



University of the Pacific
Scholarly Commons

University of the Pacific Theses and
Dissertations

Graduate School

1976

Photoevoked response of the optic tectum in larval stage XII and juvenile *Rana catesbeiana*

Michael Gale Phillips

University of the Pacific, mg.phillips@comcast.net

Follow this and additional works at: https://scholarlycommons.pacific.edu/uop_etds

 Part of the [Biology Commons](#)

Recommended Citation

Phillips, Michael Gale. (1976). *Photoevoked response of the optic tectum in larval stage XII and juvenile *Rana catesbeiana**. University of the Pacific, Thesis. https://scholarlycommons.pacific.edu/uop_etds/1900

This Thesis is brought to you for free and open access by the Graduate School at Scholarly Commons. It has been accepted for inclusion in University of the Pacific Theses and Dissertations by an authorized administrator of Scholarly Commons. For more information, please contact mgibney@pacific.edu.

PHOTOEVOKED RESPONSE OF THE OPTIC TECTUM
IN LARVAL STAGE XII AND JUVENILE RANA CATESBEIANA

A Thesis
Presented to
the Faculty of the Department of Biological Sciences
University of the Pacific

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Michael Gale Phillips

May 1976

This thesis, written and submitted by

Michael Gale Phillips

is approved for recommendation to the Committee
on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

F. R. Hunted

Thesis Committee:

Howell S. Ramos Chairman

Anne Faulkner

F. R. Hunted

Dated May 7, 1976

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
Definition of Terms	2
MATERIALS AND METHODS	3
RESULTS	8
Spontaneous E.E.G.	8
Evoked E.E.G.	11
Morphology	15
Cell Densities	20
DISCUSSION	23
SUMMARY	28
BIBLIOGRAPHY	29
APPENDIX I	31

LIST OF FIGURES AND TABLES

FIGURE	PAGE
1. Diagrammatic representation of electrode placement in larval and juvenile, <u>R. catesbeiana</u>	4
2 Schematic diagram for the photostimulation, hard copy record, and integrated record of evoked E.E.G. activity in larval and juvenile, <u>R. catesbeiana</u>	6
3 Recorded E.E.G. evoked response to photostimulation of the lateral eyes in a juvenile frog	9
4 Spontaneous E.E.G. "burst" activity in larval, Stage XII, <u>R. catesbeiana</u>	10
5 Typical spontaneous E.E.G. activity in juvenile, <u>R. catesbeiana</u>	11
6 Histogram comparing larval and juvenile, <u>R. catesbeiana</u> , E.E.G. activity to frequency of stimulation and cell density	13
7 Traces are 35 integrated E.E.G. evoked response from the optic tectum of larval, Stage XII, frogs	14
8 A plot of amplitude vs. frequency comparing the evoked E.E.G. response of larval and juvenile, <u>R. catesbeiana</u> , to photostimulation at the lateral eyes	16
9 E.E.G. evoked response in the optic tectum of juvenile frogs, integrated from 45 separate stimuli	17

FIGURE	PAGE
10 Dorsal view of the brain comparing the dimensions of larval and juvenile, <u>R. catesbeiana</u>	18
11 Cross section of the optic tectum in larval <u>R. catesbeiana</u> showing the relative cell density and distribution	21
12 Cross section of the optic tectum in juvenile <u>R. catesbeiana</u> showing the relative cell density and distribution	22

TABLE

I Group Comparison for Spontaneous and Stimulation Amplitude and Frequency	12
II Comparative Measurements	19

ACKNOWLEDGMENTS

The author expresses his appreciation to Dr. H. I. Runion for making space, equipment, advice, assistance, and friendship readily available; to Dr. F. R. Hunter for funds; to Dr. and Mrs. L. Christianson for their critical examination of the statistical methods used. I am also grateful to Mr. L. R. Upjohn and Mr. B. G. Sanford both of whom helped design the experimental station and provided much moral support throughout the project; and to Dr. A. Funkhouser who, in addition to being an outstanding mentor and friend, provided invaluable comments on and criticism of this manuscript.

This work is respectfully dedicated to Gale and Marie Phillips, my father and mother, who have always cared enough to give their very best.

INTRODUCTION

Morphologically, the larval frog optic tectum consists of nine layers of stratified cells and fibers (Larsell, 1929; and Kollros, 1953). In the optic tectum only two-thirds of the adult number of cells are present at metamorphic climax (Kollros, 1953); but by the time the animal achieves the juvenile stage, the tectum has matured to the adult form as described by Eker (1899).

Investigations of the electrophysiological function of the optic system have been limited to either single unit responses of the receptor cells or a compound recording of the optic nerve and tract. An objective step was made by Hartline (1938) when he succeeded in obtaining records from a single optic nerve fiber of the frog. Donner and Rushton (1959) subsequently described retinal response in the frog concluding that optimum photostimulation occurred at wave lengths between 500 and 580 nm. Dawson and Ralph (1974) using these wave lengths have more recently demonstrated a duration-dependent optimum photoretinal response of 10 msec. Electrophysiological evidence for the existence of a neural pathway linking the optic system with the diencephalon and pars intermedia has been compiled (Muntz, 1962 ; Dawson and Ralph, 1974). In a cytological study, Gona (1974) described connecting tracts from the lateral geniculate body to the optic tectum. The preceding information suggests that all of these areas are physically connected or are in electrical or biochemical contact with each other. If this is the case, it would be reasonable to assume that there is a correlation in the development of these areas.

The previous cytological and electrophysiological work suggests that the optic tectum matures during metamorphic climax. It was the aim of the present work to attempt to compare premetamorphic (Stage XII; Taylor and Kollros, 1946) larval and postmetamorphic juvenile, Rana catesbeiana, using a photostimulated evoked electroencephalographic (E.E.G.) response in the optic tectum and correlate that with tissue studies to verify neurophysiological maturation of the optic tectum.

DEFINITION OF TERMS

For the purpose of this paper, the term larval will refer to Stage XII, and juvenile will refer to postmetamorphic sexually immature R. catesbeiana.

The terms spontaneous E.E.G. and evoked E.E.G. are used to differentiate the experimental conditions from which the E.E.G. was recorded. Spontaneous E.E.G. refers to the E.E.G. activity recorded under "control" conditions without stimulation in a darkened Faraday room with the animals maintained at 7⁰ C. Evoked E.E.G. refers to the E.E.G. activity recorded in response to photostimulation of the lateral eyes under the same conditions.

MATERIALS AND METHODS

Larval and juvenile bullfrogs (Rana catesbeiana) were used in this investigation. The animals were collected locally and housed in large galvanized tanks with continuously flowing tap water at 17° C. Artificial lighting (12L:12D) was provided. The animals were not fed prior to use.

In order to facilitate examination and microelectrode implantation, each animal was immobilized with a combination of Finquel[®] (200 mg/l; Tricaine methanesulfonate, Ayerst Laboratories) and hypothermia (ice water at 7° C.). Recording microelectrodes consisted of insect pins electrolytically eroded to a tip diameter of approximately five microns and insulated with baked epoxy. Microelectrode placement was referenced to the parietal eye in all animals. Using this surface landmark, a grid system adapted from Kemali and Braitenberg (1969) was used. A total of four microelectrodes were employed. Two were placed 1 mm laterally while the other two were placed 5.5 mm posteriorly and 1 mm laterally of the parietal eye (Fig. 1). Microelectrodes placed laterally to the parietal eye monitored activity in the telencephalon, while microelectrodes implanted posterior to it monitored the optic tectum. Ten minutes post-implantation were allowed for stabilization before recordings were done.

The recording electrode impedance was approximately 5 Megohm each. Impedance matching to the recording amplifier was accomplished by interfacing the microelectrode via a Grass 7-HIP-5D 10 Megohm Z input network (Grass Instrument Co., Quincy, Mass.). The resulting E.E.G.

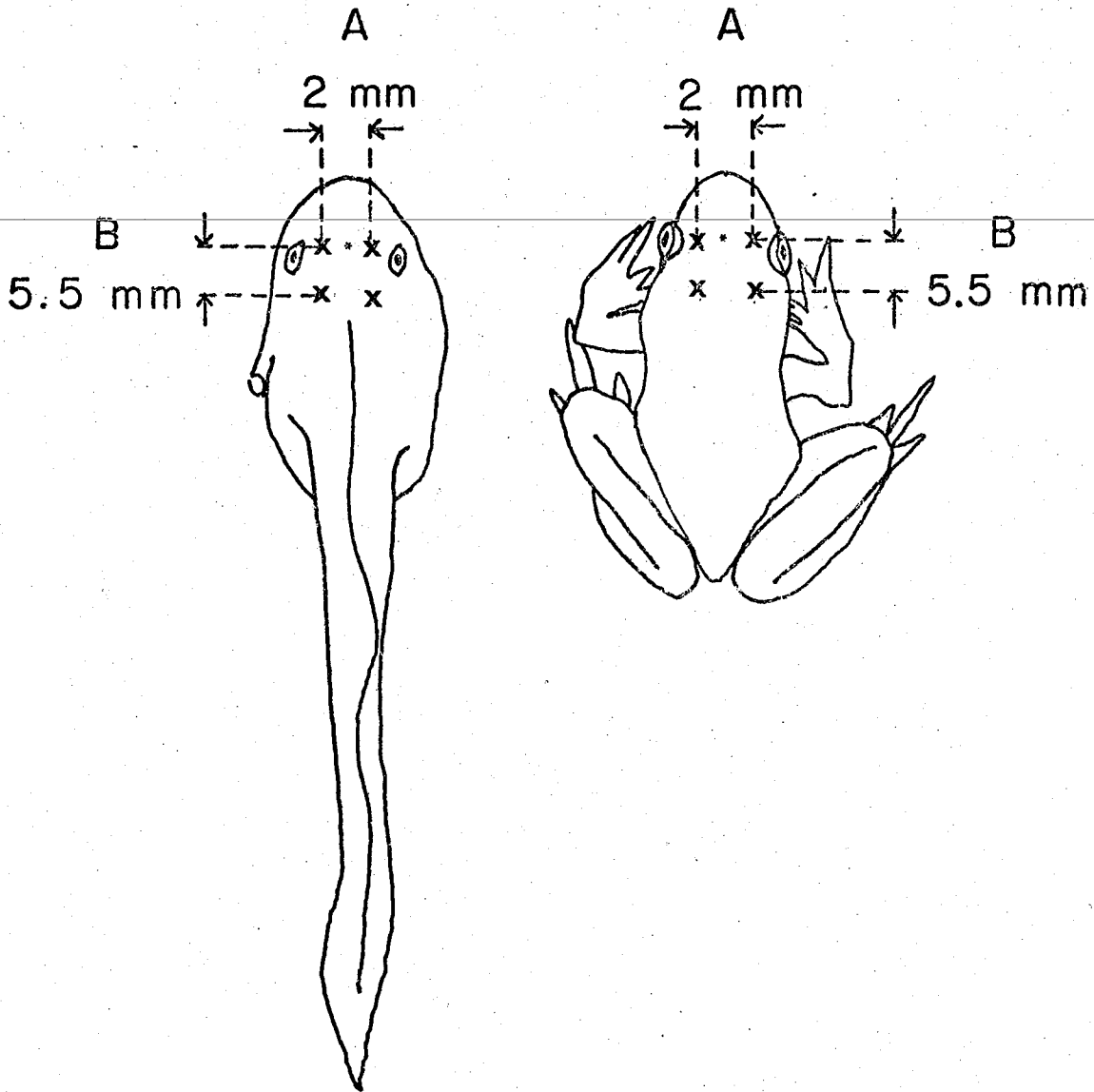


Fig. 1. Diagrammatic representation of electrode placement in larval and juvenile, *R. catesbeiana*. The X's represent the placement of the microelectrodes. (A) The microelectrodes lateral to the parietal eye. (B) Posterior microelectrodes, which are 5.5 mm posterior to the parietal eye and 2 mm apart (* equals the pineal eye).

activity recorded by the microelectrodes was processed in two stages and subsequently displayed in hard copy on a Grass 7B polygraph. The first stage was a Grass 7P3-B wide band A. C. amplifier with a 1/2 amp low frequency cut off set at 0.3 Hz. The second stage was a Grass 7D-AE wide band A. C. amplifier with a 1/2 amp high frequency cut off at 3 kHz. The information was simultaneously recorded on the polygraph and displayed on a Tektronix 504 oscilloscope as noted in Figure 2. The oscilloscope facilitated multiple trace time exposures of evoked E.E.G. potentials by photographic integration on Polaroid Type 105 film. The photostimulation, photographic, and polygraphic recording systems were synchronized by a trigger pulse derived from the photostimulators' internal pulse generator as seen in Figure 2.

Studies were conducted in a darkened Faraday room. The light source used in photostimulation of the preparations' lateral eyes was a closed system to ensure that each lateral eye received light only from the guide (4 mm O.D., non-coherent; Keystone Optic Fiber Inc., Boston, Mass.) aimed directly at it (Fig. 2).

A Xenon flash tube with a spectral range of 247.5-1262.3 nm was the light source. The output of the photostimulator was a white light of 10 msec duration which could be delivered to the lateral eyes at repetition rates from 1.10 to 9.80 Hz. The duration of a photostimulation period was typically one minute, however it varied according to the preparation's response (i.e., observed evoked cortical potentials; Fig. 3).

Fig. 2. Schematic diagram for the photostimulation, hard copy record, and integrated record of evoked E.E.G. activity in larval and juvenile *R. catesbeiana*. (1) Grass model 7B polygraph. Amplifiers are Grass models 7D-AE and 7P3-B. (2) Tektronix 504 oscilloscope. The oscilloscope facilitated integration of the E.E.G. evoked response through the use of multiple trace, time exposure photographs. With a low intensity electron beam sweep synchronized to the trigger pulse of the photostimulator (3) a time exposure can be made which records all activity traced by the beam, thus integrating its total activity. (4) Grass model 7-HIP-5D High Z input pre-amp. Note that the right hemisphere of the brain is the input to G2 and the left hemisphere is the input to G1. (5) Flexible fiber optic light guides (4 mm O.D., non-coherent; Keystone Optic Fiber Inc., Boston, Mass.) which delivered all photostimuli to the lateral eyes. The ends of the bifurcated light guide were maintained at a distance of 3 mm (± 0.5 mm) from the surface of the lateral eyes by the lead guide holder (6). (7) A ground reference electrode was maintained in the preparations' ice bath at all times. The D.C. amplifier seen at the bottom of the polygraph was used to amplify the stimulus mark pulse from the strobe.

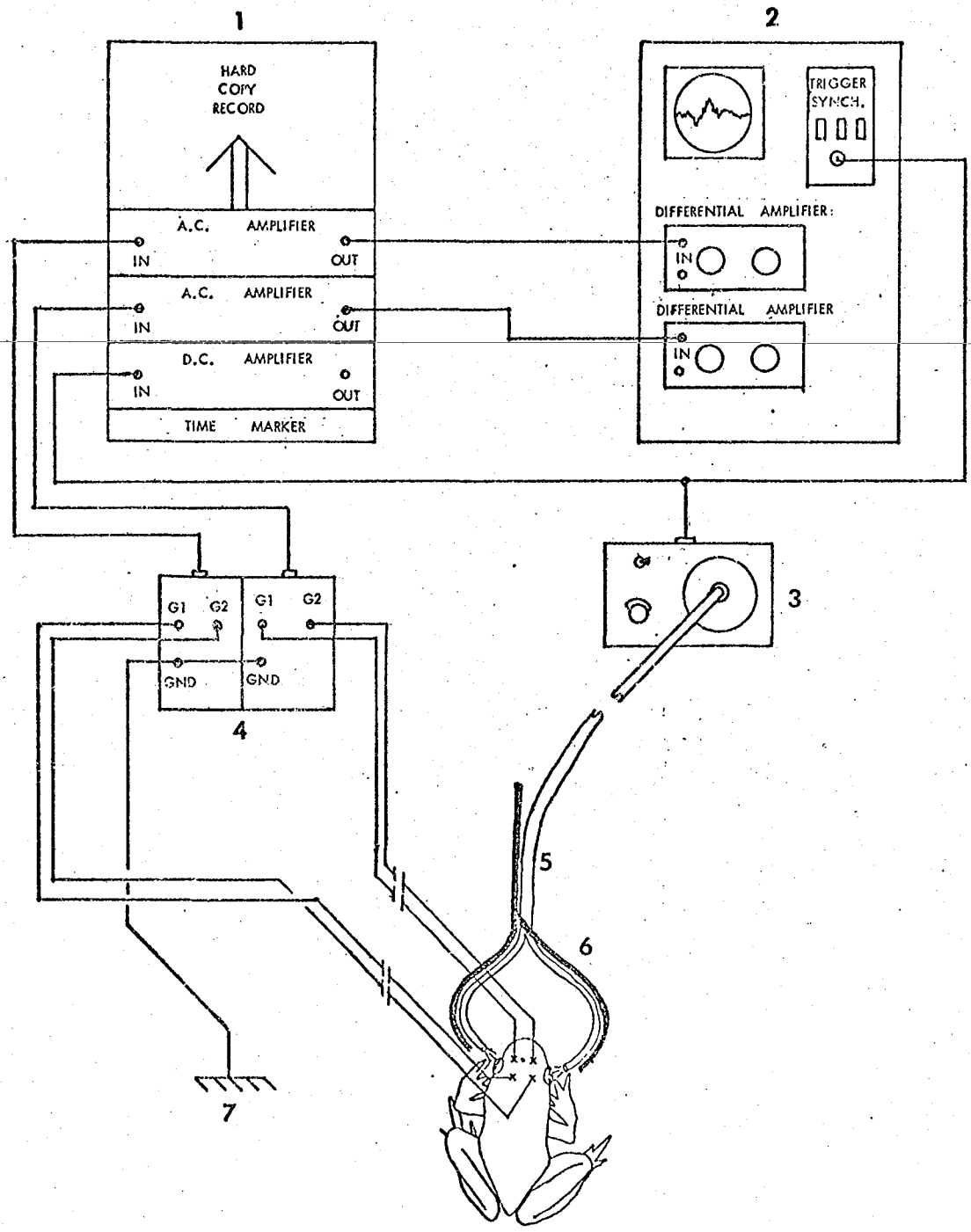


FIGURE 2

All animals were sacrificed and an autopsy was performed by removing the skull cap and exposing the dorsal surface of the brain. Measurements of the optic tectum and cerebral hemispheres were made and referenced to the major external anatomical landmarks. Representative brains from each stage were trimmed and imbedded in paraffin. They were cut in serial sections 12 microns thick and stained with iron hematoxylin. Cell densities were determined for both larval and juvenile animals using a technique developed by Kemali and Braitenberg (1969) (Fig. 6).

All data for E.E.G. amplitudes were statistically treated with the Median Test for Non-parametric data (Noether, 1976). (A Fortran IV program designed to sort the data for examination was developed and may be found in Appendix I.) The data were ranked and sorted in reference to two different parameters. First, they were ranked according to amplitude. Second, they were sorted by experimental state (spontaneous or stimulation) then again ranked according to amplitude. The Median Test was applied to the modes of the resulting ranks. Frequency data were treated in the same manner with the exception that they were done by hand as fewer data points were involved.

RESULTS

In larval and juvenile frogs after microelectrodes penetrated the surface of the optic tectum, large amplitude E.E.G. wave forms were encountered. Their amplitude and frequency were on the order of 100 μ V and 0.5 Hz. These E.E.G. wave forms were subsequently replaced by lower amplitudes which were treated as the spontaneous wave form of the individual.

Once a baseline of E.E.G. was established for a preparation, photostimulation was delivered to the lateral eyes. Of 25 preparation attempts, there were 20 animals which exhibited spontaneous activity; however, only 13 responded to photostimulation. In most cases, the lack of response was traced to electrode placement or brain damage by electrode placement.

The results presented in Figure 3 are typical of the data recorded for R. catesbeiana. The integrated E.E.G. evoked potentials in trace A of Figure 3 are achieved by photographing multiple sweeps of a very low intensity oscilloscope beam. The sweep of the beam is synchronized to start at the same place with each flash of the photostimulator. In this manner, an average voltage vs. time graph may be seen in the resulting photograph.

SPONTANEOUS E.E.G.

Larval spontaneous E.E.G. "burst" phenomena were frequently encountered. "Burst" phenomenon may be defined as a sudden increase in frequency and amplitude of E.E.G. activity. Burst activity exhibited

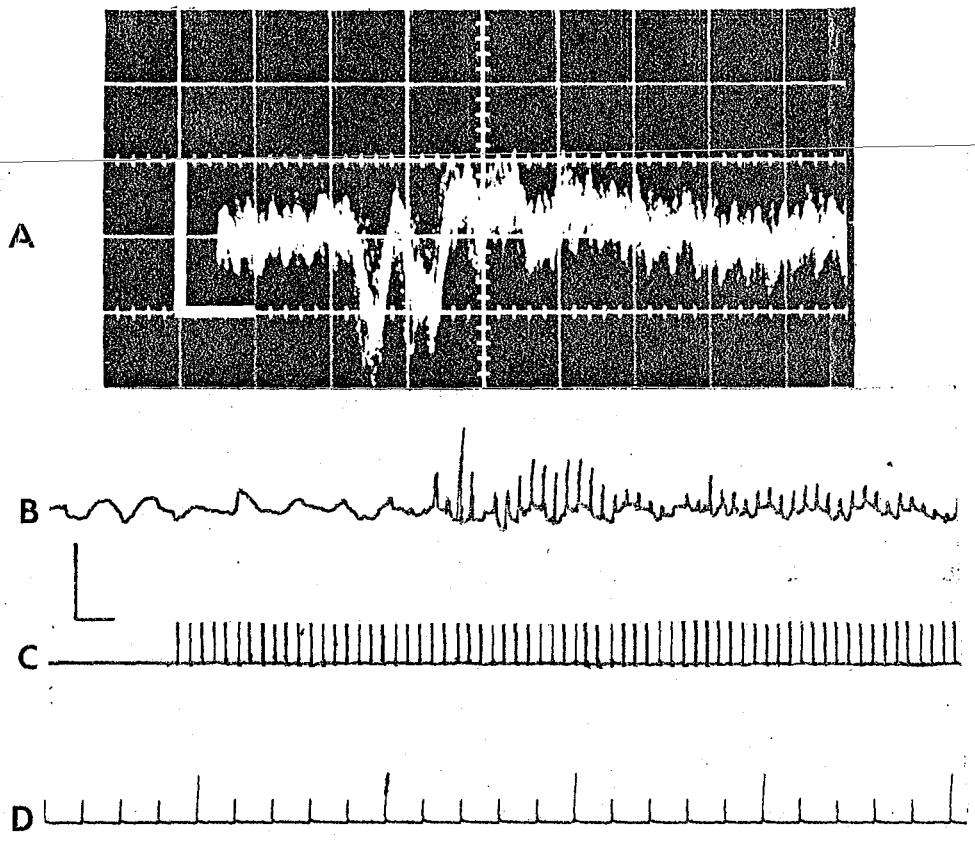


Fig. 3. Recorded E.E.G. evoked response to photostimulation of the lateral eyes in a juvenile frog. (A) Integrated activity photographed in a time exposure from a Tektronix 504 oscilloscope. Calibration equals 25 μ V and 50 msec. The integration represents the 44 separate stimulation events seen in the chart record (B). (C) Frequency of photostimulation to the lateral eyes. Calibration equals 25 μ V and 1 sec. (D) is an absolute time scale for all traces where each mark represents 1 sec.

durations of 0.5-1.5 sec, amplitudes up to $25\mu\text{V}$, and frequencies of 10-20 Hz. Figure 4 illustrates typical larval E.E.G. burst phenomenon.

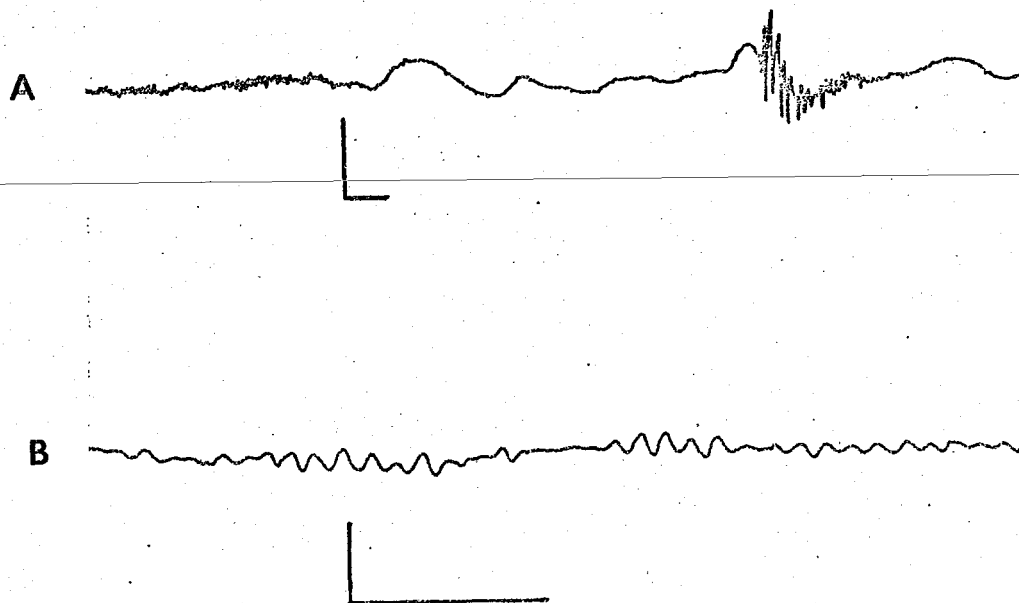


Fig. 4. Spontaneous E.E.G. "burst" activity in larval Stage XII *R. catesbeiana*. (A) "Burst" activity superimposed over slow frequency, high amplitude baseline E.E.G. (B) Isolated "burst" phenomenon with an expanded time scale. Calibration equals $25\mu\text{V}$ and 1 sec in both traces.

In Table I modal values for amplitude and frequency of larval and juvenile spontaneous E.E.G. activity are reported. The values range from $9.97\mu\text{V}$ and 1.60 Hz in larval to $13.07\mu\text{V}$ and 1.05 Hz in juveniles. These data are derived from 397 random sampled points obtained from E.E.G. activity recorded from larval and juvenile frogs. A typical example of juvenile spontaneous E.E.G. activity is shown in Figure 5.

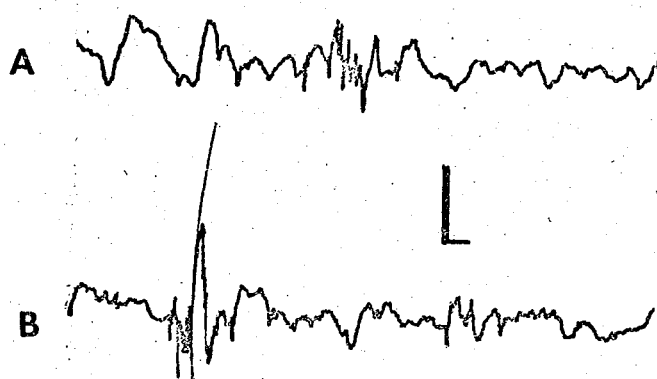


Fig. 5. Typical spontaneous E.E.G. activity in juvenile *R. catesbeiana*. (A) Spontaneous activity of the juvenile. (B) Note the spike complex to the left of center. This wave form was observed in juveniles in a frequency corresponding to the occurrence of "burst" phenomenon in larvae. Calibration equals $25 \mu\text{V}$ and 1 sec for both traces.

The relationships in amplitude, frequency, and cell density observed between larval and juvenile animals are summarized in Figure 6. The Median Test indicates that the amplitude difference between larvae and juveniles is significant ($\alpha = 0.05$), while the difference in frequencies are not ($\alpha = 0.20$). The apparent discrepancy between maximum evoked photostimulation amplitudes found in Figures 6 and 8 is due to sampling technique. The values in Figure 6 were calculated from the mode value for all data. The value represented in Figure 8 is the mode value for integrated evoked response data.

EVOKED E.E.G.

Larval preparations of the optic tectum typically respond to photostimulation of the lateral eyes as illustrated in Figure 7. From

TABLE I
 GROUP COMPARISON FOR SPONTANEOUS AND STIMULATION
 AMPLITUDE AND FREQUENCY

GROUP	AMPLITUDE (μ V)		FREQUENCY (Hz)
	Spontaneous	Evoked Response	
Larval*	17.25	18.30	0.20
	7.60	12.38	1.56
	12.16	16.78	8.00
	9.00	14.00	0.90
	9.80	35.80	5.62
	4.00	21.44	0.51
Mode+	9.97	19.78	1.60
Juvenile	22.69	32.47	0.25
	10.19	12.35	0.90
	5.06	19.88	4.33
	22.55	25.10	0.80
	9.49	45.88	0.75
	11.74	22.76	0.90
	9.76	26.40	0.80
Mode	13.07	26.41	1.05

*Each number listed within each group represents the mode of data collected for an individual of the group. A total of 6 larvae and 7 juveniles were examined.

+The mode represents the combination and ranking of all individuals within the group to form one continuous rank for all data collected. From this rank, a single mode value was selected for larvae and juveniles respectively. This was the value used to statistically compare groups.

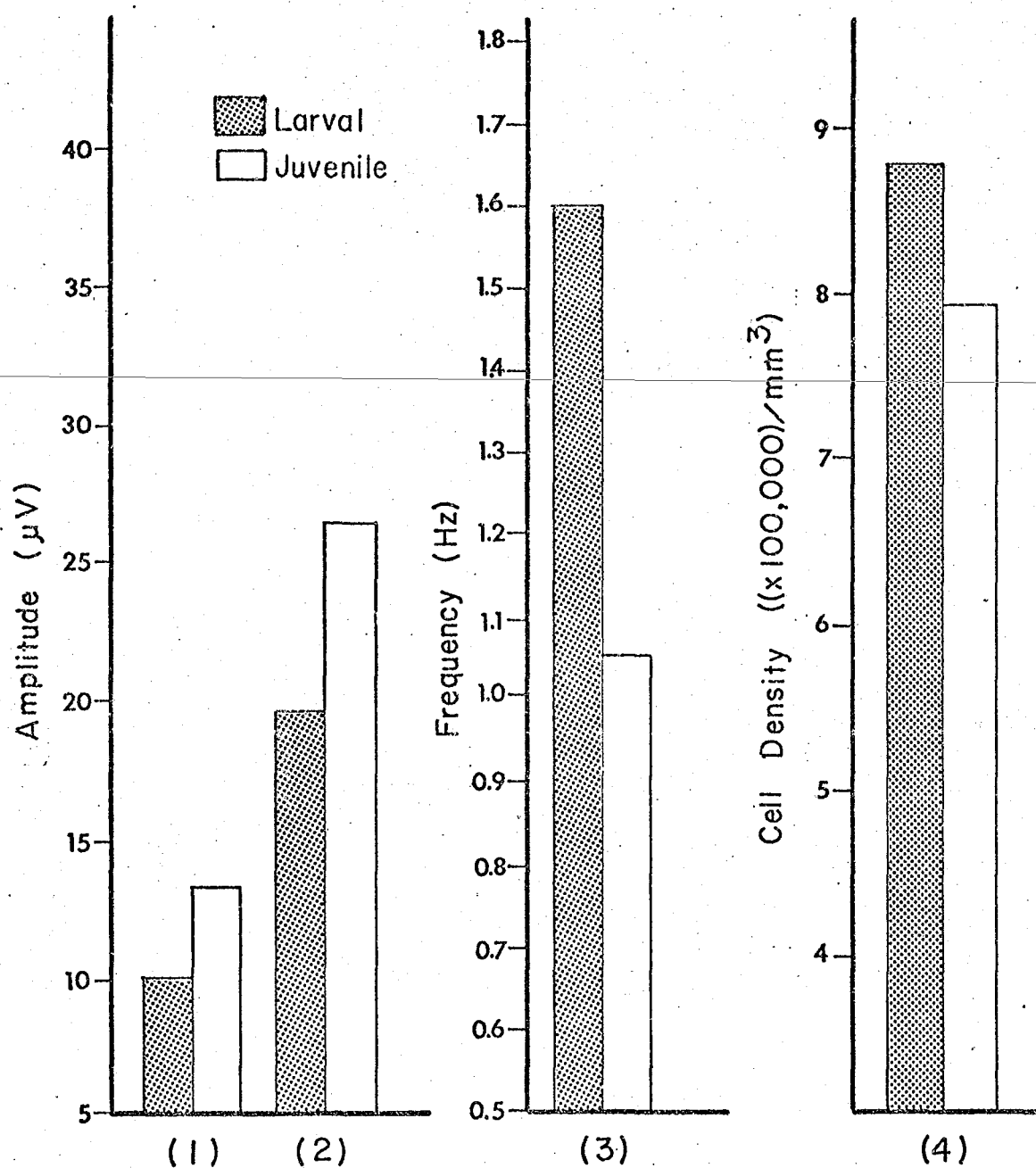


Fig. 6. Histogram comparing larval and juvenile *R. catesbeiana* E.E.G. activity to frequency of stimulation and cell density. (1) Spontaneous E.E.G. amplitude. (2) Evoked E.E.G. amplitude. (3) Frequency of stimulation which elicited the greatest amplitude in evoked E.E.G. response. (4) Cell density calculated by the Section Method B, outlined in Kemali and Braitenberg (1969).

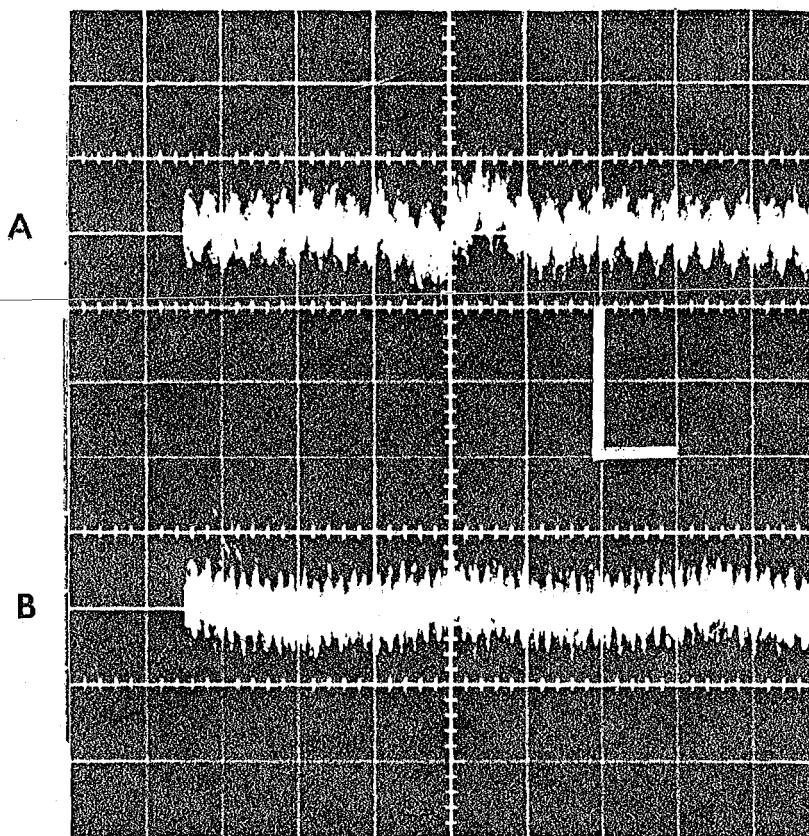


Fig. 7. Traces are 35 integrated E.E.G. evoked response from the optic tectum of larval, Stage XII, frogs. (A) E.E.G. evoked response at a stimulation frequency of 3.50 Hz. (B) E.E.G. evoked response at a stimulation frequency of 9.33 Hz. Calibration equals $25 \mu\text{V}$ and 50 msec.

Table I, the modal E.E.G. photostimulation amplitude is $19.78 \mu\text{V}$.

Observed sample maximum stimulation amplitudes and frequencies recorded were $23.10 \mu\text{V}$ at 3.50 Hz.

A photostimulation frequency of 2.40 Hz evoked a maximal response of $51.67 \mu\text{V}$ in the juvenile animals examined. This represents a 31.41%

shift in stimulation frequency and a 123.68% shift in maximal amplitude from larvae. These data are presented in Figure 8.

A typical juvenile response to photostimulation is shown in Figure 9. The evoked response of juveniles may be grouped into two categories relative to the post-stimulation elapsed time. The first group, designated alpha, occurs 50-100 msec after stimulation. The second group, beta, occurs about 250 msec after stimulation. At higher evoked response frequencies, the alpha response decreases and the beta response increases. Stimulation frequency of 1.20 Hz gave an increased alpha response. Increasing the frequency to 2.20 Hz decreased the alpha response and increases the beta response. When the stimulation frequency was increased above 3.00 Hz, both alpha and beta responses disappeared completely from the E.E.G. evoked response.

MORPHOLOGY

The overall brain morphology of larval and juvenile frogs is the same; however, as the data presented in Figure 10 and Table II indicate, growth takes place through metamorphic climax. The brain increases in length by approximately 17% and in breadth by 26%. The data for parameters 3 and 5 (the distance antero-caudally and laterally, respectively) show that the brain is oriented more posteriorly to the eyes in juveniles than larvae. The data for the distance between the centers of the optic tectum and the tympanic membrane (parameter 6 in Table II) were not taken in larvae because the membrane is absent.

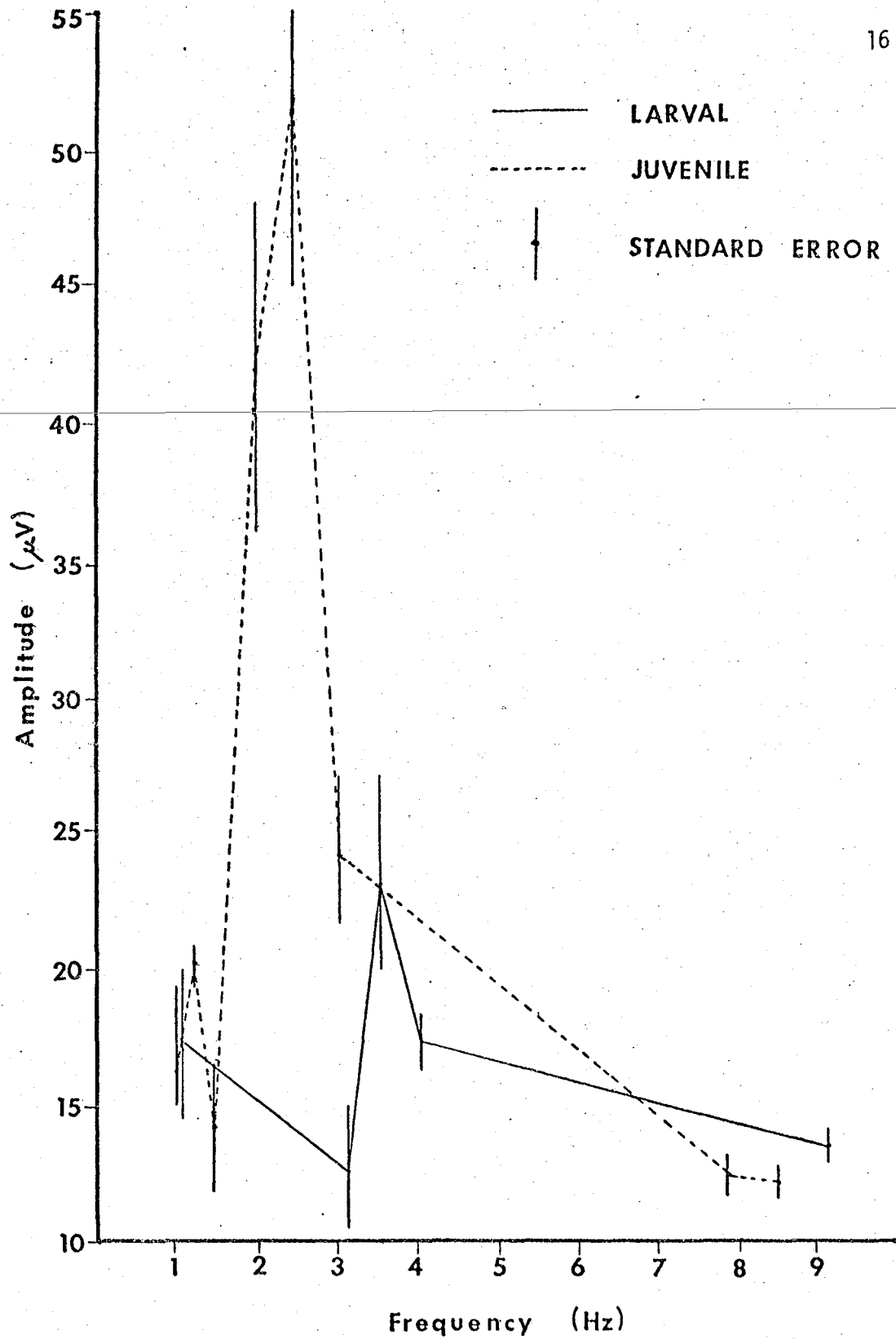


Fig. 8. A plot of amplitude vs. frequency comparing the evoked E.E.G. response of larval and juvenile R. catesbeiana to photostimulation at the lateral eyes.

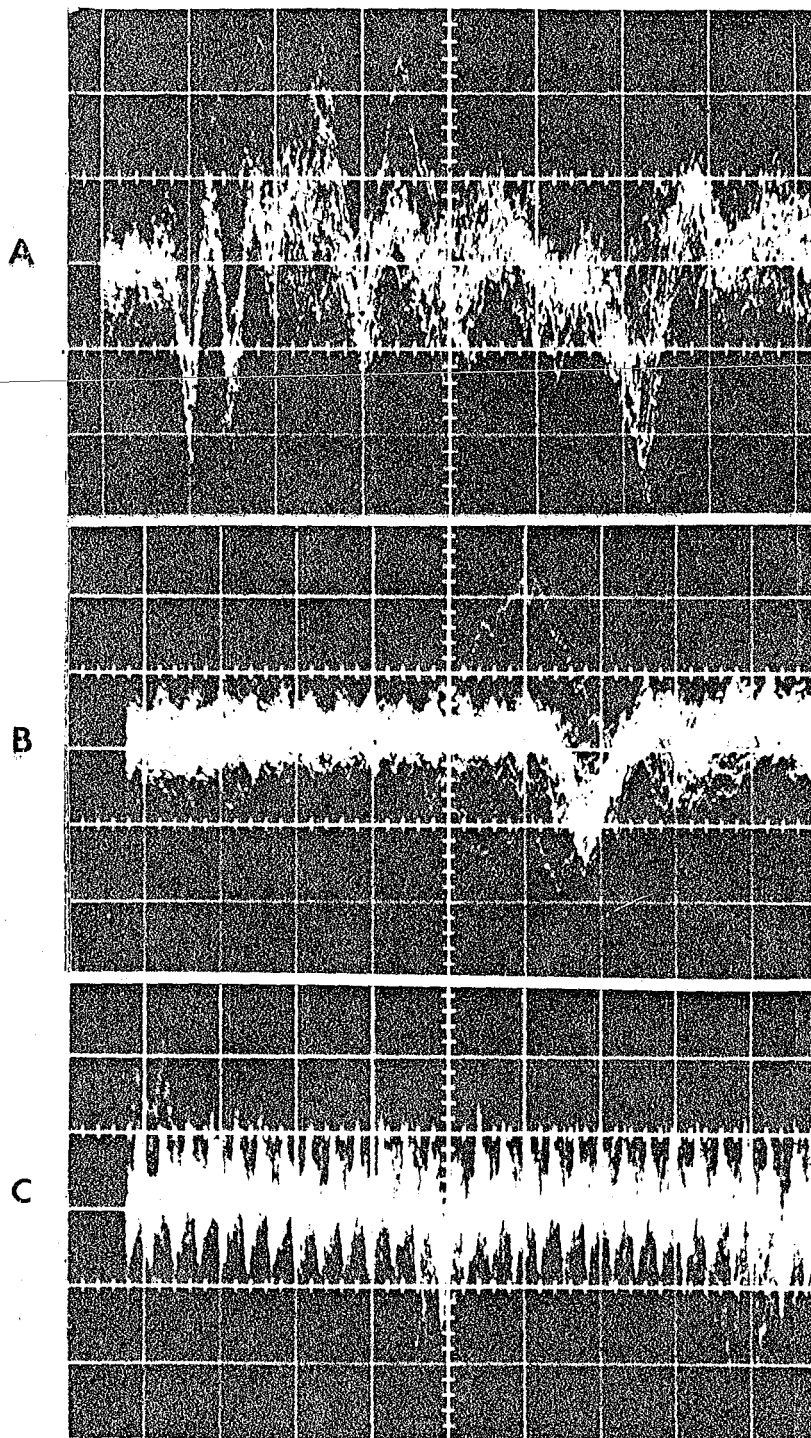


Fig. 9. E.E.G. evoked response in the optic tectum of juvenile frogs, integrated from 45 separate stimuli. (A) Stimulation frequency equals 1.20 Hz. (B) Stimulation frequency equals 2.40 Hz. (C) Stimulation frequency equals 8.00 Hz. Calibration mark on the right-hand side equals $25 \mu\text{V}$ and 50 msec.

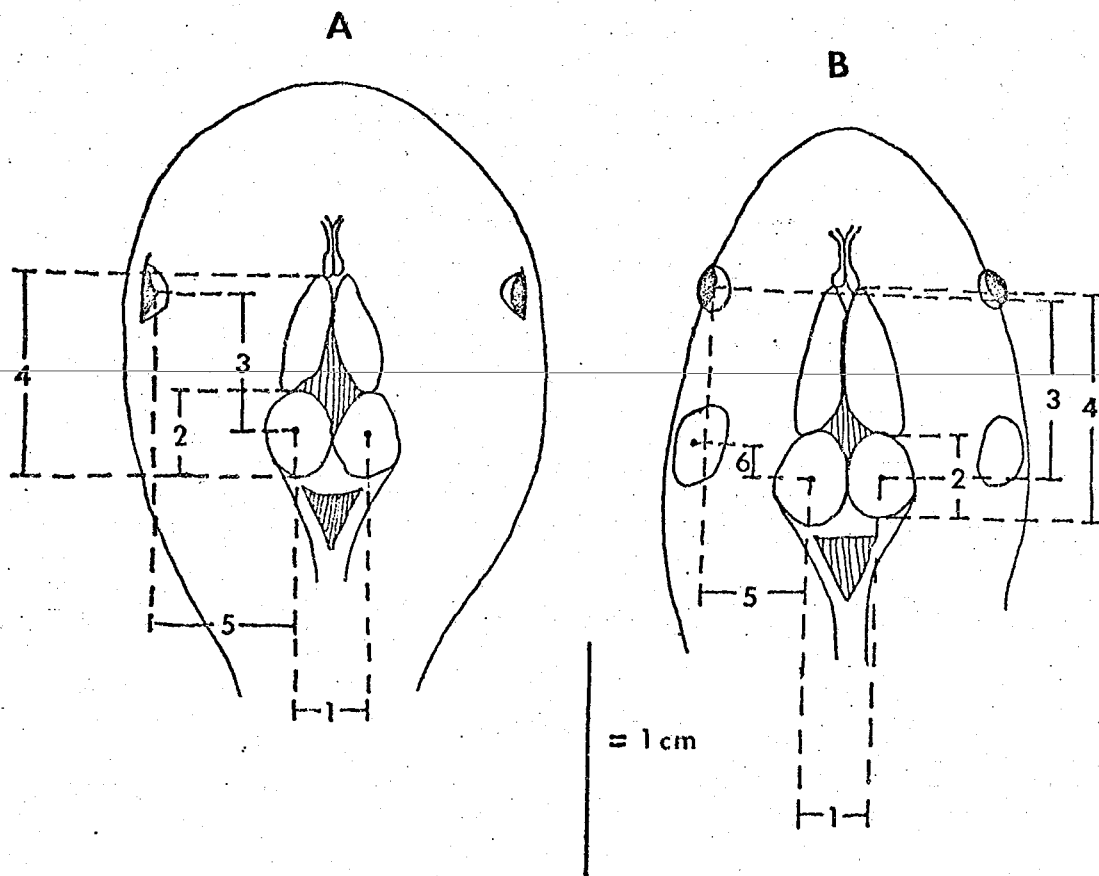


Fig. 10. Dorsal view of the brain comparing the dimensions of larval and juvenile *R. catesbeiana*. (A) Representation of larval *R. catesbeiana*. This view shows the major dimensions which were considered useful in documenting the growth of the brain. The numbered parameters describe the dimensions of the brain. 1) The distance between the midpoints of the lobes of the optic tectum. 2) The length of the optic tectum. 3) The antero-caudal distance from the center of the eye to the center of the lobe of the optic tectum. 4) The length of the brain from the posterior margin of the optic tectum to the anterior margin of the cerebral hemispheres. 5) The lateral distance between the midpoints of the eye and the lobe of the optic tectum. With parameters 3 and 5 it is possible to show changes in the intracranial orientation of the brain. Parameters 1, 2, and 4 will show intercranial relationships. (B) Note that a sixth parameter has been considered. It shows the antero-caudal distance between the midpoints of the tympanic membrane and the lobe of the optic tectum.

TABLE II
COMPARATIVE MEASUREMENTS

GROUP	PARAMETER					
	1	2	3	4	5	6
Larval	0.240	0.300	0.570	1.050	0.320	---
	0.210	0.220	0.474	0.637	0.771	---
	0.200	0.230	0.339	0.675	0.705	---
	0.207	0.257	0.505	0.770	0.720	---
	0.173	0.220	0.331	0.667	0.636	---
	0.160	0.253	0.403	0.707	0.695	---
	0.162	0.223	0.310	0.693	0.585	---
Mean	0.193	0.243	0.419	0.743	0.633	---
S.D.	0.029	0.029	0.099	0.142	0.151	---
Juvenile	0.237	0.300	0.563	0.883	0.391	0.051
	0.260	0.291	0.580	0.911	0.352	0.069
	0.313	0.295	0.425	0.896	0.547	0.083
	0.210	0.275	0.669	0.849	0.515	0.055
	0.235	0.265	0.616	0.894	0.515	0.043
	0.205	0.225	0.520	0.800	0.517	0.105
	Mean	0.243	0.275	0.562	0.872	0.473
S.D.	0.040	0.028	0.084	0.041	0.080	0.023
% Difference	25.91	13.17	34.13	17.36	-25.28	---

This table summarizes the measured distances in cm for each numbered parameter in Figure 10. The per cent differences expressed are from larval to juvenile.

CELL DENSITIES

The bases of cell densities reported below are six representative cross sections of the optic tectum from 2 larvae and 2 juveniles (Fig. 11 and 12). The average cell densities for larvae and juveniles are 887,598 cells/mm³ (SD is 69,241) and 789,288 cells/mm³ (sd is 52,971) respectively. These data compare to the datum reported for mature adults, which is 750,000 cells/mm³ (Kemali and Braitenberg, 1969). The percent difference between larval and juvenile stages is 11.08%.

Fig. 12. Cross section of the optic tectum in juvenile *R. catesbeiana* showing the relative cell density and distribution. (A) Representative cross section of the optic tectum as seen in juveniles (45X). (B) Enlargement of the area indicated in (A) showing stratification of the cells in a larger area relative to Fig. 11 (B) (100X). (C) Diagram indicating the position of the section seen in relation to the brain.

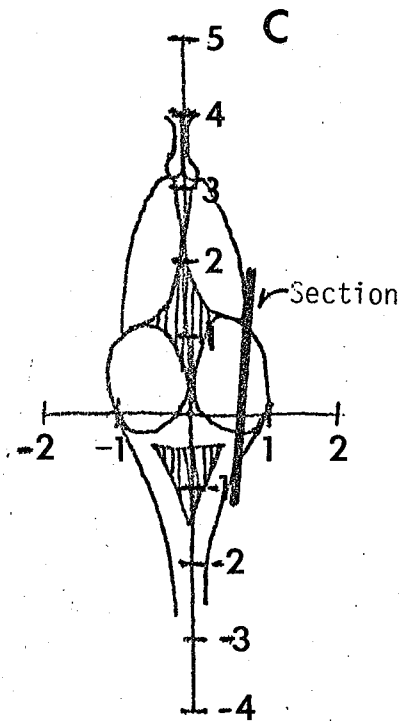
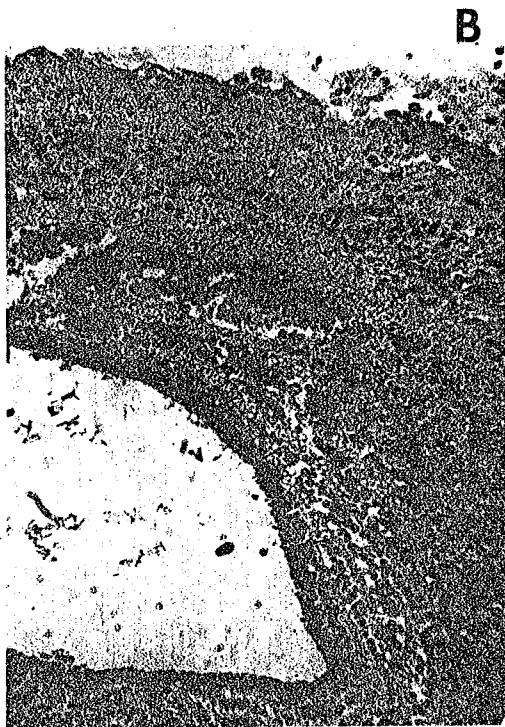
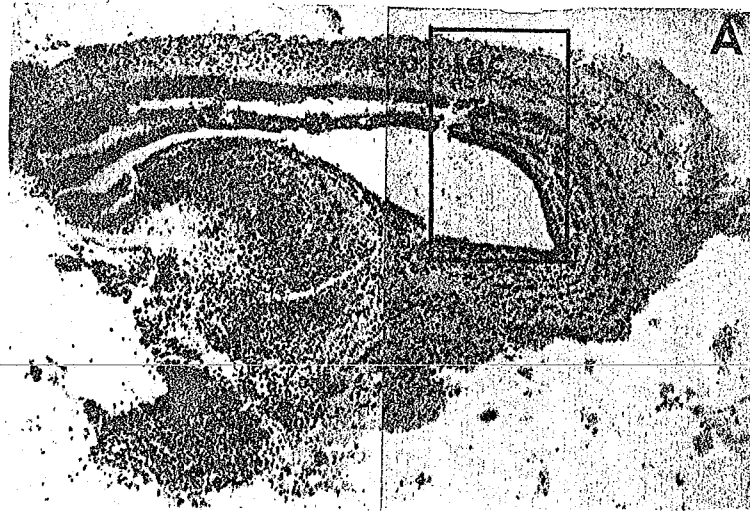


FIGURE 11

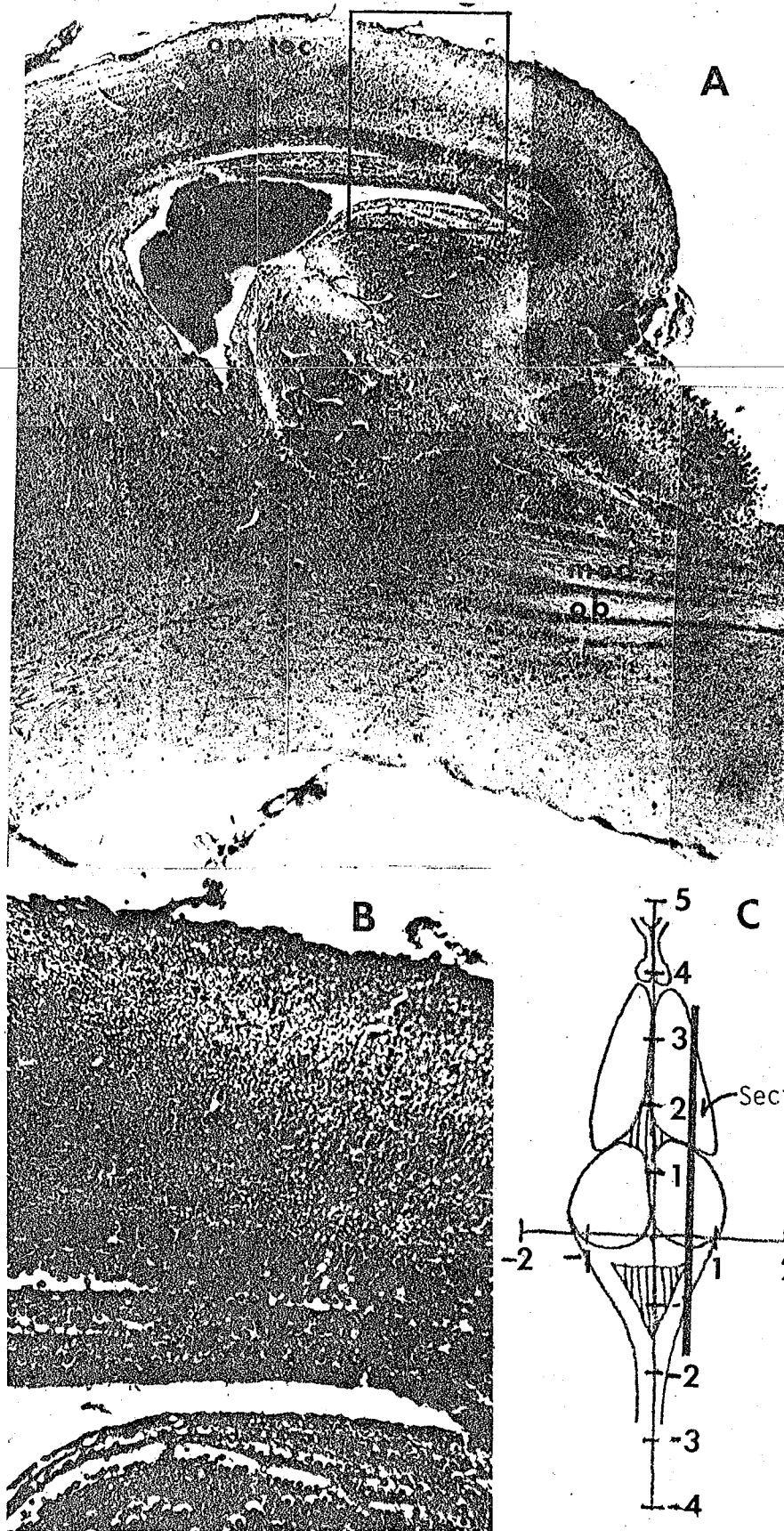


FIGURE 12

DISCUSSION

E.E.G. studies on the central nervous system (C.N.S.) of frogs are hampered by myographic activity; therefore, immobilization of the frog is desired but depression of the C.N.S. is not. For this reason a combination of Finquel and hypothermia were used rather than only one. Finquel depresses the animal quickly to allow reasonable investigation time. Hypothermia will maintain myographic depression without the progressive toxification encountered with Finquel. Hypothermia does however depress C.N.S. activity but not to the same degree as an anesthetic. Hypothermia is a natural event occurring in the normal life cycle of R. catesbeiana.

The spectra of Xenon are entire between the wave length limits set forth previously (C. R. C. Handbook, Vol. 53). The quanta of energy released with each stimulation was reasonably constant. The spectral limits encountered in the Xenon stimulus were well within the perceptual limits of the frogs optic system (cf. Donner and Rushton, 1959).

The range of selectivity and sensitivity in a recording micro-electrode is known as its "sphere of influence." Any membrane potential (MP) activity beyond that range, or limit, will not be recorded; however, any group of MP's within that range will be summed. When a quiescent preparation is stimulated, the microelectrodes record cortical E.E.G. from High Voltage Slow Activity (HVSA) to Low Voltage Fast Activity (LVFA); a response which is often called an "arousal response" (Hobson, 1967 ; Klemm, 1969). This LVFA is sometimes referred to as desynchrony. The term is derived from the hypothesis that a synchronous wave form,

such as HVSA, results from summation of MP oscillations in parallel reverberating circuits that are firing synchronously. Such synchronous reverberation would be expected to produce a high voltage because of summation of in-phase potentials. The slower frequencies observed during HVSA arise in part from voltage summation and may also be related to more temporal and spatial summation in the synchronized generation of MP fluctuations (Klemm, 1969). Although "arousal response" refers to a general increase in behavioral E.E.G. activity, the electrophysiological principle may also be applied to a tectal evoked response. The animal, through the use of a photoevoked stimulus, is driven to a simultaneous tectal cortical projection of that stimulus. The result is a evoked response which exhibits the same desynchrony and synchrony phenomena that can be seen in an "arousal response."

The physiologic mechanisms by which desynchrony is produced include the neural activating functions of the Ascending Reticular Arousal System (ARAS) in the brain stem. This system receives collateral sensory input from all major sensory channels and projects an excitatory drive diffusely upon the cortex (reviewed by Rossi and Zanchetti, 1957; Klemm, 1969). The E.E.G. arousal response parallels a simultaneous behavior arousal and is a reflection of the process by which an animal becomes attentive to stimuli and responds appropriately.

The alpha and beta response found in juveniles are possibly part of an arousal state in which the retina is integrating activity which is then passed to the tectum. Maturana, et al. (1960) have grouped

retinal ganglion cells into five classes by integrative function. They are:

- Class I - Sustained edge detection - with non-erasable holding
- Class II - Convex edge detection - with erasable holding
- Class III - Changing contrast detection
- Class IV - Dimming detection
- Class V - Darkness detection

The nature of the photostimulus used in this investigation was an on-off, constant intensity, stimulus. With this on-off photostimulus, it was expected that the response of ganglion in Classes I and II would be reduced if present at all. Class III ganglion would respond to a stimuli and Class IV would respond to off stimuli. Class V ganglion are constantly firing while in the dim light, possibly giving rise to the recorded spontaneous baseline E.E.G. from the tectal cortex. Spontaneous burst activity may partly be the result of perceived movement or light in the laboratory detected by the retina.

Larvae in comparison to juveniles show more LVFA spontaneous activity. This may be accounted for by the fact that Class I - IV ganglia respond to moving objects while Class V do not (Maturana, et al., 1960). The Class I - IV ganglia and their afferent fibers found in larvae might be immature in comparison to those found in juveniles. Larvae feeding on detritus and floating plant material in an aquatic environment have different needs of retinal perception of movement than a terrestrial juvenile feeding on flying insects and in danger from quick moving predators. Gona (1974) states that the cerebellum of the frog (R. catesbeiana) remains immature throughout premetamorphosis and the most vigorous phase

of cerebellum maturation takes place during metamorphic climax. If the cerebellum with its tracts to the reticular activating system mature at metamorphic climax, it would be reasonable to suggest that the optic tectum and the retinal ganglia and their tracts to the ARAS matures as well.

An integrated E.E.G. evoked response to photostimulation might indicate if the difference in developmental stages is indeed maturation of cell layers. This would be accomplished by detecting any consistent changes in amplitude (increase in number of MP's firing) and frequency (increase in number of synapses involved). Figure 7 shows such an increase in juvenile amplitude response over that recorded in larvae.

Several theories may explain this increase in evoked E.E.G. amplitude. Kollros (1953) has described the stratification of the optic tectum. Sidman (1967) postulated that the interaction between the Purkinje cells and the granule cells migrating past them might stimulate growth of Purkinje cell dendrites and establish synapses between them. The work of Maturana, et al. (1960), lends significance to the establishment of these synapses. They observed axons in each of the ganglion classes terminating in a separate layer of the tectum, with the exception of III and V which are mixed together so at the end of maturation they have four fundamental layers of strata of terminals. To any point on the tectum, the terminals of all layers come from the same locus in the retina (Gaze, 1958), so that there is a point to point representation of the retina on the tectum with an integration of activity for each point. Unfortunately, the strata representing each functional unit (ganglion class) have not been mapped.

Additional explanation for the differentiation of larval and juvenile evoked E.E.G. responses could be the differential myelination of afferent fibers. Class I and II ganglion axons are unmyelinated, while Class III and IV are myelinated (Maturana, et al., 1960). It is possible that the difference in conduction rates between myelinated and unmyelinated nerves will account for true difference between the alpha and beta response. Conduction velocities for the optic nerve confirm this hypothesis (Maturana, et al., 1960). The unmyelinated fibers transmit their action potentials at a rate up to 5 times slower than myelinated fibers. This difference allows ample margin for variation from the 50 msec alpha to the 200 msec beta range seen in the evoked response records.

SUMMARY

1. Spontaneous and evoked E.E.G. activity from the optic tectum were recorded and delineated for larval, stage XII, and postmet. juvenile, R. catesbeiana.

2. Data collected show an increase in spontaneous E.E.G. amplitude, a decrease in spontaneous E.E.G. frequency, and an increase in evoked amplitude from larval to juvenile stages. Several qualitative differences in recorded E.E.G. activity between larvae and juveniles were noted. These include:

- a. An increase in "spike" activity in juvenile when in an "aroused" state
- b. Larvae showed more LVFA activity than juveniles
- c. Larvae exhibited 2 frequency components while juveniles usually had 3 components
- d. Juveniles had a biphasic evoked E.E.G. response at stimulation frequencies less than 4 Hz

It was postulated that the biphasic response is correlated to the retinal ganglia functional Classes having myelinated and unmyelinated nerves.

BIBLIOGRAPHY

- Dawson, D.C. and C.L. Ralph 1974 Light-Evoked Neural Activity in the Intermediate Lobe of the Pituitary of Rana pipiens: Electrophysiology Evidence for a Neural Pathway Linking the Eyes with the pars intermedia. Neuroendocrinology. 15:267-280.
- Donner, K.O. and W.A.H. Rushton 1959 Retinal Stimulation by Light Substitution. J. Physiol. 149:288-302.
- Etkin, W. 1963 Metamorphosis Activating System of the Frog. Science 139:810-814.
- Eker, A., Gaupp, F., and R. Wiedersheim 1899 Anatomie des Froches. Zweite Aufl. Zweite Abt. Braunschweig.
- Gaze, R.M. 1958 The Representation of the Retina on the Optic Lobe of Frogs. Quart. J. Exp. Physiol. 43:209.
- Gona, A.G. 1974 Morphogenesis of the Cerebellum of the Frog Tadpole during Spontaneous Metamorphosis. J. Comp. Neurol. 146:133-142
- Hartline, H.K. 1938 The Response of Single Optic Nerve Fibers of the Vertebrate Eye to illumination of the Retina. Amer. J. Physiol. 121:415-440.
- Hobson, J.A. 1967 Respiration and E.E.G. Synchronization in the Frog. Nature 213:988-989.
- Kemali and Braitenberg 1969 Atlas of the Frogs Brain. Springer-Verlag, New York. 74 pages.
- Klemm, W.R. 1969 Animal Electroencephalography. Academic Press, New York. 292 pages.
- Kollros, J.J. 1953 The Development of the Optic Lobes in the Frog; I. The Effects of Unilateral Enucleation in Embryonic Stages. J. Exp. Zoo. 123:153-189.
- Larsell, O. 1929 The Effect of Experimental Excision of One Eye on the Development of the Optic Lobe and Opticus Layer in the Larvae of the Tree Frog (Hyla regilla). J. Comp. Neurol. 48:331-353.
- Maturana, H.R., J.V. Lettvin, W.S. McCulloch, and W.H. Pitts 1960 Anatomy and Physiology of Vision in the Frog. J. Gen. Physiol. 43:129-175.

- Muntz, W. R. A. 1962 Microelectrode Recordings From The Diencephalon Of The Frog (Rana Pipiens) and A Blue Sensitive System. J. Neurophysiol. 25:699-711.
- Noether, G. E. 1976 Intro. to Statistics: A Nonparametric Approach 2nd Ed. Houghton Mifflin Co. Palo Alto. 292 pages.
- Rossi, G. F. and A. Zanchetti 1956 The Brain Stem Reticular Formation. Anatomy and Physiology. Arch. Ital. Biol. 95:199-438.
- Sidman, R. L. 1967 Development of Interneuronal Connections In Brains of Mutant Mice. In: Physiological and Biochemical Aspects of Nervous Integration. F. D. Carlson, Ed., Prentice-Hall, Englewood Cliffs, pp. 163-193.
- Taylor, A. C. and J. J. Kollros 1946 Stages In The Normal Development of Rana Pipiens Larvae. Anat. Rec. 94:7-23.
- Weast, R. C., Ed. (1972) C. R. C. Handbook of Chemistry and Physics, 53 Ed., Chemical Rubber Co., Cleveland, Ohio.

APPENDIX I

B6700/B7700 FORTRAN COMPILATION MARK

```
FILE 5=MELINDA, UNIT=READER
FILE 6=LINDA, UNIT=PRINTER
FILE 20=LIMBO, UNIT=DISKPAC, RECORD=14, BLOCKING=30
```

```
      DIMENSION A(2000,10)
      NP=1
10  READ(5,20,END=30) A(NP,1),A(NP,2),A(NP,3),A(NP,4)
20  FORMAT(I10,F10.2,I5,I5)
      NP=NP+1
      GO TO 10
30  NP=NP-1
      WRITE(6,90)
90  FORMAT(////////,58X,"RAW DATA DISPLAY",////)
      WRITE(6,100)
100 FORMAT(35X,"N",5X,"I.D. NUMBER",5X,"DATA",5X,"EXPER. COND.",5X,
      *"MET. STATE")
      MP=1
      DO 120 I=1,NP
      WRITE(6,110) MP,A(MP,1),A(MP,2),A(MP,3),A(MP,4)
110 FORMAT(34X,I3,7X,F6.2,12X,I1,13X,I3)
120 MP=MP+1
      CALL DATASORT (A,NP,IDNO,EXCOND,MS)
      WRITE(6,80)
80  FORMAT(1H1,////////,56X,"RANKED DATA DISPLAY"////)
      WRITE(6,100)
      DO 150 J=1,NP
150 WRITE(6,40) J,A(J,1),A(J,2),A(J,3),A(J,4)
40  FORMAT(34X,I3,7X,I4,7X,F6.2,10X,I1,13X,I3)
      CALL MSSORT (A,NP,IDNO,EXCOND,MS)
      WRITE(6,80)
      WRITE(6,100)
      DO 160 J=1,NP
160 WRITE(6,40) J,A(J,1),A(J,2),A(J,3),A(J,4)
      STOP
      END
```


SUBROUTINE DATASORT (A,NP,IDNO,EXCOND,MS)

DIMENSION A(2000,10)

MAX=NP

1 J=1

FLAG=0

5 IF(A(J,2).LE.A(J+1,2)) GO TO 100

B=A(J,2)

A(J,2)=A(J+1,2)

A(J+1,2)=B

B=A(J,1)

A(J,1)=A(J+1,1)

A(J+1,1)=B

B=A(J,3)

A(J,3)=A(J+1,3)

A(J+1,3)=B

B=A(J,4)

A(J,4)=A(J+1,4)

A(J+1,4)=B

FLAG=1.0

100 J=J+1

IF(J.LT,MAX) GO TO 5

IF(FLAG.EQ.0.0) RETURN

MAX=MAX-1

IF(MAX.EQ.0) GO TO 200

GO TO 1

200 WRITE(6,210) MAX

210 FORMAT(1H0,"NO SORT . . . MAX = ",I3)

STOP

END

SUBROUTINE MSSORT (A,NP,IDNO,EXCOND,MS)

```
DIMENSION A(2000,10)
MAX=NP
1 J=1
  FLAG=0
5 IF(A(J,4),LE.A(J+1,4)) GO TO 100
  B=A(J,4)
  A(J,4)=A(J+1,4)
  A(J+1,4)=B
  B=A(J,2)
  A(J,2)=A(J+1,2)
  A(J+1,2)=B
  B=A(J,1)
  A(J,1)=A(J+1,1)
  A(J+1,1)=B
  B=A(J,3)
  A(J,3)=A(J+1,3)
  A(J+1,3)=B
  FLAG=1.0
100 J=J+1
  IF(J.LT.MAX) GO TO 5
  IF(FLAG.EQ.0.0) RETURN
  MAX=MAX-1
  IF(MAX.EQ.0) GO TO 200
  GO TO 1
200 WRITE(6,210) MAX
210 FORMAT(1H0,"NO SORT . . . MAX = ",I3)
  STOP
  END
```