared to normal untreated controls.

FIGURE 2

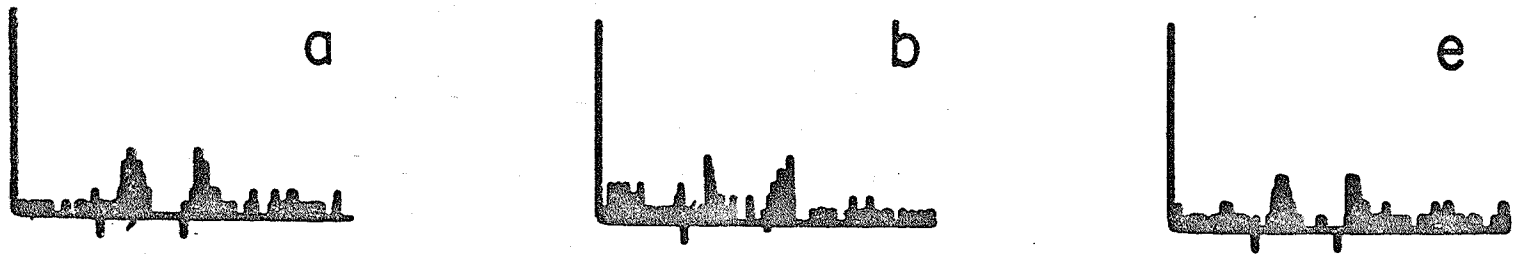
Effects of PCPA pretreatment on lateral geniculate responses to raphe (b) and tegmental (e) stimulation in cells from three different animals. For a given cell, histograms are read from left to right.

CELL NO. P-1-3-1

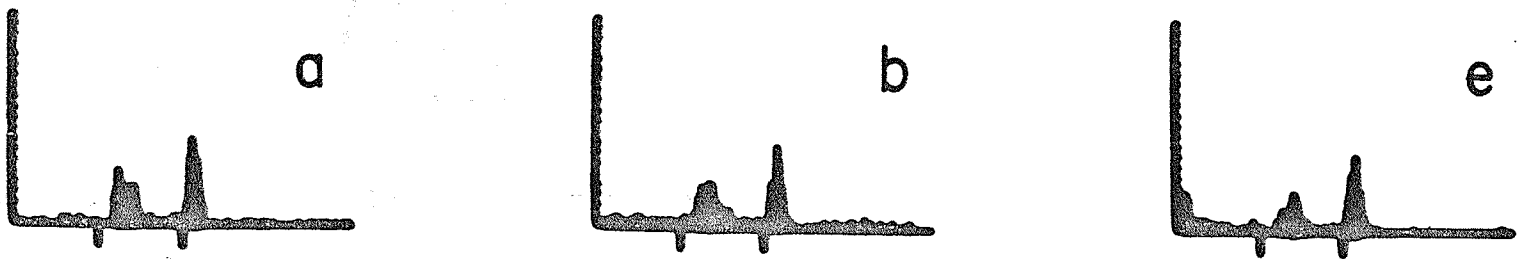
CONTROL

RAPHE STIM.

TEGMENTAL STIM.



CELL NO. P-4-1-2



CELL NO. P-7-1-5

PCPA TREATED SUBJECTS

Only 5 cells (from 2 animals) out of the entire 48 showed depression to raphe stimulation similar to that reported in the previous paper.

Effects of Stimulation in PCPA Treated Cats After 5-HTP Administration

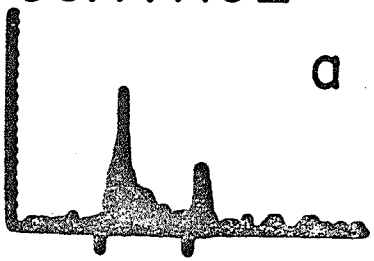
In each of the 10 PCPA-treated animals the last cell sample was retested 20-30 minutes after the slow intravenous infusion of 5-HTP. In 4 of these animals the cell was lost before the testing procedure was resumed. Figure 3 shows the effect of 5-HTP administration upon cellular responses to raphe and tegmental stimulation in one of the PCPA treated subjects. The top three histograms represent the first test sequence before 5-HTP was administered; the bottom three histograms were sampled 30 minutes after 5-HTP injection. It should be noted that prior to 5-HTP administration, this cell responded to raphe stimulation with a slight facilitation in all analysis intervals. Response to tegmental stimulation, on the other hand, was a powerful depression of activity in intervals 3, 4, and 5 corresponding to ON and OFF responses. This depression was statistically significant ($p < 0.001$). Referring to the bottom row of histograms in Figure 3, comparison of the control histograms before and after the administration of 5-HTP revealed a slight but non-significant overall depression after 5-HTP. Stimulation of the raphe nucleus after 5-HTP had two effects; a significant ($p < .05$) facilitation of activity in all analysis intervals except interval 5 (OFF response) which showed a significant ($p < 0.01$) depressant

FIGURE 3

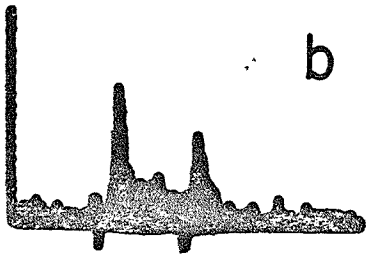
Effects of 5-HTP administration in PCPA treated cat to raphe and tegmental stimulation in PCPA pretreated subjects. Upper three histograms taken before, bottom three taken after 5-HTP.

PCPA TREATED

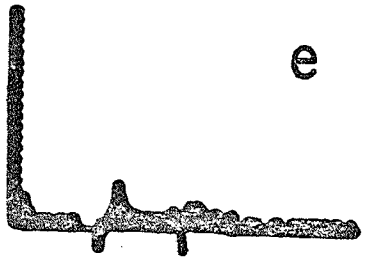
CONTROL



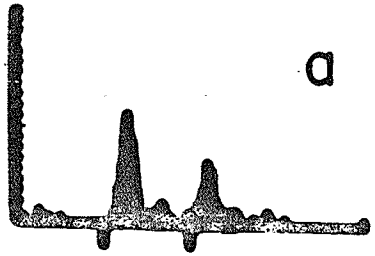
RAPHE STIM.

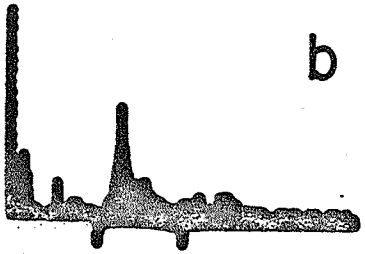


TEGMENTAL STIM.



AFTER 5 HTP (100 mg./ kg.)





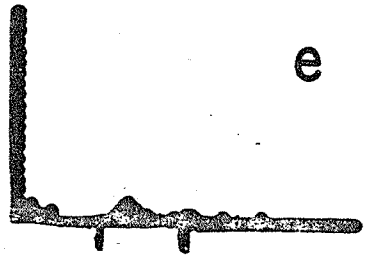
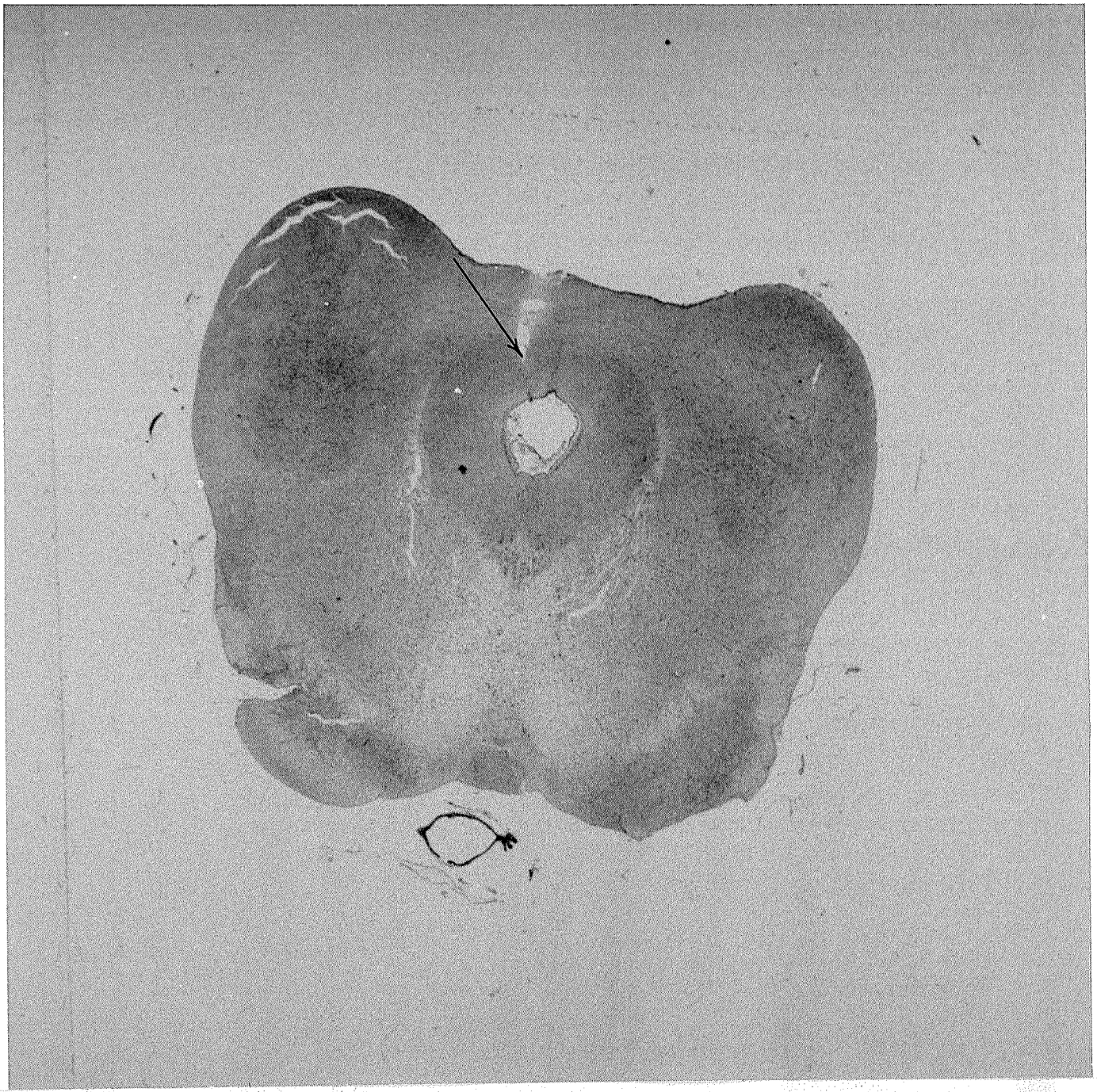


FIGURE 4

Histological section from animal described in Figure 3. Electrode tips in dorsal raphe nucleus (4a) and upper periaquaductal gray (4b).

B



effect. Response to tegmental stimulation after 5-HTP was characterized by heightening the depressant effects observed before 5-HTP administration. Since the data from this cell indicated that profound depressant responses could be elicited by stimulating the tegmental electrode in the total absence of any similar depressant effects of raphe stimulation, we were particularly curious about the accuracy of our electrode placements. Figure 4 shows the placement of both raphe and tegmental electrodes. The raphe electrode was accurately placed in the dorsal raphe nucleus while the tegmental electrode had been grossly misaligned and terminated in the dorsal aspect of periaquaductal gray (PAG) near the midline.

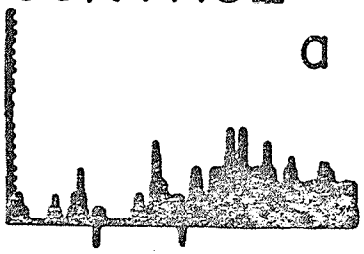
Figure 5 shows results from one of the five cells responding to raphe stimulation with residual depression in a PCPA-treated animal. It is interesting to note that tegmental stimulation also had a slight depressant effect on the cell. After administration of 5-HTP the control histogram revealed a marked facilitation of all activity significant beyond the 0.001 level. Stimulation of the raphe after 5-HTP caused a further depression of cellular discharge. Of particular interest was the observation that after 5-HTP, tegmental stimulation resulted in a considerable facilitation of activity in all analysis intervals to the extent that it completely obliterated the photic responses. Data from this cell shows that even though raphe and tegmental stimulation both

FIGURE 5

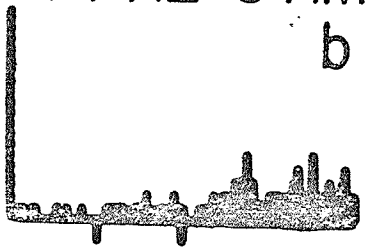
Effect of 5-HTP in PCPA treated cat administration on lateral geniculate response to raphe (b) and tegmental (e) stimulation. Cell showed depressive response to raphe stimulation prior to 5-HTP. After 5-HTP administration depression was intensified. Top three histograms taken before, bottom three after 5-HTP.

PCPA TREATED

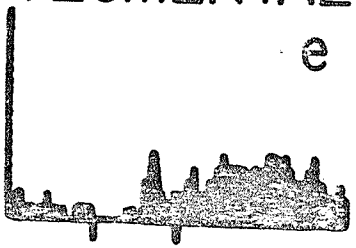
CONTROL



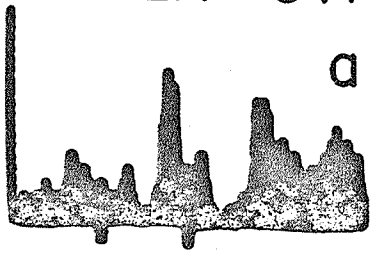
RAPHE STIM.

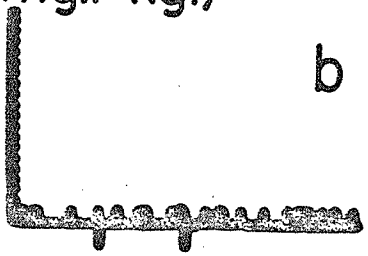


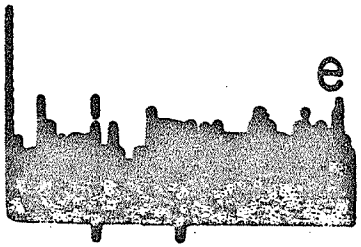
TEGMENTAL STIM.



AFTER 5 HTP (100 mg./kg.)







had a depressant effect before injection, 5-HTP was effective in heightening the depression only with raphe stimulation.

Table 1 summarizes the results of administration of 5-HTP in the PCPA-treated cats from which the six cells were sampled. For each cell the effects of raphe and tegmental stimulation were recorded before and after the administration of 5-HTP. Analyzing the data we compared each stimulation effect on the cell, interval by interval, with the appropriate control histogram A. If a significant t-ratio resulted, the appropriate effect was recorded as a depression (-) or a facilitation (+); if a non-significant t-ratio was found, a no-effect (0) was recorded for that given interval. This allowed us to look at each cell and its response to raphe and tegmental stimulation, interval by interval before and after the administration of 5-HTP. Figure 6 summarizes the effects of 5-HTP on raphe stimulation in PCPA-treated cats. The top set of curves expresses how many cells responded with depression after 5-HTP administration. The analysis intervals, represented on the X-axis were pooled into blocks of two. It can be seen that intervals 3, 4, 5, and 6, corresponding to the period during which the ON and OFF response usually occur showed more numerous depressant responses after 5-HTP than intervals 1, 2, 7, and 8. The bottom set of curves in Figure 6 indicate the number of cells responding with

TABLE #1

EFFECTS OF 5-HTP ON GENICULATE RESPONSES TO RAPHE AND TEGMENTAL STIMULATION IN PCPA TREATED SUBJECTS

SUBJECT		INTERVAL NUMBER 1		2		3		4		5		6		7		8	
		R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T
P-2	Before 5-HTP	-	0	-	0	+	0	-	-	-	0	-	-	0	0	0	0
	After 5-HTP	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
P-3	Before 5-HTP	0	0	0	+	+	+	0	+	+	+	+	0	+	0	0	0
	After 5-HTP	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+
P-5	Before 5-HTP	+	0	0	0	0	-	+	-	+	-	0	0	+	0	0	0
	After 5-HTP	+	0	+	0	+	-	+	-	-	-	+	-	+	0	+	0
P-6	Before 5-HTP	0	+	+	+	0	+	0	0	+	+	+	+	0	+	0	+
	After 5-HTP	+	+	0	+	-	+	-	+	-	+	-	+	0	0	+	+
P-8	Before 5-HTP	+	0	-	+	0	+	+	-	+	+	0	+	+	-	+	+
	After 5-HTP	+	+	+	-	-	+	0	+	0	+	-	+	0	+	+	+
P-9	Before 5-HTP	0	-	0	-	-	-	+	+	+	-	+	+	+	0	0	0
	After 5-HTP	+	+	+	+	+	-	-	+	-	+	-	+	+	-	0	-

Each column represents an analysis interval broken into 2 parts:

R = Effects of Raphe Stimulation
T = Effects of Tegmental Stimulation

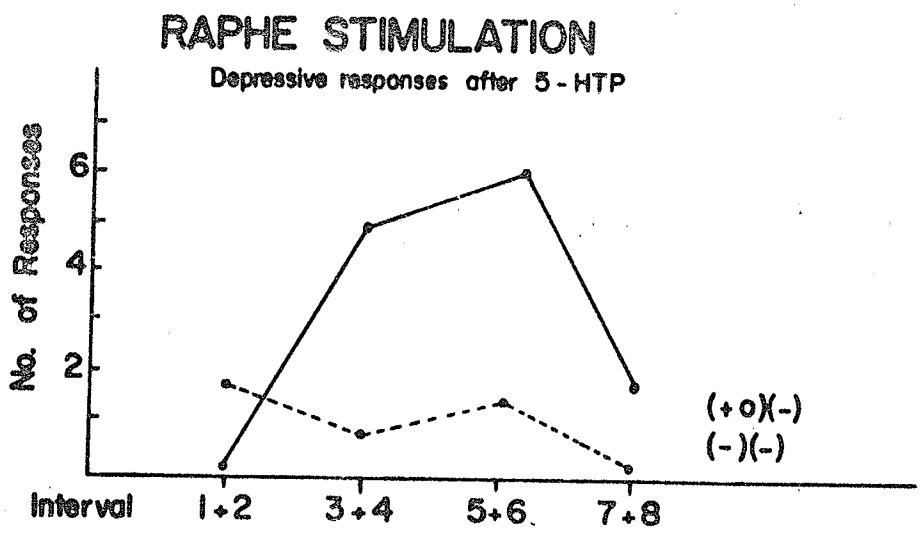
- indicates a significant depression for that interval in that animal
+ indicates a significant facilitation for that interval in that animal
0 indicates no significant response to stimulation for that interval

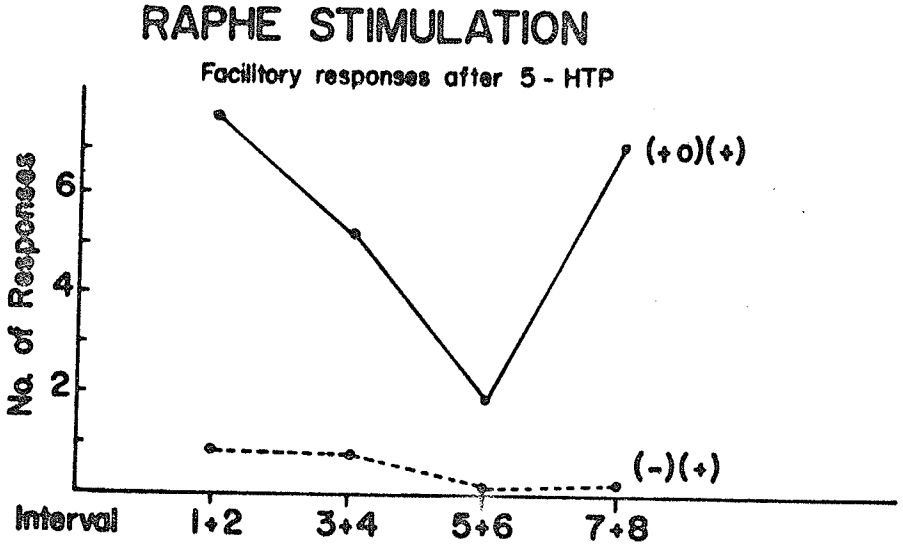
FIGURE 6

Effects of 5-HTP administration in PCPA treated cats on geniculate responses to raphe stimulation. Top graph indicates the number of cells showing depressant (significant, $p < 0.05$) responses to raphe stimulation after administration of 5-HTP. Dark lines indicates a change from either facilitation or no effect before 5-HTP to depressant response after. Dotted line shows number of responses which were depressant before and after 5-HTP.

Bottom graph shows facilitatory responses to raphe stimulation after 5-HTP. Dark line indicates number of responses which were facilitatory before as well as after 5-HTP while dotted line indicates number of responses which were depressant before 5-HTP but facilitatory after.

Data for this figure was taken from Table 1.


RAPHE STIMULATION
Depressive responses after 5 - HTP
No. of Responses
6
4
2
(+o)(-)
(-)(-)
Interval
1+2
3+4
5+6
7+8


RAPHE STIMULATION
Facilitory responses after 5 - HTP
No. of Responses
6
4
2
(+o)(+)
(-)(+)
Interval
1+2
3+4
5+6
7+8

facilitation after 5-HTP during raphe stimulation. This figure indicates that administration of 5-HTP in PCPA-treated cats had two main effects on the geniculate response to raphe stimulation. First, there was a general facilitatory effect that was most prominant during spontaneous activity and in the very late intervals after termination of the visual stimulus. This facilitatory effect was much less prominant in intervals 3, 4, 5, and 6 which correspond to the ON and OFF responses to light. The second effect of 5-HTP administration was to promote depressant responses primarily restricted to intervals 3, 4, 5, and 6 corresponding to photically driven activity. The depressant effects of 5-HTP were almost non-existent in intervals 1 and 2 corresponding to pre-stimulus spontaneous activity and intervals 7 and 8 corresponding to the latter after discharge period following termination of the visual stimulus.

Figure 7 summarizes the effects of 5-HTP administration on geniculate responses to tegmental stimulation. The top set of curves indicate the number of cells showing a depressant response after 5-HTP. The bottom set represents the number of cells responding with facilitation after 5-HTP. The analysis intervals have been pooled into blocks of two as in Figure 6.

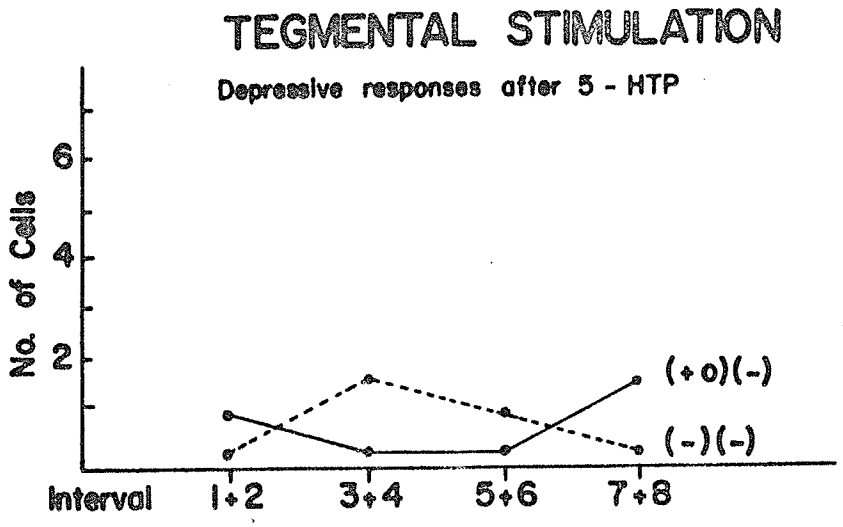
Referring to the top set of curves in Figure 7 we find that very few analysis intervals showed a significant depressant response after 5-HTP. The bottom set of curves show that the major effect of 5-HTP on geniculate response

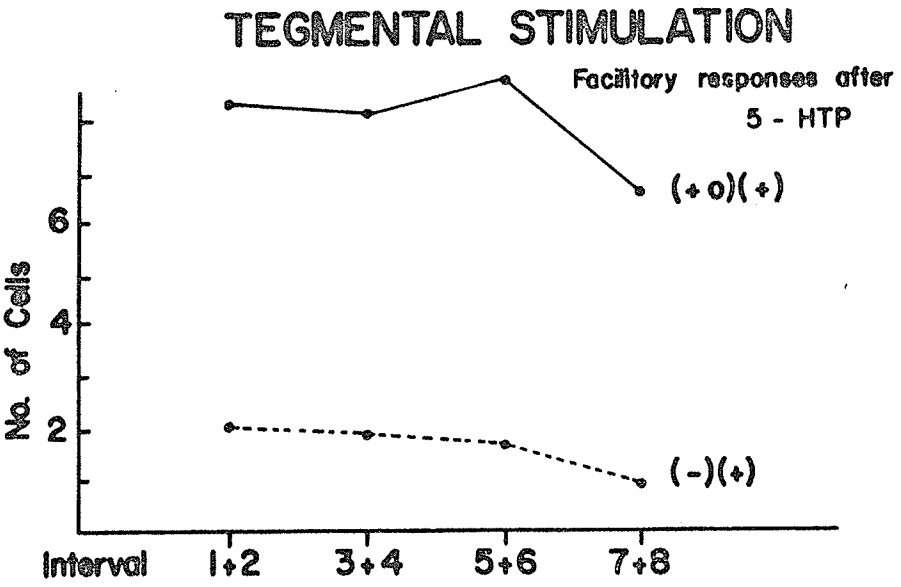
FIGURE 7

Effects of 5-HTP on geniculate responses to tegmental stimulation. Top graph shows the number of cells showing depressant responses to tegmental stimulation after 5-HTP. Dark line indicates number of responses which were either facilitatory or had no effect before 5-HTP but changed to depressant responses after. Dotted line indicates number of cells showing depressant responses to tegmental stimulation before and after 5-HTP.

Bottom graphe indicates number of cells showing facilitatory responses to tegmental stimulation after 5-HTP. Dark line indicates facilitatory responses before and after 5-HTP. Dotted line indicates depressant response before but facilitatory responses after 5-HTP.

Data for this figure was taken from Table 1.


TEGMENTAL STIMULATION
Depressive responses after 5 - HTP
No. of Cells
6
4
2
(+o)(-)
(-)(-)
Interval
1+2
3+4
5+6
7+8


TEGMENTAL STIMULATION
Facilitory responses after
5 - HTP
No. of Cells
6
4
2
(+o)(+)
(-)(+)
Interval
1+2
3+4
5+6
7+8

to tegmental stimulation was facilitation. As indicated by the solid curve, most of the intervals showing facilitation after 5-HTP also showed facilitation before 5-HTP. The results summarized in Figure 7 reveal that the primary effect of 5-HTP administration in PCPA-treated cats on the geniculate response to tegmental stimulation was to heighten facilitation. The facilitatory effects seemed to be generalized over all analysis intervals showing little of any specificity for spontaneous vs. light evoked activity. These data indicate that 5-HTP administration in PCPA-treated cats results in an apparent restoration of depressed photically driven discharge in the lateral geniculate during raphe stimulation accompanied by non-specific facilitation of discharge particularly prominent during tegmental stimulation.

Effects of AMT Pretreatment on Geniculate Responses to Raphe and Tegmental Stimulation

Five cats were pretreated with alpha-methyl-tyrosine (AMT) 10-12 hours prior to experimentation. From these five animals a total of 27 cells were tested for their response to tegmental and raphe stimulation.

Figure 8 shows cells from two different animals. In the top three histograms it can be seen that stimulation of the raphe nucleus in animal A-3 results in the typical depression of photic activity characteristic of the cells observed in untreated animals but very atypical of PCPA-treated animals. This

FIGURE 8

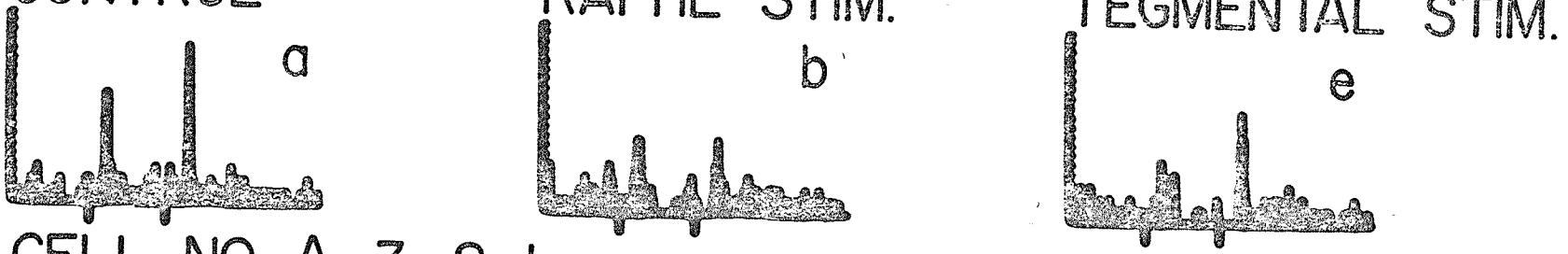
Effect of AMT pretreatment on lateral geniculate responses to raphe and tegmental stimulation in two different cells. Control histograms (a), raphe histograms (b) and tegmental histograms (e) for each cell are read from left to right.

AMT TREATED

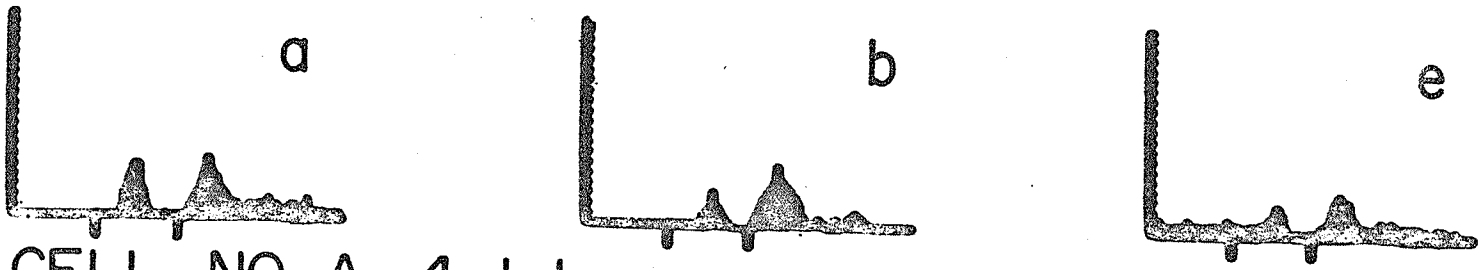
CONTROL

RAPHE STIM.

TEGMENTAL STIM.



CELL NO. A-3-2-1



CELL NO. A-4-1-1

depression was not observed during spontaneous activity. In the same cell stimulation of the tegmentum also resulted in a depression of the photic responses although not quite as strong as with the raphe. Spontaneous activity was not significantly different. In the bottom three histograms in animal A-4, stimulation of the raphe resulted in a significant depression ($p < 0.05$) of the ON response, with no depression of the OFF response. Stimulation of the tegmentum caused a significant ($p < 0.05$) facilitation of spontaneous activity (intervals 1 and 2) and a significant depression of both ON and OFF responses ($p < 0.05$).

Figure 9 illustrates a comparison of lateral geniculate responses to raphe stimulation in the two groups of pretreated cats. The bar graphs depict the percentage of cells in each group responding in each analysis interval with facilitation (white bar), depression (black bar) or no effect (hatched bars). The typical profile of selective depressant effects of raphe stimulation is observed in AMT-treated animals but not PCPA-treated animals. In the AMT animals the depression is maximal in intervals 3, 4, 5, and 6 corresponding to the ON and OFF responses of lateral geniculate cells to light.

Figure 10 compares the effects of tegmental stimulation on lateral geniculate discharge in PCPA- and AMT-treated animals. Facilitatory responses in AMT animals are less frequently observed than in PCPA-treated animals while

FIGURE 9

Comparison of AMT and PCPA treatment effects on lateral geniculate response to raphe stimulation. Top graphe shows percentage of cells in AMT treated animals responding with a significant depressant (black bars) facilitatory (white bars) or no significant effect (hatched bars) to stimulation of raphe nucleus in each analysis interval.
Bottom graph shows the same for PCPA treated animals.

RESPONSE TO RAPHE STIMULATION

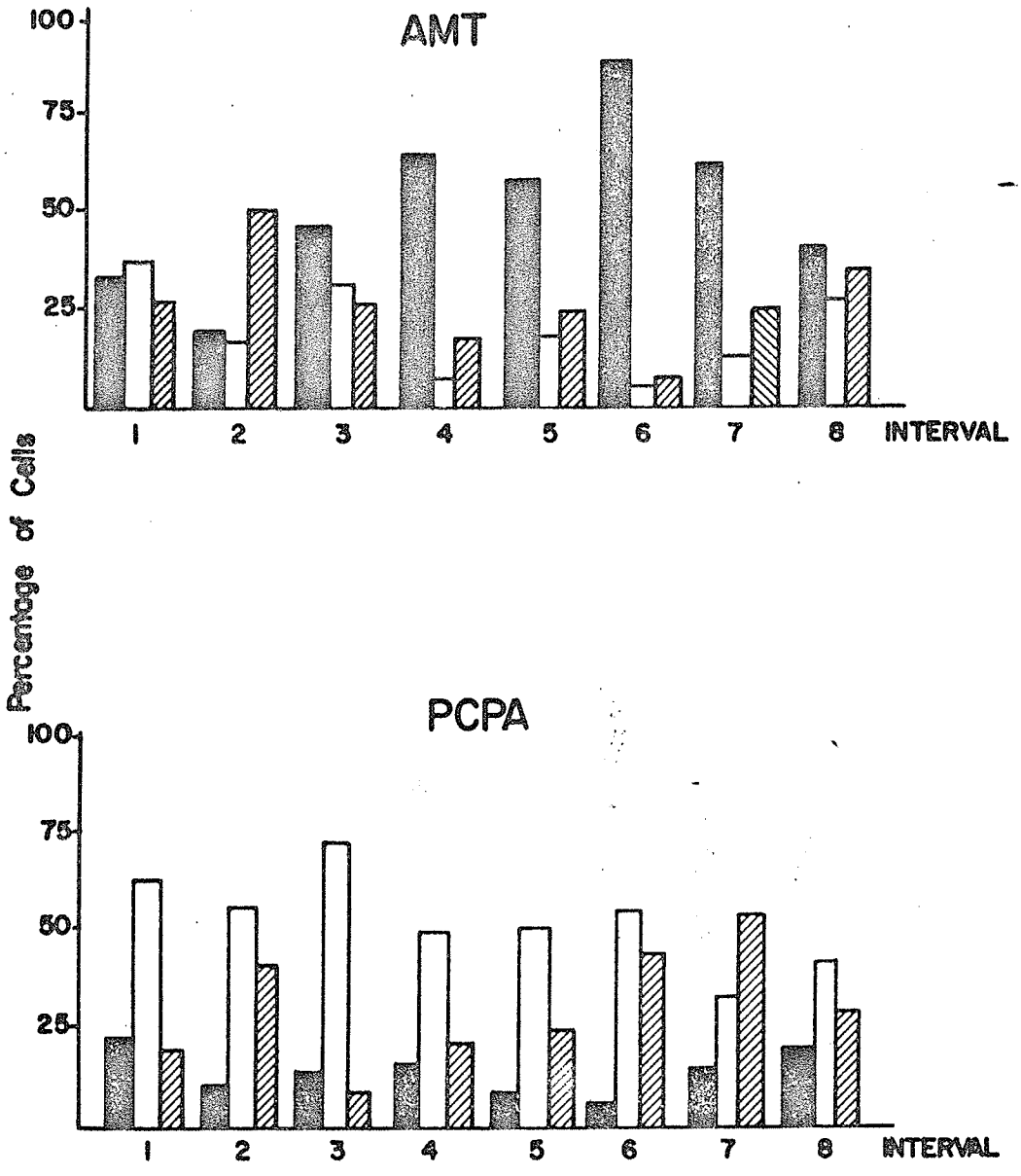
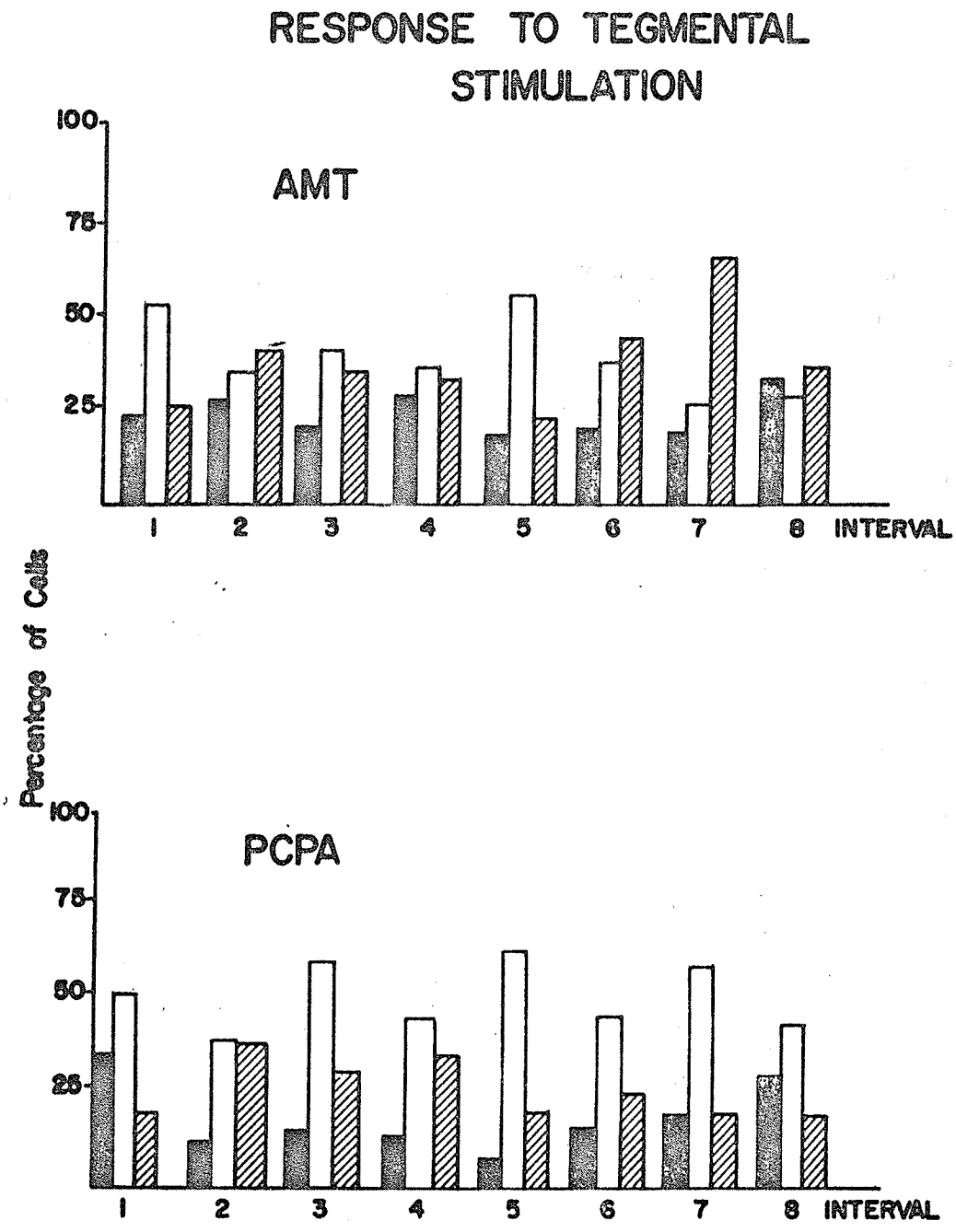


FIGURE 10

Comparison of AMT and PCPA treatment effects on lateral geniculate responses to tegmental stimulation. Top graph shows percentage of cells in AMT treated animals responding to tegmental stimulation with depression (black bars), facilitation (white bars) or no significant effect (hathced bars) in each analysis interval.
Bottom graph shows same for PCPA treated animals.


RESPONSE TO TEGMENTAL STIMULATION
AMT
100
75
50
25
1
2
3
4
5
6
7
8
INTERVAL
Percentage of Cells
PCPA
100
75
50
25
1
2
3
4
5
6
7
8
INTERVAL

depressant responses are more frequent.

The results from Figure 9 and 10 indicate that pretreatment with AMT has little effect on geniculate responses to raphe and tegmental stimulation except for a reduction in facilitatory responses. This may be contrasted with the PCPA-treated subjects where facilitatory responses are frequently observed and depressant responses are rare.

Effects of L-DOPA Administration on AMT-Treated Animals:

One cell in each of the 5 AMT-treated animals was tested for its response to stimulation after administration of L-DOPA in a similar manner previously described in the PCPA and 5-HTP experiments. The retesting procedure began 30 minutes after the infusion of L-DOPA. In two of our subjects the cell was lost before retesting could begin. Consequently the effects of L-DOPA in AMT-treated animals was only assessed in three cells.

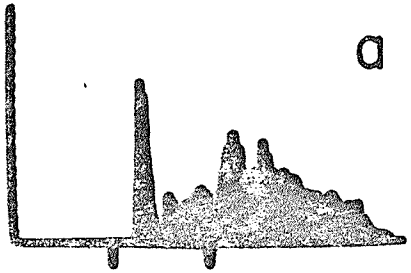
Figure 11 illustrates the effects of L-DOPA administration on lateral geniculate responses to raphe and tegmental stimulation. Prior to administratic of L-DOPA, stimulation of the raphe resulted in a significant depression ($p < 0.($ of the ON response and significant reduction of activity in intervals 7 and 8. Tegmental stimulation caused a similar reduction of the ON response accompanied by a significant ($p < 0.05$) facilitation of the OFF response (interval 5).

FIGURE 11

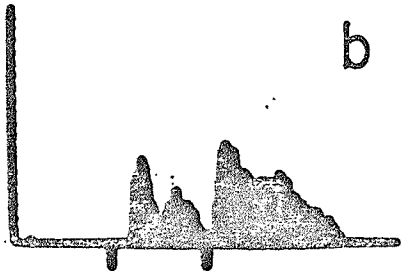
Effect of L-DOPA administration in AMT treated animal on lateral geniculate response to raphe and tegmental stimulation. Control histogram (a), raphe histograms (b) and tegmental histograms (e) are read from left to right. Upper three taken before, bottom three taken after L-DOPA administration.

AMT TREATED

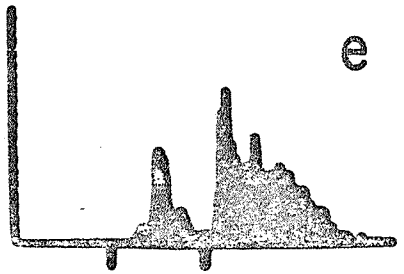
CONTROL



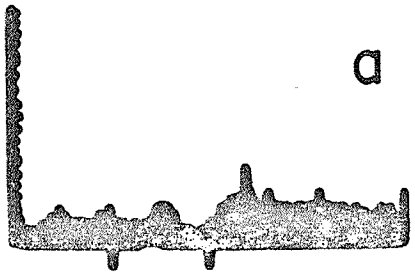
RAPHE STIM.

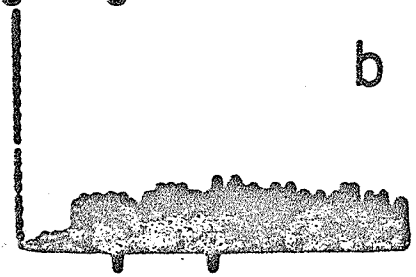


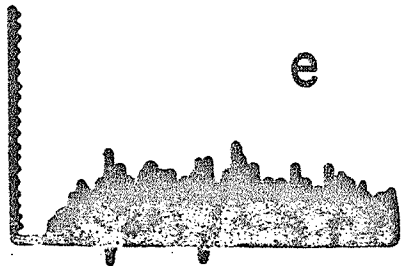
TEGMENTAL STIM.



AFTER DOPA (100 mg./kg.)







After administration of L-DOPA, the pattern of responding in the cell changed drastically. In the control histogram spontaneous activity was markedly increased ($p < 0.001$) while the photic responses were markedly reduced, to the extent that is was barely apparent that the cell was responsive to light. Both raphe and tegmental stimulation caused an increase in discharge throughout all analysis intervals such that photic patterns were obliterated. This effect was observed in the other two cells with L-DOPA although in one, the photic responses were more apparent.

Results from this limited sample suggest that L-DOPA administered in AMT-treated cats markedly facilitates unit activity in lateral geniculate cells. We were unable to ascertain whether the reduction in the photic component represented a selective depression by L-DOPA or whether it was merely obliterated by the heightened level of discharge which occurred even in the absence of light.

Effects of LSD on General Excitability of Geniculate Cells:

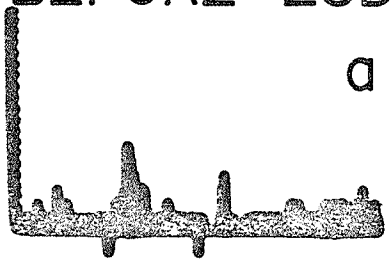
A surprising finding in this study was that intravenous LSD did not have a general depressant action on the lateral geniculate cells as reported by others, (20,36,60,91, 97). Instead, most cells responded with heightened activity, usually throughout all analysis intervals. Figure 12 shows the effect of LSD on normal discharge patterns in the absence of electrical stimulation

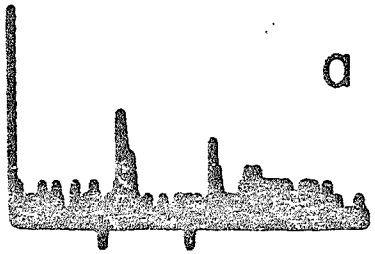
FIGURE 12

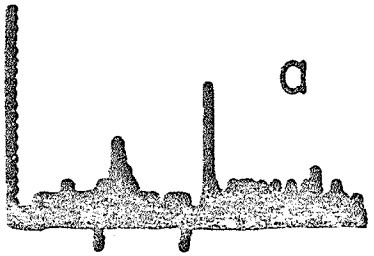
Effect of LSD on lateral geniculate activity in three cells from different animals. Non-stimulation trials; a, before LSD; A, after LSD. For a given cell the pre-drug and post-drug histograms are read from top to bottom.

ANIMAL	R-36	R-44	R-26
DOSE	100 ug/kg.	50 ug./kg.	100 ug./kg.

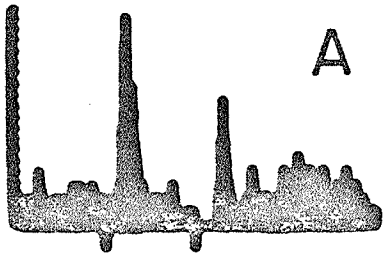
BEFORE LSD

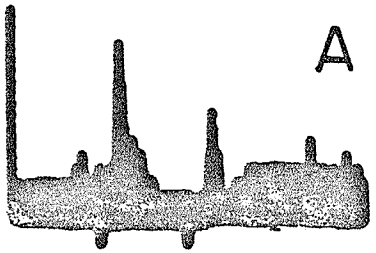


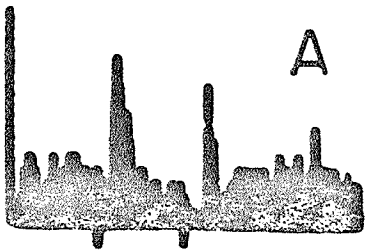




AFTER LSD





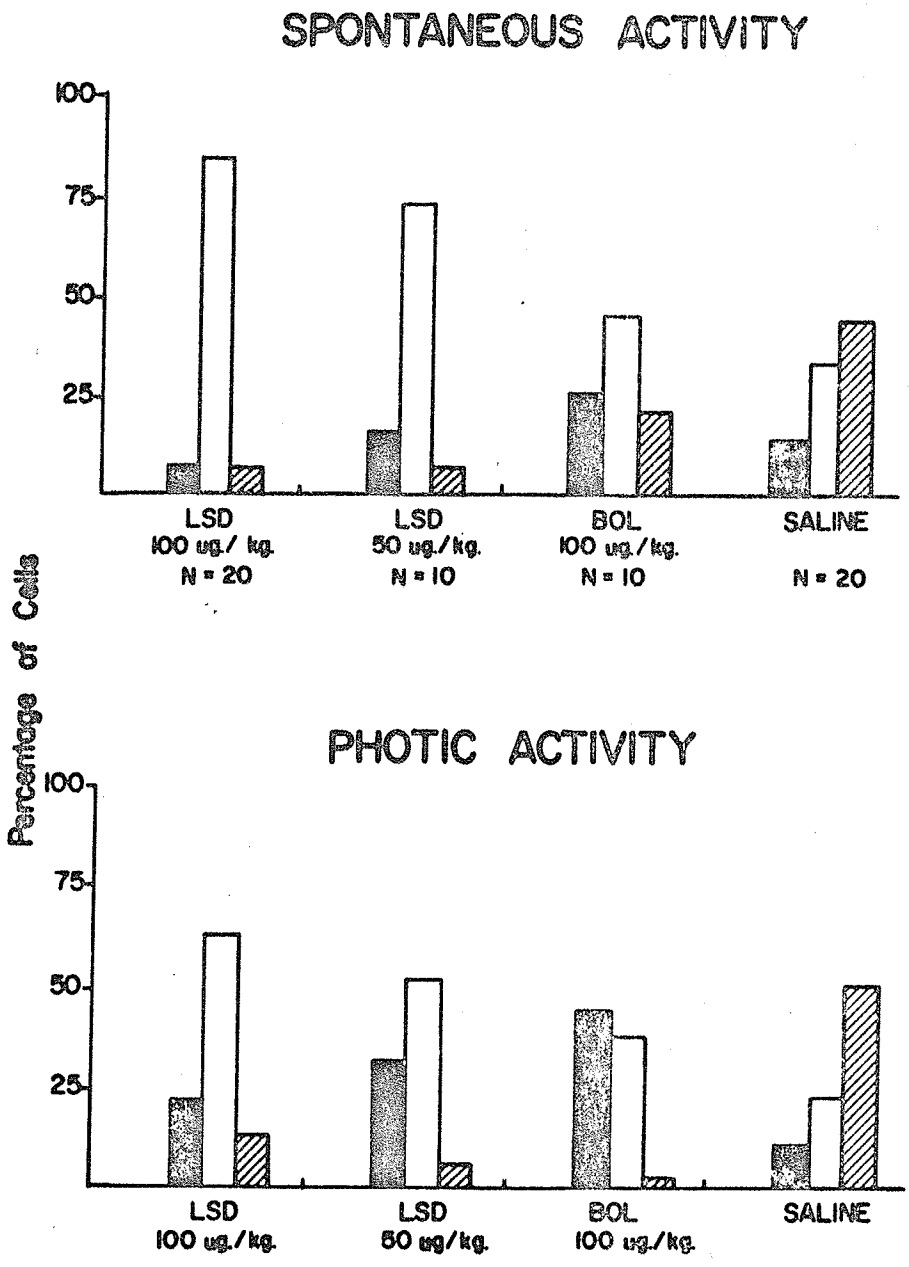


in cells from three different animals. The top row of A-histograms were taken before injection of LSD, while the bottom three were taken after. These histograms show that 50 ug/kg or 100 ug/kg doses increase photic activity considerably. Spontaneous activity was facilitated in some cells only slightly (R-44) and very significantly in others (R-26). In animal R-36 the increase in photic activity is almost threefold. Since this was an unexpected finding, we did detailed comparisons of LSD, BOL, and saline effects on the level of spontaneous and light-evoked activity in the absence of any electrical stimulation (comparing pre- and post-drug trial histograms A). To do this we pooled the two spontaneous activity analysis intervals (1 and 2) and compared the mean number of spikes occurring in those intervals before and after drug administration; then we pooled the remaining six analysis intervals corresponding to light evoked and afterdischarge activity and again compared mean number of spikes in the pooled sample before and after treatment. In this way we obtained an index of general excitability during spontaneous and photically driven activity without regard to particular temporal patterns of discharge. Figure 13 summarizes our findings.

Spontaneous and light-evoked activity were heightened significantly in the majority of the cells with 50 and 100 ug doses of LSD. A facilitation of spon-

FIGURE 13

Comparison of LSD (two doses) BOL and saline on mean rate of firing in lateral geniculate cells during spontaneous and photic activity in the absence of electrical stimulation. Number of cells (expressed as a percentage) showing significant depression after drug treatment indicated with black bars; significant facilitation, white bars; no significant effect, hatched bars. Intervals 1 and 2 pooled for spontaneous activity, intervals 3, 4, 5, 6, 7, and 8 pooled for photic activity.


SPONTANEOUS ACTIVITY
100
75
50
25
LSD
100 ug./ kg.
N = 20
LSD
50 ug./kg.
N = 10
BOL
100 ug./kg.
N = 10
SALINE
N = 20
Percentage of Cells
PHOTIC ACTIVITY
100
75
50
25
LSD
100 ug./kg.
LSD
50 ug/kg.
BOL
100 ug./kg.
SALINE

taneous activity with a reduction in photic activity was most commonly observed in cells tested with 100 ug/kg BOL. The most frequent finding with saline was a lack of any significant change in spontaneous or photic activity.

Effects of LSD on Geniculate Responses to Raphe and Tegmental Stimulation:

In 50 ug/kg and 100 ug/kg doses, LSD reduced the effectiveness of raphe stimulation in producing depression of photically driven activity in geniculate cells. Figure 14 illustrates the effects found in 7 out of 10 cells with 50 ug/kg of LSD. The top three histograms show the response before, the bottom three after, LSD administration.

Before LSD, raphe stimulation had the usual depressant effect on photically driven activity. Tegmental stimulation had a depressant effect on the initial peak on the ON response and a slight facilitatory effect on spontaneous activity.

After administration of LSD, raphe stimulation was less effective in depressing photically driven discharge. It was interesting to find, however, that tegmental stimulation showed increased efficacy in depressing photic activity. Upon histological examination of electrode placements in this animal it was found that the "tegmental" electrode was located on the lateral boundary of the PAG, perhaps accounting for its depressant effects.

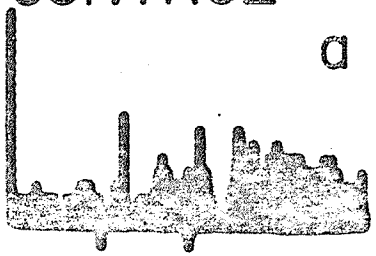
This finding suggests that the reduced effectiveness of electrical stim-

FIGURE 14

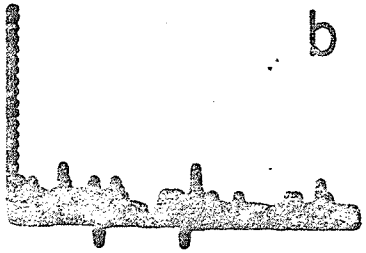
Effect of LSD (50 ug/kg) on lateral geniculate response to raphe and tegmental stimulation. Top three histograms (a, b, c)taken before, bottom three (A, B, E) taken after LSD.

BEFORE LSD

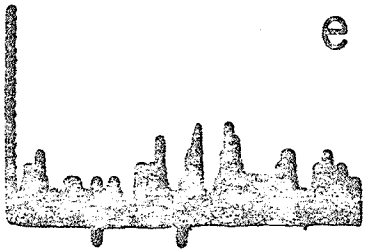
CONTROL



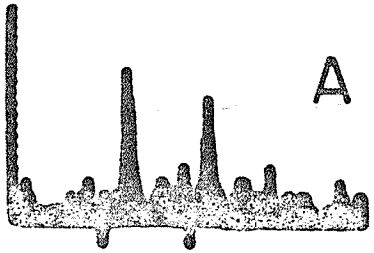
RAPHE STIM.

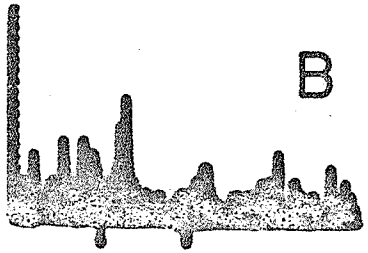


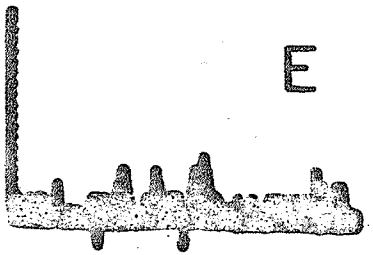
TEGMENTAL STIM.



AFTER LSD (50 ug./kg)







ulation in depressing photic activity under LSD in specific to the raphe area and not surrounding regions.

Partial or complete reversal of raphe induced depression of photic activity was observed in 16 out of 20 cells tested with 100 ug/kg of LSD. Figure 15 shows that before LSD injection, this cell responded to raphe stimulation with significant depression of the ON and OFF responses, while tegmental stimulation induced a slight acceleration of spontaneous activity accompanied by a slight reduction in the initial peak of the ON response. After LSD, spontaneous and photic discharge were markedly enhanced. Raphe stimulation had no effect on the ON response while still depressing the OFF response. Similar effects were observed with tegmental stimulation. Histological examination of this animal found the tips of the electrodes to be in the dorsal raphe nucleus and in the medial PAG near the dorsal raphe area.

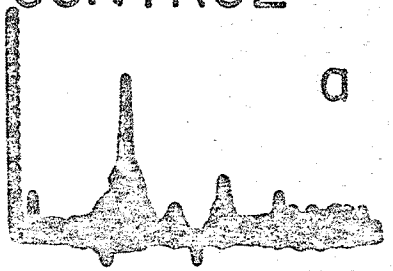
In cats where the tegmental electrode was located in the lateral midbrain area, LSD increased the facilitatory effects of stimulation on lateral geniculate cells. Figure 16 illustrates this effect. This was a partucularly interesting experiment since histological examination revealed that the electrode intended for the dorsal raphe nucleus was misaligned and ended up almost two mm. lateral to the midline. The tegmental electrode, on the other hand, was

FIGURE 15

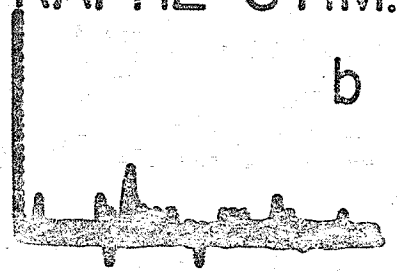
Effect of LSD (100 ug/kg) on lateral geniculate responses to raphe and tegmental stimulation. Top three histograms (a, b, e) taken before, bottom three (A, B, E) taken after LSD.

BEFORE LSD

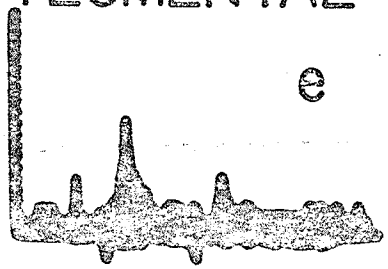
CONTROL



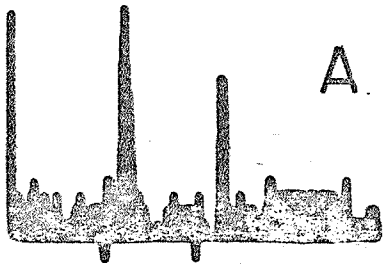
RAPHE STIM.

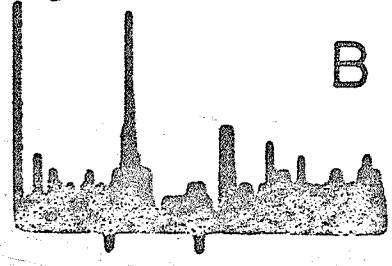


TEGMENTAL STIM.



AFTER LSD (100 ug./ kg)





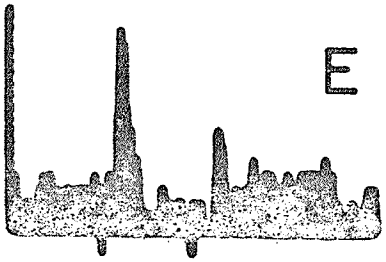


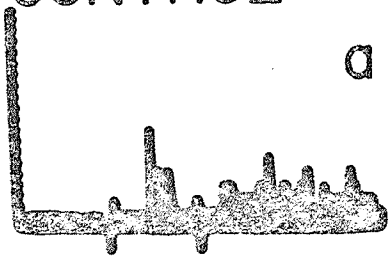
FIGURE 16

Effect of LSD on geniculate response to raphe and tegmental stimulation in aminal where electrode missed raphe nucleus. Top three histograms (a, b, e) taken before, bottom three (A, B, E) taken after LSD.

BEFORE LSD

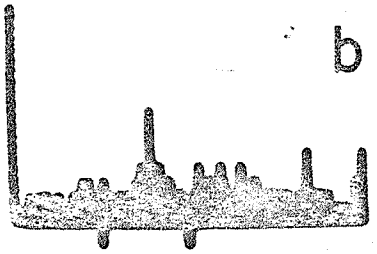
CONTROL

a



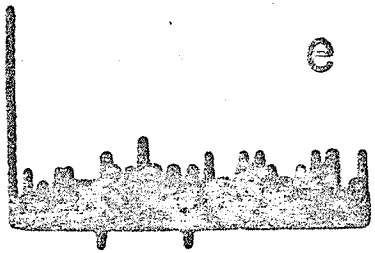
RAPHE STIM.

b



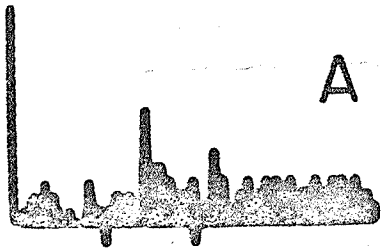
TEGMENTAL STIM.

e

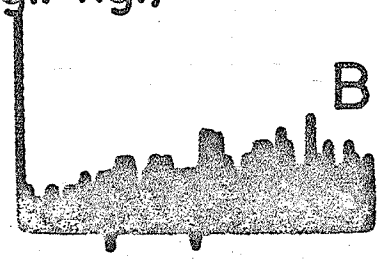


AFTER LSD - (100 ug./kg.)

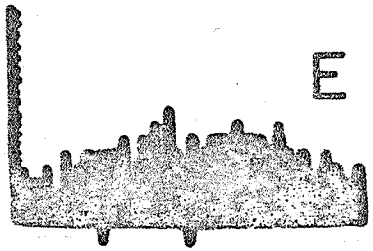
A



B



E



placed just central to the superior colliculus near the central tegmental area. Prior to LSD injection, stimulation through the misaligned "raphe" electrode caused a facilitation of spontaneous activity with no significant effect on the photic component. Tegmental stimulation caused a marked facilitation of activity throughout all analysis intervals, obliterating the photic response. After LSD stimulation of both loci resulted in a gross facilitation of spike activity in all analysis intervals to the extent that any photic-related discharge was undetectable. In other animals where the tegmental electrode was placed in lateral regions, LSD caused a similar increase in stimulation inducing facilitation of geniculate activity, although in some cases photically driven activity was still quite apparent in the histogram records.

Figure 17 illustrates the effect of BOL on lateral geniculate responses to stimulation. Prior to BOL injection, raphe stimulation resulted in a diminution of the photic response; tegmental stimulation caused a generalized facilitation of activity in all analysis intervals. After 100 ug/kg of BOL general activity including the photic response was significantly increased ($p < 0.05$). In contrast to the finding with LSD, the depressant properties of raphe stimulation were still pronounced. Tegmental stimulation caused a significant increase in activity ($p < 0.05$) in all analysis intervals after BOL to the extent that ON and OFF responses were obliterated. In this respect LSD

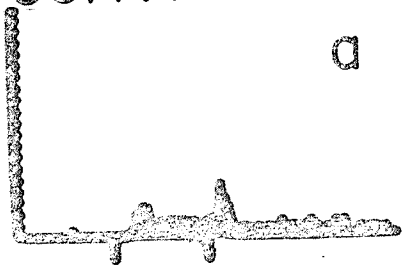
FIGURE 17

Effect of BOL (100 ug/ kg) on lateral geniculate response to raphe and tegmental stimulation. Top three histograms (a, b, e) taken before, bottom three (A, B, E) taken after LSD.

BEFORE BOL

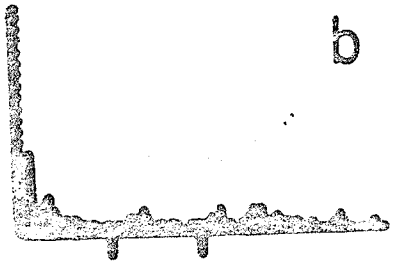
CONTROL

a



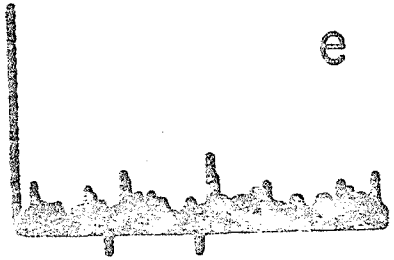
RAPHE STIM.

b



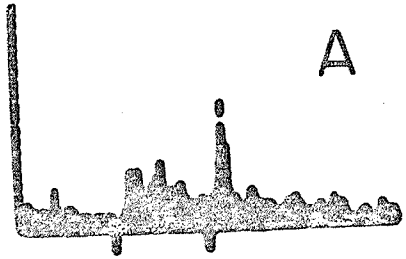
TEGMENTAL STIM.

e

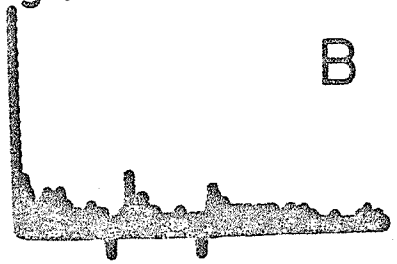


AFTER BOL (100 ug./kg.)

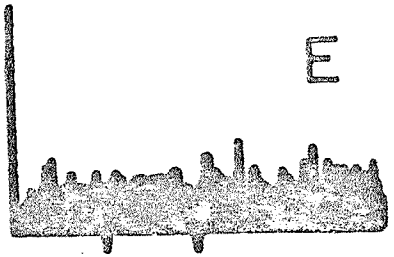
A



B



E



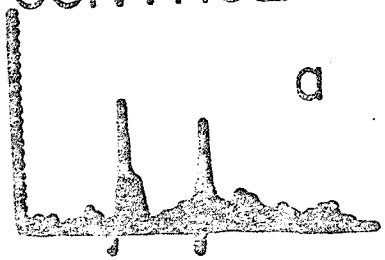
and BOL were similar; they differed, however, in that BOL did not antognize the depressant effects of raphe stimulation.

Figure 18 shows the lack of any significant change in the geniculate response to raphe and tegmental stimulation in a saline-treated subject. This cell responded as usual to raphe stimulation with a depression of photic activity both before and after the administration of saline. There were no cells in the saline sample that significantly changed their response to stimulation after injection.

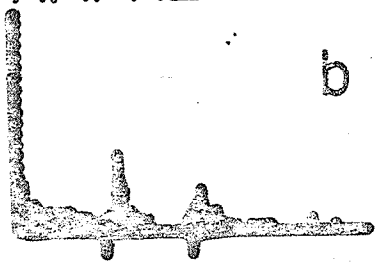
FIGURE 18

Shows lack of effect of saline injections on lateral geniculate response to raphe and tegmental stimulation. Upper three histograms (a, b, e) taken before, bottom three (A, B, E) taken after saline.

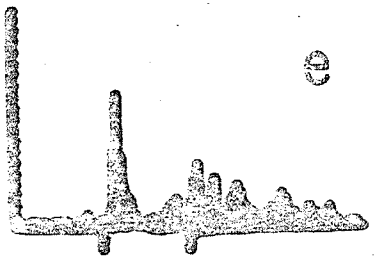
BEFORE SALINE
CONTROL



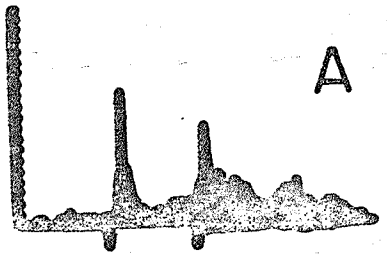
RAPHE STIM.

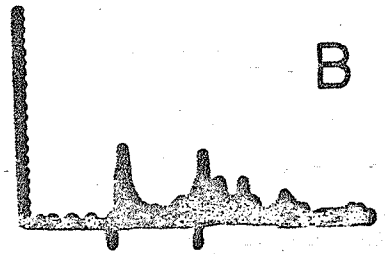


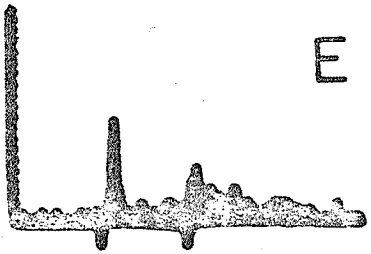
TEGMENTAL STIM.



AFTER SALINE







DISCUSSION

The most significant finding in this series of experiments was that depression of photic activity in geniculate cells during raphe stimulation, characteristic of control animals, was essentially absent in PCPA treated subjects but not AMT treated subjects. This finding supports the notion that this depression is dependent on functional pools of serotonin, thought to be released synaptically from cells of the raphe nucleus. Geniculate cells in PCPA treated cats respond to raphe stimulation with a generalized facilitation or no effect at all. Cells responding to raphe stimulation with facilitation usually also responded to tegmental stimulation with facilitation although this was not always the case. In one cell (Fig. 17) raphe stimulation resulted in a generalized facilitation of discharge in all analysis intervals while there was powerful depression induced by tegmental stimulation. Histological examination revealed that the "tegmental" electrode was really in the upper periaquaductal gray (PAG) of the midbrain. In most cells responding with depression to tegmental stimulation in non-treated animals the electrode was found to be in the vicinity of the PAG also. These observations suggest that there may be other inhibitory pathways from the brainstem to the LGN. Since depressive responses to tegmental stimulation in PCPA treated cats occurred in the absence of similar responses to raphe stimulation it would appear that these other inhibitory pathways do not require functional pools of serotonin to exert their effect. This may also account for the finding that 5 cells in two PCPA animals showed depressive responses to raphe stimulation. If there are inhibitory pathways close to the dorsal raphe nucleus perhaps in the PAG it is possible that current spread from the tip of the raphe electrode might account

for these results. A possible alternative explanation is that there may be enough residual serotonin left in these PCPA treated cats to occasionally exert effects. If current spread is an important factor in our experiments it might account for the frequently observed facilitory responses to raphe stimulation, particularly in PCPA treated subjects. We have shown that when the tegmental electrode is actually situated in or near the central and lateral tegmental field, geniculate cells respond to stimulation primarily with facilitation. If current were to spread from the midline outward during raphe stimulation one might expect activation of some proportion of these facilitory responses to raphe stimulation are prominant in PCPA treated subjects but less frequently observed in normal controls. First it is conceivable that all cells in the LGN are not equally influenced by excitatory and inhibitory input. Numerous investigations (32, 57, 58, 124) indicated that there are functional subpopulations of lateral geniculate cells. One might expect that depending on the cell type, excitatory or inhibitory influences from the brainstem may predominate over the other. Second and perhaps more important, is that in untreated animals excitatory effects produced by current spread from the raphe electrode are opposed by the depressive effects resulting from stimulating the raphe fibers themselves. In the PCPA treated animal the serotonin dependent depressive effects would be absent and the excitatory effects due to current spread are left relatively unopposed. Since in this experiment current strength was not manipulated as an experimental variable it is impossible to determine whether current spread was an important factor in the mixed effects of electrical stimulation; however, in future experiments this should be an easy hypothesis to test.

In an effort to see of the lack of depressive responses to raphe stimulation in PCPA cats could be reversed by restoring functional pools of

serotonin we tested a limited number of cells after administration of 5-HTP, the immediate metabolic precursor of serotonin.

In some of our animals it was not possible to hold the cell long enough to complete the test so we are necessarily restricted by a small sample. Nevertheless some definite trends emerged from these experiments. First, more cells showed inhibitory responses to raphe stimulation after administration of 5-HTP. Of paramount interest is the observation that most of these cells did not show inhibitory responses before 5-HTP. Second, there were a number of facilitory responses to both raphe and tegmental stimulation after 5-HTP. It should be emphasized that those cells showing a change from facilitation or no effect before 5-HTP to depression after, did so primarily during photic activity. In other words, in those cases where 5-HTP restored depressive responses to raphe stimulation, the depression occurred in the analysis intervals which show maximal depression in normal untreated animals. In the previous experiment we showed that raphe induced depression showed considerable specificity for photic activity in geniculate cells as opposed to spontaneous activity. If these effects are dependent upon released serotonin one would expect that when serotonin is restored in the PCPA treated animal the same profile of depressive effects would be observed. Our data, although obtained from a rather small sample lend support to this idea.

The facilitory responses to raphe stimulation after 5-HTP might be explained in one of two ways. It could be argued that some subpopulation of raphe fibers exert an excitatory effect on geniculate cells which is dependent on serotonin release. Alternatively it might be hypothesized that large doses of 5-HTP have a non-specific excitatory effect. This seems more likely on three counts. First, the facilitory responses were more numerous

to tegmental stimulation than to raphe stimulation; second, some of our cells showed a considerable increase in discharge rate in the non-stimulated control trials (see Fig. 18); third, if serotonin had a prominant excitatory effect on geniculate cells one might expect to see a larger proportion of facilitory responses to raphe stimulation in the non-treated control animal. It is interesting to note that injection of the precursor DOPA in AMT treated animals resulted in an enormous non-specific elevation of activity in both control and stimulation trials in the three animals tested. It is possible that both 5-HTP and DOPA have some non-specific actions when administered in large doses. Since both are converted to their appropriate amine by a non-specific decarboxylase enzyme it is possible that they are taken up and released synaptically at inappropriate sites. Snyder (121) has presented some biochemical evidence for this. Our data indicate that the specific depressive effects of raphe stimulation, are restored in PCPA treated subjects by 5-HTP only which also acts to promote an increased general excitability of geniculate cells, a property shared by and more predominant with DOPA. A better understanding of the overall effects of 5-HT could have been provided by additional experiments where it was administered alone in the absence of PCPA.

In summary, we have shown that the specific depressive effects of raphe stimulation in geniculate cells can be clocked by pretreating our animals with PCPA, a serotonin depletor, but not AMT, a catecholamine depletor. The finding that these depressive effects can be restored in PCPA treated animals with subsequent 5-HTP administration with the same profile as they occur in controls gives considerable support to the hypothesis that the effects are mediated by the release of serotonin when the raphe nucleus is stimulated.

Effects of LSD and BOL

One of the most intriguing findings in this study was that general activity in lateral geniculate cells is increased after LSD in 50 ug/kg and 100 ug/kg doses. This finding is contradictory to a number of other studies (20, 36, 42, 60, 97, 126). Evarts and Bishop found substantial depression of geniculate potentials evoked by optic nerve stimulation (20, 42). In their experiments they used barbiturate anesthesia while in our experiments an unanesthetized preparation was used. Horn and McKay (60, 91) found that small doses of LSD (25-100 ug) excited some cells and depressed others in urethan anesthetized animals. As the dose was increased depression became more prominant. Mouriz-Garcia studied the effects of LSD on geniculate cells in an unanethetized preparation and found four cells to exhibit an increase in mean discharge rate and three others to show mixed responses (94). No totally depressive responses were seen. Thus it seems that anesthesia is a crucial variable in studying LSD effects at the lateral geniculate, possible sensitizing the geniculate to depressive effects. Several studies on LSD effects on sensory evoked potentials have bearing on this question. Those studies conducted using an anesthetic found LSD to cause depression of evoked potential waveform (20, 42); in unanesthetized subjects LSD causes an enhancement of waveforms (70, 75). Several iontophoretic studies have found LSD depression of lateral geniculate activity (36, 97, 126); however, since depression has been found at practically all areas tested including the olfactory bulb, cortex, hippocampus and brainstem (3, 18, 23, 24, 103), the physiological significance of these findings must be questioned. It has been suggested for instance that iontophorectically applied LSD has a local anesthetic action on neurons (21, 22). Aghajanian demonstrated that intravenous as well as iontophoretically applied LSD have depressive actions on cells in the raphe nucleus of

the rat (3, 4, 43, 55); however, these experiments were carried on under chloral hydrate anesthesia. The possible contributions of this anesthetic to the depressive effects observed must not be overlooked. The possible interactions between LSD and various anesthetic agents must be studied in more detail, however evoked potential studies in unanesthetized animals and our results suggest that the physiological actions of LSD might be studied more clearly in conscious preparations.

In our previous experiments we have shown that electrical stimulation of the raphe nucleus causes a depression of lateral geniculate activity. This depression is maximal when the cell is being photically driven and minimal or absent when the cell is spontaneously firing. These effects of raphe stimulation are not observed in cats treated with PCPA but can be restored with subsequent administration of 5-HTP, suggesting serotonin release to be a mediating factor. On the basis of these experiments we hypothesized that if LSD was a serotonin synergist the effects of raphe stimulation would be intensified after treatment; whereas if LSD as a serotonin antagonist, the effects of raphe stimulation on geniculate cells would be diminished. Our data support the latter hypothesis. Doses of 50 ug/kg or 100 ug/kg resulted in a much diminished depressive response to raphe stimulation. In addition the photic response in control histograms in geniculate cells after LSD was usually substantially larger - an effect one would expect if LSD blocked the action of 5-HT in limiting the input to geniculate cells from the optic nerve. These data support the notion that LSD interacts in this part of the visual system as a serotonin antagonist. BOL, a potent peripheral serotonin antagonist did not share these effects.

This idea gains support from other investigation in a study by Kawai and Yammamoto (69). It was shown that stimulation of the optic nerve evoked

field potentials in the superior colliculus incubated in vitro. Addition of 5-HT to the incubating medium caused a reduction in these potentials, an effect blocked by LSD but not BOL. Stimulation of the raphe nucleus depresses the discharge evoked in lamina 5 cells to nocioceptive stimulation in the cat (84).

This effect was blocked by LSD and PCPA. It should be noted that these observations occurred in cats immobilized with flaxedil. This is particularly interesting since it mimics the results of our experiments on the visual system. In both cases raphe stimulation with very similar stimulation parameters depressed a stimulus specific discharge in a sensory cell in normal animals but not animals pretreated with PCPA or given LSD.

Evidence from some iontophoretic studies support the notion that LSD acts as a serotonin antagonist in the CNS. Roberts found the iontophoretically applied LSD blocked about half of the neurons responding to 5-HT with an excitatory effect; however, this property was shared by a number of substances which are not psychoactive (103). Boakes and his co-workers (21, 22, 23) have found that LSD applied iontophoretically antagonizes brainstem neurons responding to 5-HT with excitation. BOL showed very little antagonism of these neurons. These workers have also shown however, that LSD mimics the effect of 5-HT in cells responding to this amine with depression, giving rise to speculation that LSD can act as agonist or antagonist of serotonin depending on the particular synapse involved.

A number of other studies have implicated LSD as a mimetic of 5-HT rather than an antagonist. Samamin et al (108) have found that raphe stimulation depresses somatosensory evoked potentials in the urethane anesthetized rats and LSD potentiated this depression. It is worth questioning whether the opposite results might have been observed in the absence of anesthesia. Sheard and Aghajanian found that stimulation of the raphe nucleus causes rats

to dishabitutate to an auditory stimulus (118). Sensory hishabituation is a frequent behavioral effect of LSD (71, 72). Aghajanian has interpreted these results as indicative of a serotonin synergistic role of LSD. To support his hypothesis he cites results of other experiments in his laboratory (3, 4, 43, 55) showing that LSD and 5-HT depress raphe neurons when administered iontophoretically. He suggests that although LSD inhibits raphe firing, it acts like serotonin at 5-HT receptor sites and may be involved in a feedback system to reduce raphe firing. If LSD exerted its effects by mimicing 5-HT at postsynaptic receptor sites while at the same time depressing the firing of raphe neurons one would expect to see a decreased turnover of 5-HT with LSD. A number of biochemical studies have demonstrated a rise in 5-HT levels accompanied by a fall in 5-HIAA, the primary metabolite of 5-HT; thus conforming this notion. These biochemical findings however could also support a 5-HT antagonist interpretation of LSD. Chase et al (31) have shown LSD to partially block stimulation induced release of serotonin in brain slices. If LSD were to do this in vivo it would reduce the effectiveness of serotonin at postsynaptic sites, reduce its turnover and increase its endogenous levels. The possibility that LSD is a serotonin agonist or antagonist at different synapses within the CNS cannot be eliminated. Further research is necessary to provide definitive answers; however, our experiments indicate that it may act as an antagonist in the visual system.

An important finding in our experiments was that in addition to the antagonism of geniculate responses to raphe stimulation, in many cells LSD caused a facilitation of background discharge particularly during spontaneous activity (see Fig. 28). LSD seemed to have complex effects on geniculate responses to stimulation of loci in the lateral midbrain tegmental area. In some cases the response was grossly facilitated and other cases it was mildly depressed. We demonstrated in the previous experiment that the geniculate

responses to tegmental stimulation was highly dependent on the location of the electrode.

Further, no effort was made to elucidate the identity of transmitter substances mediating these effects. Consequently, it is difficult to establish what mechanisms might be responsible for LSD effects on spontaneous discharge and response to tegmental stimulation in geniculate cells. Typical facilitory responses to tegmental stimulation were observed in PCPA treated animals, consequently it is unlikely that 5-HT plays an important role. It is interesting to note that injections of DOPA in AMT treated animals had a profound facilitation of background discharge similar to that observed in some cases with LSD. This coupled with the observation that there are catecholamine nerve terminals in the lateral geniculate (6, 49), suggest that catecholamines may be involved in regulating LGN excitability and perhapsLSD interferes with these transmitter substances causing changes in background discharge which we observed in the form of LSD induced bursting. LSD has been demonstrated to exert action of catecholamine receptors in the peripheral nervous system (95) and causes a fall in noradrenaline levels in the brain (45). Although studies linking LSD with catecholamines are less numerous than those linking it with serotonin it is not unreasonable to propose that LSD exerts its central effects by virtue of a unique pattern of interaction with a number of synaptic transmitter systems. Further research into this proposition may reveal a more clear understanding of the mechanism that LSD causes considerable changes in lateral geniculate discharge.

Our results on the action of LSD on the lateral geniculate and its relevance to the sensory effects of this substance are unkown. The lateral geniculate appears to be involved in rudimentary coding of visual information (61, 62, 63) transmitting output to cells of the striate cortex where

specific patterns of excitation and inhibition from the LGN cells define the characteristics of receptive fields of cortical cells. These cells in turn appear to be involved in the synthesis of a mental map of the visual environment since it has been demonstrated that changes in shape and movement of visual stimuli profoundly influence the firing of these cells. The appropriate question to ask here is how altered discharge in the lateral geniculate nucleus may or may not influence cortical cells to misread the coded visual message as it arrives from this relay nucleus. It seems that drug induced changes of mean rate of firing or changes in general excitability would not distort vision, since changes in general level if discharge are often seen in repeated samples of normal cells, changes of arousal and attention and fluctuations in stimulus intensity (80, 85). Clearly, these kinds of events do not produce "hallucinations" or misreading of the visual environment. Instead, there may be considerable utility in studying changes in temporal patterns of discharge during both light evoked and spontaneous periods. Since receptive field properties of cortical neurons depend on appropriate profiles of excitatory and inhibitory input from the LGN it seems likely that change in temporal patterns of discharge would convey different information to the cortex. Unfortunately, a close inspection of changes in patterns cannot be attained in experiments where summed, averaged, or total amount of responses are the primary form of data collected. More sophisticated analysis of time intervals between spikes according to specific input would be more informative.

If the geniculate is considered in terms of a relay nucleus receiving input not only from specific afferent channels (optic nerve) but also various brainstem pathways presumably serving various modulating, regulating and gating functions, then it seems conceivable that a substance exerting dif-

ferential effects on afferent channels as well as regulating channels, might induce abnormal patterns of temporal discharge in LGN neurons that is sufficient to distort the message received at the cortical level. We have found that LSD antagonizes the raphe system which seems to be involved primarily in regulating actual afferent input from the optic nerve to the geniculate. We have also found LSD to modify discharge patterns during spontaneous activity. Whether the interaction of these two effects is responsible for its well known sensory effects cannot be determined from these experiments; however, it should make for some intriguing research efforts in the future.

GENERAL SUMMARY AND CONCLUSIONS

We have attempted to determine whether LSD effects on the lateral geniculate are in part brought about by virtue of its actions on serotonin containing pathways originating from the brainstem and synapsing within the geniculate.

In the first set of experiments we studied the effects of electrical stimulation of the mesencephalic raphe nucleus, thought to be the primary source of serotonin fibers in the brain, on lateral geniculate discharge. In an effort to assess the degree of anatomical specificity of stimulation effects we also studied the effects of electrically stimulating various loci in and around the lateral tegmental area of the mesencephalon.

In the second set of experiments we employed the use of the substances p-chlorphenylalanine (PCPA) and alpha-methyl-p-tyrosine (AMT), the action of which is to deplete endogenous stores of serotonin and catecholamines respectively, by inhibiting their synthesis. By pretreating our experimental subjects with these agents we tested the hypothesis that the effects of stimulation of the raphe nucleus on lateral geniculate cells was dependent upon functional levels of endogenous serotonin. In an attempt to determine if the effects of stimulation in PCPA and AMT treated subjects could be reversed by restoring functional levels of serotonin and catecholamines we injected some subjects with 5-hydroxytryptaphan, the immediate precursor of serotonin, and L-DOPA, the immediate precursor of catecholamines.

Next we administered LSD in two different doses and studied its effects on the lateral geniculate responses to electrical stimulation of the raphe nucleus and surrounding midbrain structures in an effort to see whether the stimulation effects were selectively antagonized or mimicked by this psychotomimetic agent. To determine if the effects observed under LSD were unique

to its psycho-active properties we also tested animals injected with 2-brom-lysergic acid (BOL) a potent serotonin antagonist in peripheral preparations but devoid of psychic effects.

These experiments led to the following observations:

1) Stimulation of the raphe nucleus causes a depression of cellular firing in lateral geniculate cells.

2) This raphe induced depression is maximum when the geniculate cells are photically driven and minimal when the cell is spontaneously firing. Cells which are not responsive to light are not affected by raphe stimulation.

3) Stimulation of various areas of the midbrain lateral to the raphe nucleus in the diffuse reticular area yield a mixture of facilitory and depressive effects which are not specific for photically driven geniculate activity.

4) Depression of photic activity during stimulation of the raphe nucleus does not occur in PCPA treated animals but is restored by subsequent injections of 5-HTP. Pretreating subjects with AMT does not prevent the specific depression of photic activity during raphe stimulation.

5) General level of responsiveness in geniculate cells after LSD is increased rather than depressed in unanesthetized animals.

6) LSD but not BOL selectively antagonizes the depressive effects of raphe stimulation.

7) LSD exerted a general facilitory action on geniculate activity during spontaneous discharge which was often characterized by intermittent high frequency bursting.

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