THE SEXUAL BEHAVIOR OF ANURA

2. NEURAL MECHANISMS CONTROLLING MATING IN THE MALE LEOPARD FROG, *RANA PIPIENS*

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INTRODUCTION

ALTHOUGH SOME of the very early concepts of the neural mechanisms controlling sexual behavior were developed from experimentation on Anura, almost all the recent investigators in this field have concentrated on mammals. The rapid progress made in the last two decades in this aspect of mammalian research has not been paralleled in the lower vertebrates. In Anura, few advances have been made in this subject since the close of the last century.

Some of the earlier investigators probably chose the frog because lesser technical problems were involved in brain experiments on this animal, but a more important reason for investigating the neural mechanisms of Amphibia is now apparent. It is generally accepted that the amphibian brain closely resembles the generalized prototype from which the higher vertebrate brains developed (Kuhlenbeck, 1921; Herrick, 1933a). Many of the ancient pathways of the amphibian brain are still recognized in the mammalian and human brains (Papez, 1929; Ariëns Kappers, Huber, and Crosby, 1936). Relatively speaking, the frog brain is organized along very elementary lines, and this is reflected in the simple and extremely stereotyped mating patterns which these animals exhibit. Hence information concerning the mechanisms of the innate behavior patterns of these primitive vertebrates should be of considerable value in interpreting mammalian and human brain function (Beach, 1942a) and in formulating general theories of the evolutionary changes in the nervous control of this very important aspect of behavior.

For many years Dr. C. J. Herrick has been engaged in an exhaustive study of the morphology of the amphibian brain, and during the course of this work he has formulated a number of hypotheses concerning the function of the structural elements which he described in such detail. However, as Herrick (1933a) himself expresses it, "These are inferences drawn from the anatomical arrangements, and for most of them there is as yet little direct experimental proof." The present experiments were intended, in part, to examine some of these concepts from the behavioral point of view.

While the anuran brain exhibits a considerable amount of specialization as compared with that of the urodele, the technical advantages gained by using the frog, particularly *Rana pipiens*, were such as to make this species particularly desirable, at least for an introductory investigation.

In developing the project we attempted to utilize, wherever possible, recent methods and techniques. Foremost among these is the pituitary injection method developed by Rugh (1935) which made available to us an abundant supply of sexually active leopard frogs for eight consecutive months in the year. Other techniques such as the use of suction and electrocautery for producing brain lesions, and the sectioning and reconstruction of the operated brain enabled us to localize more precisely than heretofore the functionally important areas in the frog brain. Finally, the results of hours spent learning and quantifying the normal mating patterns (Noble and Aronson, 1942) proved to be an invaluable aid in interpreting the effect of brain injury upon sexual behavior.

The present study was designed to survey in a general way the effects of brain deprivations on various phases of the mating pattern. Intensive investigation of any particular phase was left for future investigation now contemplated.

ACKNOWLEDGMENTS

This study was initiated under the guidance of Dr. G. Kingsley Noble, late Curator of the Departments of Herpetology and Experimental Biology of the American Museum of Natural History and Visiting Professor of Biology of the Graduate School of New York University. Dr. Noble's inexhaustible energy, unending enthusiasm, and inspiration are gratefully remembered. Sincere thanks are due to Dr. Frank A. Beach for many valuable suggestions made during the final course of this research, and also for his very helpful criticism of the manuscript. Thanks

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LITERATURE

ROBERT BOYLE (1663), renowned physicist and natural philosopher, was among the earliest experimentalists to investigate the behavior of frogs following the extirpation or destruction of the brain. Boyle's experiments, and the studies of a number of investigators during the following century (reviewed by Eckhard, 1883), demonstrated the importance of the brain of the frog to the maintenance of various vital functions such as heart beat, respiration, and locomotion. More than a century later the great naturalist Spallanzani (1786) demonstrated that during the breeding season clasping male frogs and toads would suffer excessive mutilation of their bodies and hind limbs without releasing the females. When forcibly separated from the females, these mutilated males soon reclasped. Spallanzani decapitated clasping males, and found that they still remained in amplexus for several hours, and separated only when near death. On at least two separate occasions oviposition occurred while the decapitated frogs were coupling with normal females. These headless males fertilized the eggs, and viable tadpoles were recovered.

Flourens (1824) was the first to report the loss of all spontaneous movement resulting from forebrain extirpation, observing also that the removal of only one cerebral hemisphere caused no apparent behavioral changes. At about the same time Desmoulins (1825), who was also studying the physiology of the anuran brain, came to the opposite conclusion, namely, that the decerebrate frog still exhibits spontaneous activity. His conclusion was reached after observing the swimming activity of forebrainless animals in deep water. Here are found the beginnings of a controversy which has continued to the present day. Desmoulins also showed that the decerebrate frogs were not blind, for they could be stimulated to jump accurately through a slot in a board held obliquely in front of them.

Most of the early investigators (Patton, 1846; Vulpian, 1866; Goltz, 1869; Onimus, 1870; Steiner, 1885; Stroud, 1899) agreed with the viewpoint of Flourens. It is of interest to note that after Vulpian had extirpated the forebrains of a group of sexually active males which had just been separated from females, he observed that one of the decerebrate animals reclasped a female. He interpreted this to be the result of the female's accidentally swimming underneath the male and stimulating the male's clasp reflex, that is, a simple local reaction rather than a general response of the decerebrate male towards the female.

Goltz (1869) confirmed Spallanzani's findings relative to the tenacity of the male's clasp. He observed that the clasp reflex could be initiated by stimulation of the ventral pectoral skin and that frogs deprived of this skin area no longer clasped. Moreover, a male could be induced to clasp the experimenter's finger when it was rubbed against the pectoral skin. However, according to Goltz, a male would soon release the finger, whereas when the pectoral region was stimulated by the back of a female, a lengthy clasp usually ensued. Males deprived of the cerebral hemispheres distinguished between the experimenter's finger and a female as efficiently as an intact male; but following ablation of the entire brain, the animal attempted to embrace and continued to clasp without discrimination, any object which touched his pectoral skin. Goltz concluded that a tonic embrace of the finger is not obtained in the intact animal because the brain transmits inhibitory impulses to the clasp reflex center located in the spinal cord at the level of the brachial plexus, and at the same time the brain sends excitatory impulses to many other centers in the spinal cord.

From other experiments investigating the functions of the forebrain Goltz (1869) stated that after various types of extirpation, frogs lose "voluntary or spontaneous locomotion." Such frogs will not snap at insects, and during the breeding season decerebrate males will not pursue females. In agreement with Desmoulins (1825) Goltz reported that these forebrainless animals were not totally blind, since they avoided obstacles when prodded to leap. Likewise, the decerebrate males were still sexually active since they clasped firmly when placed on the backs of females.

In Goltz's investigation of the croaking reflex he observed that stimulation of the skin of the back at a point just caudal to the axillae readily elicited the croak. The observer's fingers or a similar smooth surface touching the skin proved to be an adequate stimulus. This reflex was produced more uniformly in decerebrate frogs than in normals, since the former never croaked unless stimulated in the above manner, whereas unoperated males sometimes croaked without any apparent stimulus. It was also demonstrated that transection through the optic lobes abolished the croak.

Finally, Goltz reported that males which had been castrated during the breeding season continued to approach and clasp females.

Bechterew (1884) and Steiner (1885) observed that removal of the midbrain roof did not interfere with the croak reflex. When the base of the optic lobes was invaded, the operates invariably croaked loudly at the moment the injury was inflicted, after which they could never again be stimulated to vocalize.

Steiner (1885) demonstrated that removal of the pallial part of the forebrain did not interfere with the so-called spontaneous behavior. He localized the mechanism for spontaneity in the subpallium.

Tarchanoff (1887) removed the heart, lungs, various parts of the liver, the intestine, stomach, kidneys, and testes in male frogs without producing any immediate slackening of the clasp; release of the female occurred only when the operated male neared death. When the seminal vesicles (caudal expanded portion of the Wolffian duct) were opened and drained, or surgically removed, the male frogs immediately lost all sexual activity, and released. Tarchanoff refilled the vesicles with water or milk and noted an immediate resumption of sexual activity. These observations led to the conclusion that afferent stimuli responsible for the sexual clasp arose from the seminal vesicles which were distended by the accumulation of semen. As a final proof, Tarchanoff ligated all peripheral nerves leading to the seminal vesicles and obtained an immediate cessation in sexual activity.

In a second investigation Tarchanoff (1887) utilized the observations of Spallanzani (1786) and Goltz (1869) to the effect that painful peripheral stimuli applied to a clasping male eventually resulted in relaxation of the clasp. Brain transection at the caudal end of the cerebral hemispheres did not affect relaxation of the clasp according to Tarchanoff. When the transections were made at the caudal level of the optic lobes, or just caudal to the cerebellum, or at the caudal end of the medulla oblongata, clasping males rarely released even after the most intense and lasting peripheral stimulation. From these observations it was concluded that impulses resulting from the painful peripheral stimulation reach the dorsal thalamus and tectum. From this point they are relayed to the spinal clasp center in the form of inhibitory impulses. To verify this hypothesis, Tarchanoff exposed the brain of a clasping male and punctured various areas with a sharp needle. Punctures of the cerebral hemispheres and medulla oblongata did not modify amplexus, but a single, properly placed puncture of the dorsal thalamus or optic lobes caused immediate relaxation of the clasp.

Using approximately the same methods, but working with toads, Albertoni (1887) arrived independently at essentially the same conclusion, namely, that the optic lobes are inhibitory centers which play upon the primary clasp center in the anterior end of the spinal cord.

In all the investigations up to 1887, the frogs were tested shortly after operation. In the experiments of Schrader (1887) ample time was allowed for the wounds to heal. The experimental frogs were induced to hibernate over the winter and were first tested the following spring. Contrary to the findings of the majority of previous investigators, Schrader observed that decerebrate frogs exhibited spontaneous activity. Thus Schrader supported the earlier contentions of Desmoulins. These decerebrate frogs snapped at food quite readily, and sought females with the same ardor as the intact animals. However, when the optic thalamus was extirpated in addition to the forebrain, a considerable reduction in spontaneous activity occurred.

To harmonize his observations with the opposing results of previous investigators, Schrader postulated that these workers must have inadvertently damaged the diencepha-

lon while removing the forebrain, and that the loss of spontaneity which they observed was in reality due to the thalamic injury. This conclusion was accepted by Fritz Edinger (1913) in his review of the physiology of the central nervous system of the frog.

Schrader contradicted Goltz's interpretation of the neural mechanism mediating the croak, holding that destruction of the optic lobes did not abolish this behavior. The medulla oblongata was designated by Schrader as the "center" for the croak reflex. Schrader placed the inhibitory or modifying "center" of the spinal clasp reflex in the anterior part of the medulla, rather than in the midbrain where the earlier authors had concluded it was situated.

Schrader's contentions were supported by Loeser (1905) who noted that decerebrate frogs readily caught flies. A week after Loeser transected the brains of male frogs just rostral to the medulla oblongata the croak reflex returned.

Steinach (1910) observed that the inhibitory center for the clasp reflex was located in the optic lobes, but he found additional inhibitory centers in the medulla oblongata, since removal of the latter caused a clasp even stronger than normal. Steinach believed that the peripheral impulses for the clasp reflex arose mainly from the stimulation of the thumb pads, and only secondarily from the pectoral skin as Goltz (1869) had reported.

Whereas Tarchanoff (1887) claimed that the internal stimuli for sexual activity arose through distension of the seminal vesicles and was a purely nervous phenomenon, Steinach (1894, 1910) found that impulses arose primarily from the testes and were hormonal. This conclusion was based upon the observation that castration reduced the clasp, and the injection of testicular substance increased this response. Steinach (1910) even thought that implanted brain tissue, particularly midbrain of the male, would strengthen the clasp.

The experiments of Busquet (1910) located the inhibitory center for the clasp reflex in the cerebellum, and this investigator attempted to demonstrate the independence of the clasp reflex from internal secretions by drawing off all the frog's blood and substituting saline solution. No reduction of the clasp was noted. Hence it was concluded that the clasp did not depend upon hormones carried in the blood stream. Busquet (1910a) observed that the clasp can be elicited any time of the year following cerebellar injury, and that the clasp mechanism is not present in females and immature males.

Burnett (1912) remarked that the decerebrate frogs which he observed exhibited a reduction in spontaneous activity. On the other hand they caught flies more readily than normals, since the intact animals generally spent their time in attempts to escape from captivity. Burnett concluded that "the reflex excitability of the decerebrate frog is heightened owing to the loss of inhibitory influences from the higher centers."

Baglioni (1913) reviewed some of the earlier literature and reported in addition some further experiments on the clasp reflex (1911). When he stimulated the optic lobes of a clasping male with a faradic current, the clasp became even stronger. When the optic lobes were narcotized by placing on them a cotton pledget soaked in "stovaine," clasping soon ceased. He therefore concluded that the optic lobes were an irritative rather than inhibitory center for the spinal clasp reflex. Baglioni hypothesized that during the breeding season the testes produced internal secretions which, by stimulating the optic lobes to produce excitatory stimuli, acted upon the spinal clasp center to cause the heightened clasp. He also drew a distinction between the sexual clasp and the clasp produced by midbrain or bulbar injury, which he believed to possess a locomotor function.

Blankenagel (1931) studied in somewhat greater detail the effects of forebrain extirpation, although not particularly in relation to sexual activities. He allowed the wounds to heal thoroughly before making his observations, and was able to refute Schrader's (1887) and Loeser's (1905) claims that decerebrate frogs do not lose their "spontaneity" of locomotion. Blankenagel localized in the subpallium the region necessary for spontaneous locomotion. He also found that decerebrate frogs did not seek food despite starvation for as long as a whole year. According to Blankenagel the region necessary for active food get-

ting is located in the posterior end of the subpallium.

Confirming an earlier observation by Goltz (1869), Blankenagel (1931) observed that the croak can be elicited more regularly in the decerebrate frog than in the intact animal. The spontaneously occurring croak heard in the ponds during the summer (which is undoubtedly the sex call as described in our account of mating behavior) was never emitted by the decerebrate male.

Diebschlag (1934) also refuted Schrader's (1887) claims relative to the loss of "spontaneity" after cerebral extirpation. He attempted to explain the discrepancy between his own findings and those of Schrader, first by noting as did Steiner (1885) and Blankenagel (1931) that if the base of the cerebrum is left intact, there will be no noticeable decrease in spontaneous activity, and second by suggesting that Schrader probably did not remove all the forebrain in the operated frogs designated as decerebrates. Whereas Blankenagel recognized in the base of the forebrain one mechanism for spontaneous activity and another for feeding, Diebschlag considered the irresponsiveness of the decerebrate frog towards food simply as a manifestation of the loss of "spontaneity."

Turning to sexual activities, Diebschlag reported that the ability to recognize the female as a result of a specific tactile stimulus is absent in the decerebrate male.

Recent preliminary findings by Noble and Kelman (unpublished) indicate that the spawning movements of the male frog during oviposition appear to be entirely normal after total forebrain extirpation. Fertile eggs may be recovered after an oviposition of a normal female when clasped by a forebrainless male.

To summarize the results of the above investigations, the following major generalizations appear to be warranted despite numerous contradictions:

1. There appears to be a reduction in "spontaneous activity" after forebrain deprivation, but this change is not evident if the base of the forebrain remains intact. Non-responsiveness to food and to the female appear to be manifestations of this reduction in "spontaneity."

2. A neural mechanism essential to the occurrence of the clasp (primary clasp center) is located at the rostral end of the spinal cord.

3. The activity of the primary clasp center is modified by secondary clasp centers located possibly in the thalamus, midbrain, cerebellum, and/or medulla oblongata.

4. Following extensive midbrain lesions, frogs do not croak. The mechanism for vocalization appears to be located in the base of the tectum.

5. Forebrain deprivation does not modify the spawning movements of the male during oviposition.

The relationship of the nervous system and sexual behavior in other vertebrates will not be considered here since a number of reviews of this literature have been published in recent years (Stone, 1939; Bard, 1940; Young, 1941; Beach, 1942; 1942a; 1944).

MATERIALS AND METHODS

THE READER IS REFERRED to an earlier paper by Noble and Aronson (1942) for the methods of maintaining the intact frogs and for obtaining estrous (ovulated) females. Operated males were isolated in eight-liter aquaria containing one-half centimeter of water which was changed daily. A flat stone at one end of each aquarium provided a dry area.

OPERATIVE PROCEDURES

Four operative techniques were used to remove various regions of the brain, namely, low pressure suction (using an ordinary faucet aspirator), high pressure suction (approximately 10 gr./sq. cm.), electrocautery, and combinations of the above. Two types of operations were performed, extirpations and transections. With one exception, which will be noted later, suction was used for the transections, and to secure complete separation a segment at least 1 to 2 mm. in thickness was removed anterior to the level to be studied.

Bleeding was minimized in many cases by applying a 1:100 solution of adrenalin chloride. Where large amounts of brain tissue had been removed, soft bone wax was used to fill the cranial cavity.

In the first group of experiments, the males were anaesthetized with a 3 per cent solution of urethane. Immediately following the operation they were injected with one or two anterior pituitary glands from females, and tests were started on the following day. Throughout the period of observation each operate was injected daily with one or two pituitaries. In the subsequent experiments it was found more expedient to start the pituitary treatment the day preceding the operation. The surgery was then performed without anaesthesia, and tests were begun one to two hours after the operation.

The dorsal approach was used for the majority of the operations. The skin was incised longitudinally along the midline and tied laterally with hooks. Next, the frontoparietal bone was carefully chipped away under a dissection microscope with a fine pair of scissors. Special care was taken to avoid tearing the laterally situated orbitonasal arteries. When it was necessary to expose the diencephalon, some bleeding usually occurred. During operations in which bleeding was marked, low pressure suction was used to keep the field clear while the bone was being removed as rapidly as possible. With the suction still clearing the field of blood, the desired lesion was made, after which the bleeding was controlled by cotton pledgets, adrenalin, or bone wax as previously described.

Lesions in the preoptic area, hypothalamus, and tegmentum were accomplished by means of a ventral approach. With the frog securely tied on its back, and its mouth clamped widely open, a median longitudinal incision was made in the mucous membrane lining the roof of the oral cavity. The optic chiasma which was visible through the semitransparent os parabasalis was used as a landmark. The desired region of the parabasal bone was chipped away with a fine pair of scissors, and the underlying chondrocranium was removed with the same instrument. In removing the preoptic area attempts were made (which were usually, but not always, successful) to avoid severing the laterally overlying anterior rami of the cerebral carotid arteries (Gaupp, 1899). The pituitary injections mentioned above were used to increase the sexual activity of the operated frogs, since it has been shown that without such treatment only one-third of the males in captivity are sufficiently stimulated to clasp estrous females (Noble and Aronson, 1942).

OBSERVATIONS OF BEHAVIOR

As already stated, the observations were begun within one hour to one day after the operation. In most instances the tests were completed within one week, after which the operates were killed by decapitation. Many of the operates with very extensive lesions died before all the desired tests had been completed. The procedures used, together with other details of method, are given in the descriptions of the various experiments which follow.

DETERMINATION OF THE EXTENT OF THE BRAIN INJURY

The heads were fixed in 10 per cent formalin, after which the brains were removed and the blood clots and meninges carefully

dissected away. Both the dorsal and ventral aspects of the brains were photographed, and in addition a sketch and written description of the gross aspects of the lesion were made. The brains were embedded in paraffin, sectioned transversely at 15 μ and stained with gallocyanin (Einarson, 1932).

For control and general reference, an average size *Rana pipiens* brain was similarly treated. Every seventh or eighth section from the olfactory bulbs to the medulla oblongata was projected, and outline diagrams were drawn indicating the nuclear masses and other outstanding landmarks. These sketches were reduced, assembled, duplicated, and employed as standard charts upon which the lesion in each operated brain (as determined by microscopic examination) was plotted.

The description of the topography of the normal *Rana pipiens* brain, the lesion in each operated brain, and the drawings of the normal and experimental brains were made in part from these sketches.

RÉSUMÉ OF THE NORMAL MATING PATTERN

IN THE FOLLOWING BRIEF OUTLINE of the sexual behavior of *Rana pipiens*, emphasis is placed on those phases of the male's behavior which were tested after brain deprivations. For further details, the reader is referred to the description of the normal mating pattern by Noble and Aronson (1942).

In the spring when the frogs are in the breeding ponds, the croak most frequently heard is known as the sex call. It can be represented phonetically as an "ir-a-a-a---a-a-h," starting softly, and gradually growing louder as the vocal sacs become inflated. In captivity the sex call is sounded only occasionally.

When the back of the male is touched at a point just caudal to the axillae, a vocalization is generally emitted which is known as the warning croak. It can be described as "ir-a-a-a-h----ir-a-a-h---ir-a-a-h---," etc., which continues as long as the stimulation of the back is maintained. The anestrous female also emits a warning croak when her back is touched, but the female's warning vocalization is not so loud as that of the male. When one male attempts to clasp another male or an anestrous female, the frog being clasped emits the warning croak as soon as its back is touched, and this response is partially responsible for the clasping male's prompt release. This is one of two major factors which prevent males from remaining in amplexus with other males or with anestrous females. The second factor is the relative girths of the slender male and spent female as contrasted to the swollen size of the egg-laden estrous female. The larger the girth of the clasp object (within certain limits) the more likely is the clasping male to retain his clasp.

The sexually active male attempts to clasp a male, female, or any object of the approximate size and shape of a frog. There is little discrimination in these attempts among males, anestrous females, estrous females, or pairs. If an estrous female is clasped, amplexus is maintained until the oviposition is completed. Amplexus is pectoral, with the ventral surface of the male pressed closely against the back of the female. The male's back is slightly arched convexly, and his lower extremities remain tightly flexed. The male remains in this position until the spawning begins. Within a few minutes to several hours after the onset of amplexus, the female exhibits peculiar backward movements which we have termed "backward shuffling." Alternate periods of backward shuffling, rest, and swimming activity occur. As the time for the spawning approaches, the female assumes the oviposition posture. Her thighs extend laterally at a 45° angle, and the shanks are turned inward at the same angle, forming a diamond-shaped enclosure in which the eggs are deposited.

As the female assumes the oviposition posture, the male moves forward slightly and rotates the shanks of his hind limbs somewhat downward and inward.

The female starts the oviposition by a sharp abdominal contraction followed by a concave arching of her back. The male responds to these movements by spreading his legs slightly and arching his back convexly (upstroke), followed by a down stroke in which he straightens out his arched back and presses his legs against the female's abdominal walls. We have termed this complex series of motions of the male an "ejaculatory" or "spawning" movement. With each of these ejaculatory movements, a cluster of eggs is emitted from the female's cloaca and sperm are ejected from the male's cloaca. There are on the average 16 spawning movements during each egg laying, but in individual cases the total may vary from 10 to 23. The mean duration of the oviposition is about four minutes, with a range of two to eight minutes.

After all the eggs are deposited, the male usually swings his hind limbs from side to side (pre-release motions) and finally releases the female on one or the other side. After the spawning is terminated and the female abandons the egg-laying posture, she generally moves away from the eggs. In most cases the male's release of the female occurs within one minute after the end of oviposition.

MAJOR NUCLEAR MASSES OF THE FROG BRAIN

THE HISTOLOGICAL DETAILS of the anuran brain have been described by a large number of investigators, and two of these studies (Herrick, 1921, 1925) were based partly on *Rana pipiens* material. However, the information is quite scattered, the terminology employed by the various investigators varies considerably, and the illustrations are not particularly suitable for the present study. For these reasons it has been considered advisable to present a brief review of the major nuclear masses in the forebrain, diencephalon, and midbrain of the leopard frog.

In the following descriptions we have attempted to adopt the most widely accepted terminology. In general it follows that of Herrick (1933) and of Ariëns Kappers, Huber, and Crosby (1936). The descriptions and illustrations are based on a cross-section series cut at 15 μ and stained with gallocyanin (Einarson, 1932).

The reader is referred to the two volumes by Ariëns Kappers, Huber, and Crosby (1936) for a more detailed discussion of the anuran brain and for an excellent review of the literature.

The forebrain consists of two cerebral hemispheres and an unevaginated telencephalon medium. At the frontal end of each cerebral hemisphere are situated the olfactory bulbs which can be delimited from the remaining hemispheres by a sulcus limitans bulbi olfactorii.

The cerebral hemispheres are divided through most of their rostrocaudal extent into a dorsally situated pallium and a ventral subpallium by cell-free medial and lateral zona limitantes (figs. 1 to 3, z. lim. m. and z. lim. l.) and their corresponding ventricular and external sulci (figs. 1 to 3, s. lim. hip., s. endorh., s. sep. pall., and s. lim. lat.). The medial segment of the pallium is known as the primordium hippocampi (figs. 1 to 5, prim. hip.), the lateral segment as primordium piriforme (figs. 1 to 5, prim. pir.). Dorsally there is situated the primordium palli dorsalis (figs. 1 to 5, prim. pall. d.). In *Rana pipiens* this condensation of cells cannot be sharply delimited from the primordium hippocampi medially, or the primordium piriforme laterally.

The septum comprises the medial sector of subpallium and is readily divisible into medial and lateral septal nuclei (figs. 1 and 2, n. sep. m., n. sep. l.). The extension of the septum caudal to the interventricular foramen is known as the pars fimbrialis septi (fig. 3, fim. sep.).

The lateral sector of the subpallium has been subdivided in two different ways. Röthig (1912), Ariëns Kappers and Hammer (1918), Ariëns Kappers (1921), and others recognize a paleostriatum and a more dorsally situated epistriatum. Herrick (1921) following the lead of Gaupp (1899) considers the rostral part of the lateral sector of the

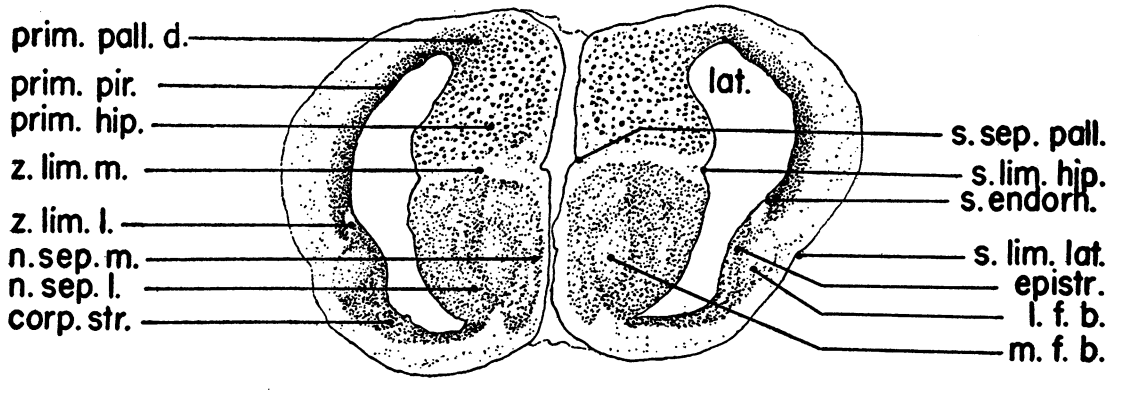

FIG. 1. Transverse section through the middle of the cerebral hemispheres of *Rana pipiens;* gallocyanin; ×24. (All sections cut at 15μ.)

subpallium to be the primordium of the corpus striatum, while the more caudally lying cell group at the level of the interventricular foramen is designated as the homologue of the mammalian amygdala. However, Herrick (1921) does carry a few strands of cells of the corpus striatum to the same caudal level. Herrick (1933, p. 164) points out that his subdivisions of the ventrolateral quadrant of the hemisphere overlap those of Ariëns Kappers. However, it seems possible from a purely topographical point of view to recognize three more or less discrete areas, namely, a rostrally lying corpus striatum or paleostriatum (figs. 1 and 2, corp. str.), a dorsal epistriatum (figs. 1 and 2, epistr.), and a caudal amygdala (fig. 3, amyg.).

The medial forebrain bundle (figs. 1 to 6, m. f. b.), the lateral forebrain bundle (figs. 1 to 6, l. f. b.), and three major components of the anterior commissure can be clearly recognized in gallocyanin-stained material. The major subdivisions of the anterior commissure are (1) the hippocampal commissure (fig. 3, hip. com.), (2) the decussation of the medial forebrain bundle (figs. 2 and 3, dec. m. f. b.), and (3) the decussation of the lateral forebrain bundle (fig. 3, dec. l. f. b.). Associated with each of the two decussations just mentioned are condensations of cells known as the bed nuclei of the respective decussations (figs. 2 and 3, n. dec. m. f. b., n. dec. l. f. b.). The small masses of cells, oval in cross section, are known as the bed nuclei of the hippocampal commissure (figs. 3 and 4, n. hip. com.).

The ventral aspect of the diencephalic-telencephalic boundary is not clearly defined. Some authors consider the preoptic area to be part of the telencephalon, and place the ventral di-telencephalic boundary at the dorsal ridge of the chiasma. Others regard this region as diencephalic, placing the ventral di-telencephalic boundary at the anterior commissure. In the present report we shall follow Herrick (1933) and place the preoptic area "somewhat arbitrarily" in the telencephalon, bearing in mind, as Herrick does, the functional relationship of the preoptic area with the diencephalon.

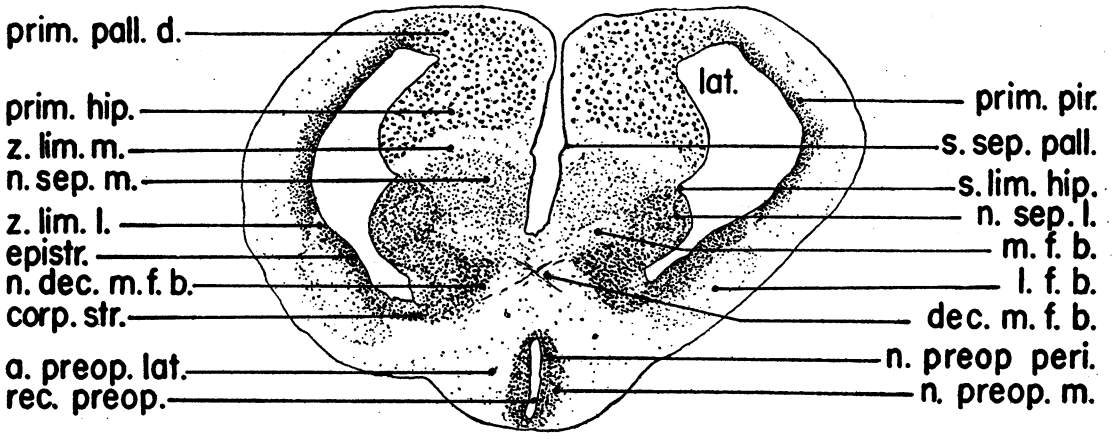

FIG. 2. Transverse section through the anterior part of the preoptic area of *Rana pipiens;* gallocyanin; ×24.

The structure of the preoptic area of *Rana pipiens* follows the description of Crosby and Woodburne (1940) for *Rana catesbeiana*. The condensation of cells surrounding the preoptic recess (rec. preop.) is known as the nucleus preopticus and is composed of three subdivisions, namely, a periventricular part (figs. 2 and 3, n. preop. peri.), a medial part (figs. 2 and 3, n. preop. m.), and a magnocellular part (fig. 3, n. preop. mag.). In *Rana pipiens* the pars magnocellularis is markedly reduced both in size and differentiation when compared with *Rana catesbeiana* or *R. clamitans*. Its cells are only slightly larger than the

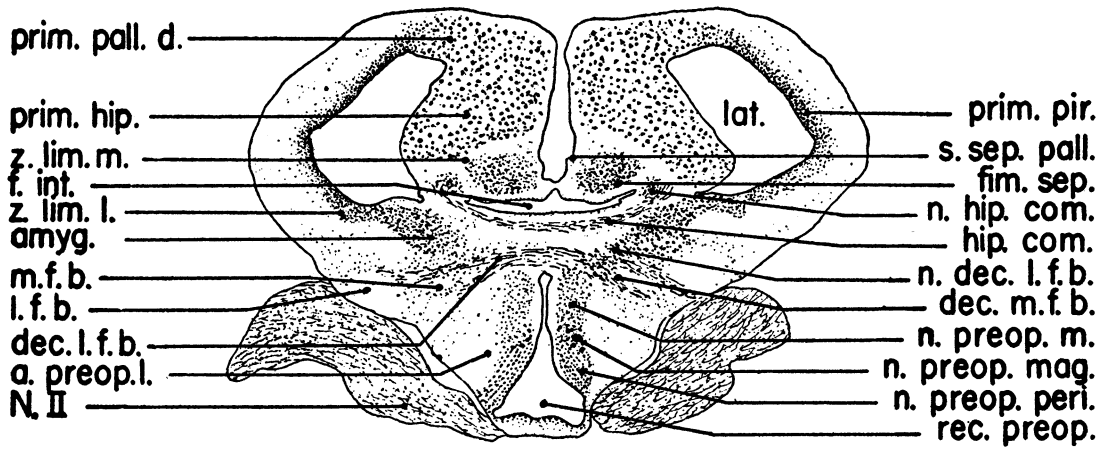

FIG. 3. Transverse section through the anterior commissure of *Rana pipiens;* gallocyanin; ×24.

adjacent cells of the nucleus preopticus periventricularis and nucleus preopticus medialis. Following Crosby and Woodburne (1940), we have designated the fibrous area and scattered cells lateral to the preoptic nuclei as the lateral preoptic area (figs. 2 and 3, a. preop. l. or a. preop. lat.).

It should be noted that the preoptic nucleus extends caudally over the chiasma to its dorsal tip where the nucleus preopticus merges with the nucleus suprachiasmaticus of the hypothalamus. In the experiments here reported, we have considered this edge of the preoptic area between the anterior and dorsal edges of the chiasma as a transitional zone between telencephalon and diencephalon. When, in our experiments, the preoptic area was ablated from its anterior edge as far caudally as the rostral edge of the chiasma, we classified the destruction as a complete ablation of the preoptic area.

The diencephalon is divisible into four major components, namely, the epithalamus, the dorsal thalamus, the ventral or subthalamus, and the hypothalamus. In cross sections, the limits of these regions are clearly marked by ventricular sulci (figs. 5 to 7, s. subhab., s. med., s. vent.).

The epithalamus consists of the pineal stalk and associated structures, the habenular commissure (fig. 6, hab. com.), the habenular nucleus (fig. 6, n. hab.), and the sub-

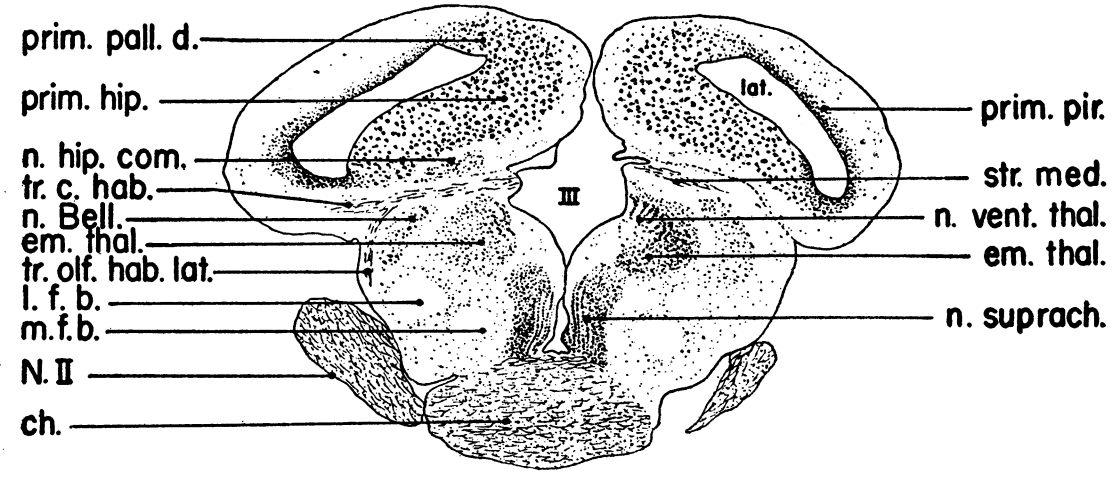

FIG. 4. Transverse section through the emenentia thalami of *Rana pipiens;* gallocyanin; ×24.

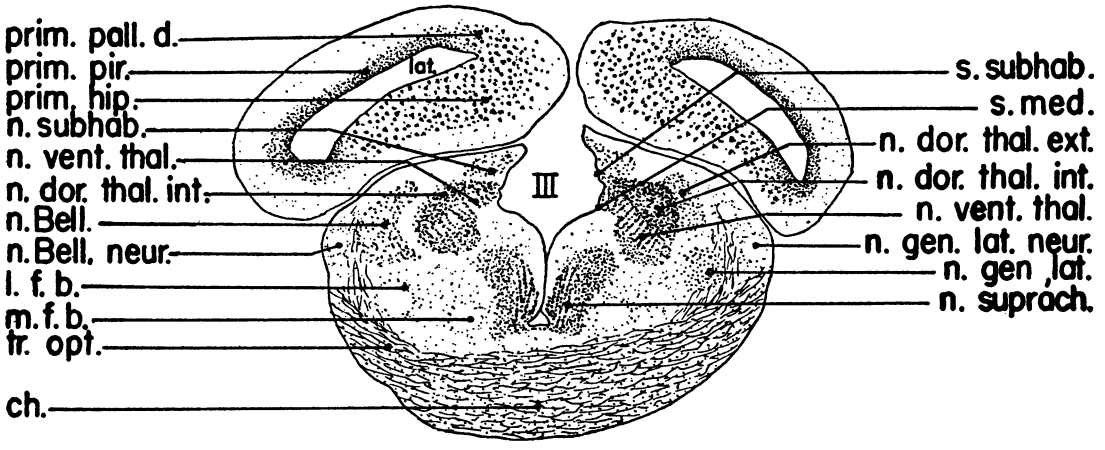

FIG. 5. Transverse section through the anterior part of the diencephalon of *Rana pipiens;* gallocyanin; ×24.

habenular nucleus (figs. 5 and 6, n. subhab.).

The dorsal thalamus is divisible into anterior, middle, and posterior portions. The anterior portion contains the nucleus of Bellonci (figs. 4 and 5, n. Bell.), the nucleus geniculatus lateralis (fig. 5, n. gen. lat.), and two concentrations of neuropil associated with the foregoing nuclei (fig. 5, n. Bell. neur., n. gen. lat. neur.). These collections of neuropil are faintly seen in sections stained for cellular detail. The boundary between the middle portion (figs. 5 and 6) and posterior portion of the dorsal thalamus (fig. 7) is not easily recognized in transverse sections. The cellular concentrations in the middle and posterior portions are easily separated into a pars interna (figs. 5 to 7, n. dor. thal. int.) in which the cells are arranged in a somewhat linear fashion, and a more scattered pars externa (figs. 5 to 7, n. dor. thal. ext.). At the caudal end of the dorsal thalamus a pretectal area (fig. 8, a. pret.) and a nucleus of the posterior commissure (fig. 9, n. post. com.) can be identified.

The emenentia thalami (fig. 4, em. thal.) which is the most rostral portion of the diencephalon is recognized as an anterior extension of the ventral or subthalamus. The cellular concentrations of the subthalamus are also divisible into a linearly arranged pars

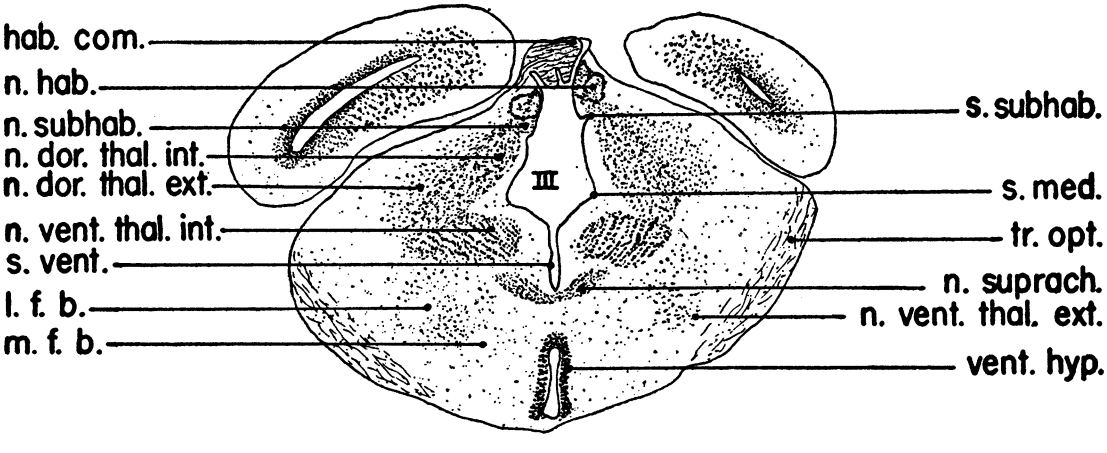

FIG. 6. Transverse section through the middle of the diencephalon of *Rana pipiens;* gallocyanin; ×24.

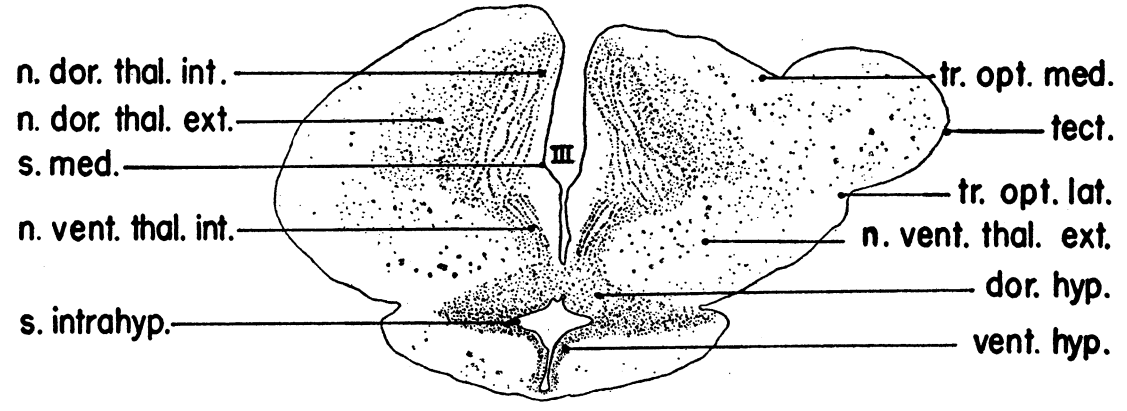

FIG. 7. Transverse section through the posterior part of the diencephalon of *Rana pipiens;* gallocyanin; ×24.

interna (figs. 6 and 7, n. vent. thal. int.) and a scattered pars externa (figs. 6 and 7, n. vent. thal. ext.).

The most anterior component of the hypothalamus is the nucleus suprachiasmaticus (figs. 4 to 6, n. suprach.). As already stated, this nucleus is continuous rostrally with the preoptic nucleus. The nucleus suprachiasmaticus is replaced caudally by the pars ventralis hypothalami (figs. 6 to 8, vent. hyp.). Farther caudally, the pars dorsalis hypothalami (fig. 7, dor. hyp.) intervenes between the ventral thalamus and the pars ventralis hypothalami and is separated from the latter by the intrahypothalamic sulcus (fig. 7, s. intrahyp.).

Crosby and Woodburne (1940) recognize a number of subdivisions of these hypothalamic areas in *Rana catesbeiana*. Since these components are not easily discernible in *Rana pipiens*, they need not be considered further.

The mesencephalon consists of the tectum opticum or superior colliculi (figs. 7 to 12, tect.), the inferior colliculi or tori semicirculares (figs. 10 to 12, inf. col.), and ventrally the tegmentum (figs. 8 to 12, teg.).

Huber and Crosby (1933, 1933a) recognize six fundamental layers in the vertebrate tec-

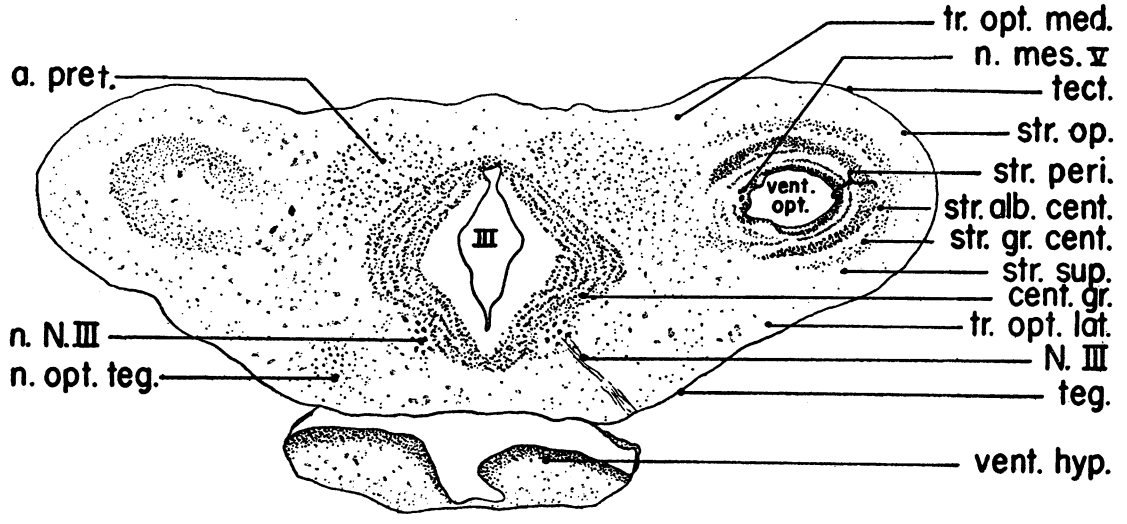

FIG. 8. Transverse section through the anterior end of the tectum mesencephalicum of *Rana pipiens;* gallocyanin; ×24.

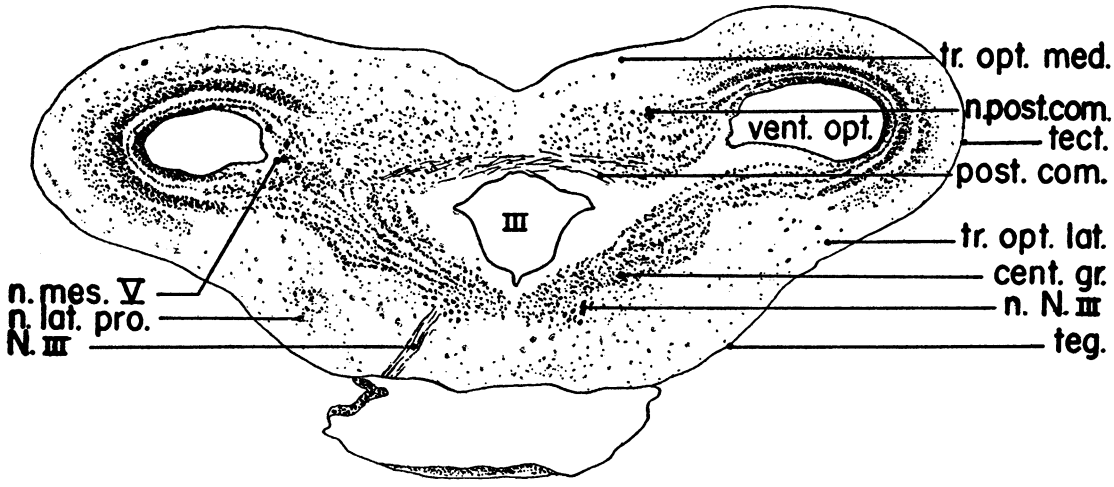

FIG. 9. Transverse section through the posterior commissure of *Rana pipiens;* gallocyanin; ×24.

tum. In the tailless Amphibia, according to Ariëns Kappers, Huber, and Crosby (1936) there are recognized (labeled in fig. 8): (1) stratum opticum (str. op.), (2) stratum fibrosum et griseum superficiale (str. sup.), (3) stratum griseum centrale (str. gr. cent.), (4) stratum album centrale (str. alb. cent.), (5 and 6) stratum griseum periventriculare intermingled with fibers of the stratum fibrosum periventriculare (str. peri.). Towards the anterior end of the tectum the large scattered cells of the nucleus mesencephalicus trigeminus (figs. 8 and 9, n. mes. V) are recognized.

Caudal to the inferior colliculi, the ganglion isthmi (fig. 12, gang. isth.) is easily identified by its border of deeply staining cells. In the tegmentum, the large cells of the oculomotor nucleus (figs. 8 and 9, n. N. III) can be delimited from the similarly appearing cells of the trochlear nucleus (fig. 10, n. N. IV) by a region relatively free of large motor cells. At the anterior level of the oculomotor nucleus, the nucleus opticus tegmenti (fig. 8, n. op.

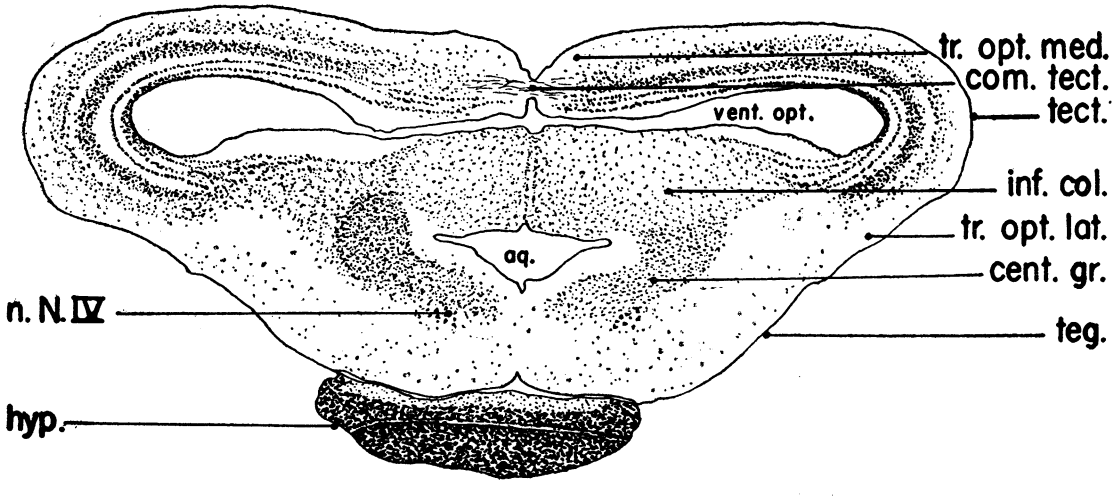

FIG. 10. Transverse section through the anterior part of the inferior colliculus of *Rana pipiens;* gallocyanin; ×24.

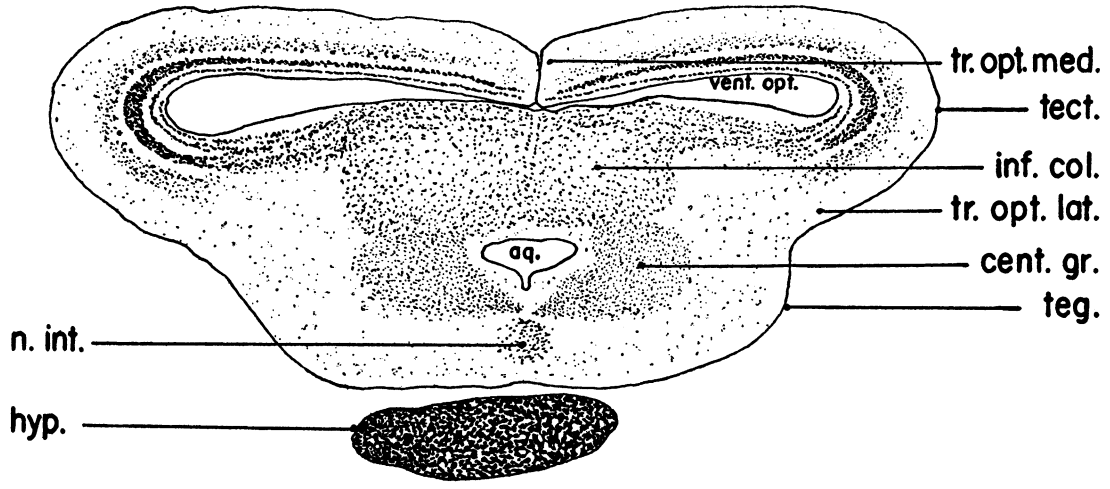

FIG. 11. Transverse section through the interpeduncular nucleus of *Rana pipiens;* gallocyanin; ×24.

teg.) can be identified. Farther caudally, the nucleus lateralis profundus (fig. 9, n. lat. pro.) is seen to be embedded in the fiber bundles of the cerebral peduncles. The interpeduncular nucleus (fig. 11, n. int.) is situated along the midline near the caudal end of the tegmentum.

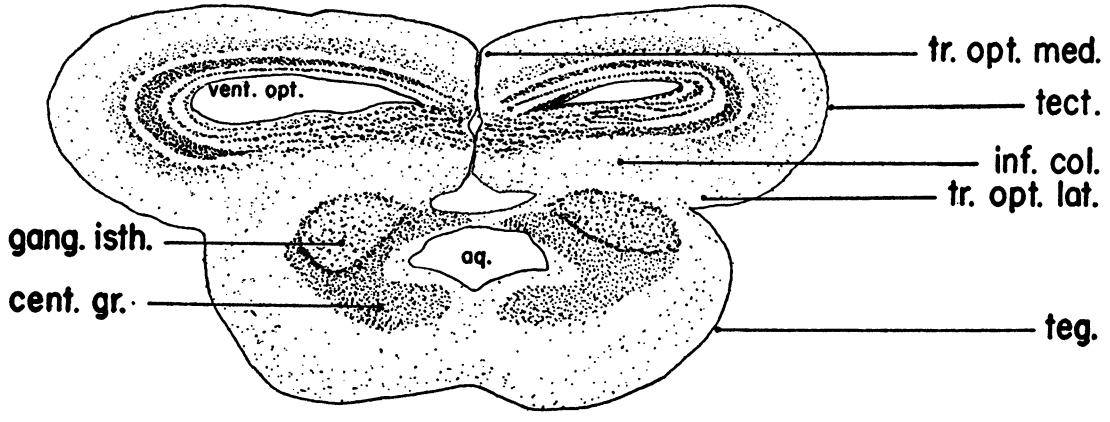

FIG. 12. Transverse section through the ganglia isthmi of *Rana pipiens;* gallocyanin; ×24.

EXPERIMENTAL PROCEDURES AND RESULTS

SWIMMING RESPONSE OF THE MALE TO THE FEMALE

THE MALE FROG'S TENDENCY to swim towards and attempt to embrace a female was tested in aquaria 30 cm. by 40 cm. by 25 cm. filled with 10 cm. of water at approximately 23° C. Estrous (fully ovulated) females, to be used as clasp objects, were obtained by injecting the females two or three days prior to the test with two female pituitaries. These females were placed at random in the test tanks with the experimental males.

The control males were injected with a female pituitary one day before being tested. Most of the experimental males received the pituitary treatment on the day preceding the operation, and tests were started approximately one hour after the surgical treatment. A few of the males received the pituitary injection and were operated upon the day before the observations began.

The controls (intact males) were given a two-hour test with the estrous females. If they showed the response by swimming over to the female and clasping her, they were forcibly separated after two to five minutes and were retested. The operated frogs were similarly tested for two hours. In most cases, if they did not show the reaction, the test was continued for an additional period, generally two more hours. In some instances when the operates did not show the response, they were given a second pituitary injection and were retested on the following day. With few exceptions, those that did not react during the first two hours failed to exhibit the response during the additional time allowed them.

Table 1 summarizes the results of this experiment. The percentages of males exhib-

TABLE 1

EFFECTS OF BRAIN INJURY UPON THE SWIMMING RESPONSE OF OPERATED MALES TO OVULATED FEMALES

Operation	No. of Males Tested	No. of Males Exhibiting Swimming Response[1]	Percentage of Males Exhibiting Swimming Response	Record Numbers of Operated Males[2]	
				Response Exhibited	Response Not Exhibited
Intact males (controls)	24	23	95.9		
Extensive injury to forebrain. Preoptic areas intact	11	10	90.9	63, 65, 67, 742, 813, 910, 915, 929, 935, 942	75
Forebrain ablated, excepting the preoptic area	10	10	100.0	2, 8, 12, 51, 58, 68, 70, 79, 92, 125	
Forebrain ablated excepting caudal three-fourths to one-quarter of the preoptic area	13	7	53.8	32, 48, 60, 89, 104, 105, 120, 939	78, 82, 95, 96, 98, 120
Forebrain completely ablated (including from 75% to 100% of the preoptic area)	19	5	26.3	52, 119, 121, 127, 140	46, 49, 55, 72, 73, 76, 77, 83, 84, 94, 103, 106, 115, 155
Preoptic area ablated or extensively injured	12	10	83.3	11, 16, 17, 62, 215, 918, 920–923	74, 80
Hypothalamus mostly ablated	9	7	77.8	85, 88, 93, 97, 831, 906, 924	86, 91

[1] At least once during the test period.

[2] For descriptions of the individual operations and summaries of the behavioral data, see page 117.

iting the swimming reaction after (1) extensive injury to the forebrain, leaving the preoptic area intact; (2) complete ablation of the forebrain with the exception of the preoptic area; and (3) ablation or extensive injury to the preoptic area, leaving the rest of the forebrain intact, did not differ significantly from the controls. The per cent differences between the controls and operates are less than twice the standard error.

When the forebrain was ablated, including from 75 per cent to all the preoptic area, only 26.3 per cent of the males responded, the deviation from the record of the controls being clearly significant. When the forebrain was extirpated with the exception of the caudal three-quarters to one-quarter of the preoptic area, 53.8 per cent of the operates showed the response. This difference is probably reliable since it is greater than two and one-half times the standard error. This group consisted of operates which were intermediate as regards the amount of preoptic area removed with the cerebral hemispheres; the percentage of males exhibiting the swimming response also fell into intermediate position.

Following complete removal of the hypothalamus, and after complete ablation or extensive injury to the preoptic area, the percentages of males that reacted to the estrous females did not differ significantly from the controls (less than twice the standard error).

The conclusion which is drawn from these data is that complete loss of the forebrain (including the preoptic area) causes a considerable reduction in the number of males that will swim towards and clasp the estrous female. If, on the other hand, the cerebral hemispheres alone are extirpated (leaving the preoptic area intact) or the preoptic area is removed (leaving the cerebral hemispheres intact), a normal swimming reaction is exhibited. Finally, loss of most of the hypothalamus does not appear to affect the swimming response.

Removal of the preoptic area in addition to the cerebral hemispheres often resulted in some injury to the anterior edges of the dorsal thalamus, ventral thalamus, and epithalamus. However, we were unable to correlate the loss of the swimming reaction with the extent of the diencephalic injury. It should be understood that this does not eliminate the possibility that injury to the thalamus or epithalamus might affect the swimming response adversely.

On the other hand, in those operations in which the preoptic area was spared, remnants of the cerebral hemispheres and varying

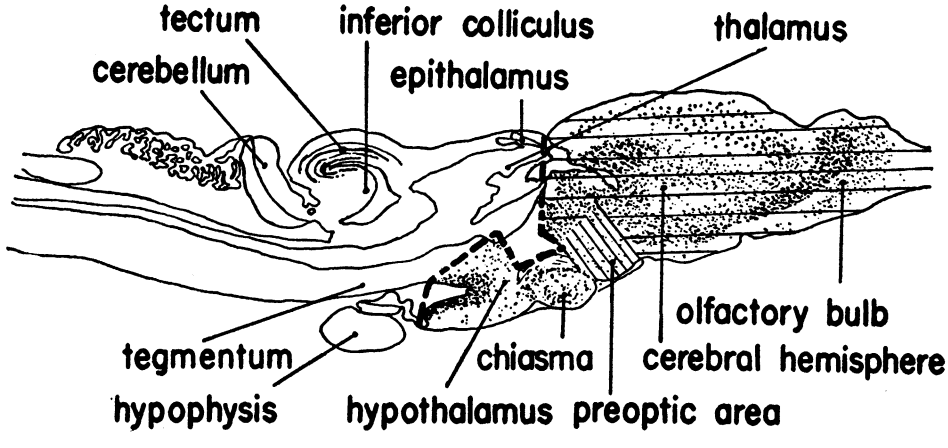

FIG. 13. Diagrammatic sagittal section through the brain of *Rana pipiens*. The regions situated anterior to the heavy broken line were explored while studying the swimming reaction of the male towards the estrous female. Either the preoptic area (delimited by oblique cross hatching) or the remainder of the cerebral hemispheres (delimited by horizontal cross hatching) must remain intact if this behavior is to be elicited readily.

amounts of the anterior commissure and related bed nuclei sometimes remained. In these cases no relation was found between the extent of the remaining cerebral tissue and the swimming reponse to the estrous female. Whether the presence of the anterior commissure or slight cerebral remnants, or invasion of the diencephalon alters the swimming reaction will have to be determined by further experimentation.

Figure 13 illustrates the areas of the brain that were explored in this experiment (anterior to the heavy broken line) and the portions that appear to be essential for the mediation of the swimming reaction of the male towards the estrous female. Either the

TABLE 2

EFFECTS OF BRAIN INJURY UPON THE WARNING CROAK OF THE MALE

Operation	Males Tested	No. of Males Responding	Percentage of Males Responding	Record Numbers of Operated Males[1]	
				Response Exhibited	Response Not Exhibited
Intact males (controls)	25	25	100.0		
Forebrain ablated	20	20	100.0	20, 24, 34, 46, 47, 49, 50, 52, 103, 106, 115, 121, 123, 126–128, 135, 140, 143, 322	
Hypothalamus mostly ablated	8	8	100.0	85, 97, 344, 403, 831, 845, 924, 945	
Forebrain and thalamus extirpated	10	9	90.0	53, 54, 108, 341, 412, 413, 422, 741, 911	402
Thalamus ablated, plus extensive injury to posterior third of forebrain	11	11	100.0	100, 112, 114, 116–118, 324, 331, 743, 805, 815	
Forebrain and diencephalon ablated. Tecta largely ablated; tegmentum invaded; slight injury to inferior colliculi	2	2	100.0	411, 433	
Extensive injury to tecta. Slight invasion of inferior colliculi. Two operates included ablation of cerebellum	3	3	100.0	212, 304, 333	
Extensive injury to tegmentum. Three included ablation of hypothalamus	4	3	75.0	724, 832, 928	704
Forebrain and diencephalon ablated. Tecta and inferior colliculi ablated or mostly ablated. Lesions extended into tegmentum. Two operates included ablation of cerebellum	13	0	0.0		242, 414, 424, 431, 441, 443, 521, 531, 532, 712, 722, 723, 731
Tecta and inferior colliculi largely ablated. Three operates included damage to thalamus. Two included ablation of cerebellum	11	1	9.1	241	45, 101, 214, 233, 311–314, 321, 903
Extensive lesions in hypothalamus, inferior colliculi, and tecta	4	0	0.0		711, 714, 721, 733

[1] For descriptions of the individual operations and summaries of the behavioral data, see page 117.

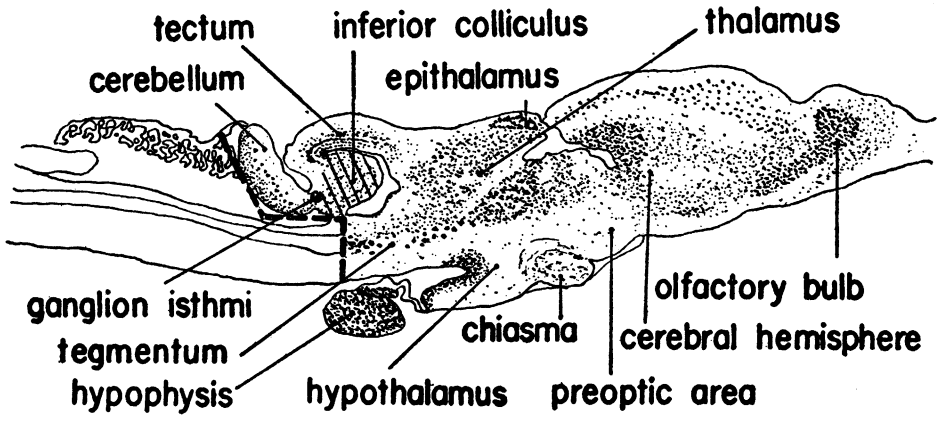

FIG. 14. Diagrammatic sagittal section through the brain of *Rana pipiens*. The regions situated anterior to the heavy broken line were explored while studying the warning croak. The cross-hatched area delimits the inferior colliculi which must remain intact if this behavior is to be elicited.

cerebral hemispheres (horizontal cross-hatch) or the preoptic area (oblique cross-hatch) must remain intact if this response is to be elicited readily.

EMISSION OF THE WARNING CROAK

The warning croak was elicited readily by touching the back of the male with the thumb and forefinger at a point just caudal to the axillae. In the control group, the response occurred an average of 1.6 seconds after the application of the stimulus, and all the 25 control males responded within seven seconds.

In testing the operated males for the warning croak, each animal was stimulated continuously for at least five minutes. Frogs that failed to croak were given a brief rest period, and were then retested at least once more.

The results of this experiment are summarized in table 2. Examination of this table reveals that complete forebrain and diencephalic extirpation did not interfere with the response. Similarly, after extensive injury or ablation of the tectum and cerebellum, the response could still be obtained, even when the lesions extended a considerable distance into the tegmentum. Slight injury to the inferior colliculi (tori semicirculares) did not interrupt the warning croak. However, with only one exception, operations involving extensive injury to the inferior colliculi abolished this response completely.

In figure 14 the cross-hatched area represents the region of the frog brain which appears to be necessary for the mediation of the warning croak. The regions that were explored in this study are anterior to the heavy broken line.

The record of operated male 433 (fig. 23) illustrates the persistence of the warning croak following a transection through the midbrain at a level just rostral to the nuclei of the oculomotor nerve with the tecta completely ablated, but with only slight damage to the inferior colliculi. The performance of male 314 (fig. 21) exemplifies the abolition of the warning croak following the removal of most of the tecta and the entire inferior colliculi. Results obtained with male 928 (fig. 26) are an example of extensive hypothalamic and tegmental injury which did not interrupt the warning croak. The operation of male 901 (not included in table 2) consisted of a midsagittal incision from the medulla oblongata to the dorsal thalamus. This lesion did not abolish the warning croak. In operated male 711, the destruction of the inferior colliculus on one side only was sufficient to abolish the warning croak.

SEX CALL

When *Rana pipiens* are kept in captivity, very few males emit the sex call, although this limited group may call repeatedly over a period of several hours. In our experiments

homoplastic pituitary injections increased the proportion of calling males, but the group still appeared to be too small to warrant quantitative records of the sex call following brain injury. However, some operated males were heard to sound the sex call, and whenever the individual could be definitely identified, the call was recorded. These data appear to be of sufficient interest to be reported here.

The sex call was heard following various lesions of the forebrain (males 1, 2, 12, 41, 51, 67, 70, 125, 302, 813, 816, 923, 929, and 936), after complete ablation of the forebrain (male 115), and after partial or complete hypothalamic injury (males 403, 906, 924, 930, 941, 946). The call was also sounded by males suffering various diencephalic and midbrain injuries which did not reach the inferior colliculi (males 109, 212, 801, 815, 822).

Operated male 233 (fig. 20) is a particularly interesting case. This male could not be stimulated to sound the warning croak even after numerous repetitions of the test, yet for two successive days he repeatedly emitted the sex call. Examination of the brain revealed complete ablation of the tecta and inferior colliculi with the ganglia isthmi, cerebellum, and adjacent structures remaining uninjured.

While no definite conclusion can be drawn from these data, it does appear that the essential mechanism controlling the sex call is located somewhere in the midbrain. By the process of elimination it is suggested that the ganglia isthmi might form an essential link in this mechanism.

CLASP REFLEX

Although quantitative measurements of the clasp reflex were not taken, qualitative observations of the effects of various invasions and transections of the brain on this behavior were noted. In the intact frog, the reflex can be demonstrated in two ways. First, if the pectoral skin and ventral surfaces of the frog's forelimbs are gently stroked with the fingers, the frog will clasp the fingers and will continue to do so as long as the stroking continues. Secondly, if the experimenter grasps a male by the hind limbs and places it on the back of an estrous female, the clasp will occur. If the frog thus manipulated is a sexually active male, it will arch its back, adjust itself squarely on the back of the female, and remain clasping with a powerful grip. If, on the other hand, the male being tested is not sexually active, the clasp reflex will cease as soon as the experimenter releases the test animal.

Females exhibit this clasping behavior, but the muscular complex involved is considerably weaker than in the male, thus accounting for a relatively feeble grip. Occasionally females are seen clasping other females (Noble and Aronson, 1942). Males exhibit the clasp reflex throughout the year, but it is much weaker immediately after the breeding season. Again, this seems to be due, at least in part, to a reduction of the brachial muscular apparatus.

Males in which the brains had been transected at the level of the cerebellum or anterior end of the medulla oblongata exhibited a clasping pattern which was considerably different from that just described for the intact animal. The slightest stimulation of the pectoral skin or ventral surfaces of the forelimbs in these operates initiated a strong clasp reflex. Such preparations remained clasping one's finger or inanimate objects for long periods, the duration of the clasp appearing to depend on muscular strength rather than on any relaxation of the clasp. Even after the clasp object was removed, these operates generally held their forelimbs in a clasping position for some time. This clasping posture ceased as soon as the frog was placed on the substratum.

Since the slightest stimulation of the pectoral skin caused the forelimbs to assume the clasping position, it was usually necessary to spread the forelimbs apart forcibly when attempting to place the operated male on estrous females. These males did not arch their backs or attempt to adjust themseves squarely on the back of the female, but they held on tightly in whatever position they were placed. If they slid off to one side, as often happened, no correction was attempted.

Following transection through the caudal end of the medulla oblongata (spinal animal), the frogs exhibited a similar clasp pattern, and therefore this behavior is referred to as a spinal clasp reflex.

Males with forebrain lesions or with com-

plete ablation of the forebrain showed no deviation from the normal in their clasping behavior. With extensive lesions in the diencephalon, the clasp pattern resembled slightly that of the spinal clasp reflex described above. As the lesions were placed farther and farther caudally in the midbrain and cerebellum, the behavior resembled more and more the spinal clasp pattern.

Thus it appears that invasions of the diencephalon, midbrain, cerebellum, and medulla oblongata modify the clasping behavior to resemble closely that of the spinal frog.

SPAWNING BEHAVIOR

To test the spawning behavior of operated males they were placed on estrous (ovulated) females and were allowed to clasp. The observations were carried on in aquaria 30 cm. by 40 cm. by 25 cm. filled with about 8 cm. of water at approximately 23° C. The intact males normally exhibit one spawning movement following each oviposition movement of the female. In the execution of these spawning motions, a limited amount of variation is seen. If the operated frog responded to all the

TABLE 3

EFFECTS OF BRAIN INJURY UPON THE SPAWNING RESPONSES OF THE MALE

Operation	No. of Males Tested	Av. No. of Tests (Spawnings) per Male	Operates Showing Normal Spawning Behavior		Record Numbers of Operated Males[1]	
			No.	Percentage of Total Tested	Showing Normal Spawning Behavior	Showing Abnormal Spawning Behavior
Intact males (controls)	37	1.1	37	100.0		
Forebrain completely ablated (including from 75% to 100% of the preoptic area)	13	1.7	13	100.0	34, 46, 47, 49, 123, 126–128, 133–135, 143, 322	
Hypothalamus mostly ablated	12	1.9	12	100.0	85, 86, 88, 93, 97, 343, 344, 403, 845, 924, 941, 945	
Forebrain and/or thalamus ablated or extensively injured	7	1.1	7	100.0	14, 53, 413, 801, 802, 815, 823	
Extensive lesions in tecta and inferior colliculi. Three included ablation of cerebellum	5	1.0	5	100.0	233, 241, 312, 333, 903	
Extensive lesion in forebrain, diencephalon, and/or midbrain, involving anterior half of tegmentum only	6	1.0	6	100.0	36, 342, 414, 433, 734, 832	
Hypothalamus ablated or extensively injured. Extensive invasion of tegmentum. Two included damage to inferior colliculi, tecta, and thalamus	6	1.0	0	0.0		542, 702, 713, 714, 724, 733
Lesion in caudal half of tegmentum	1	2.0	0	0.0		603
Extensive injury or ablation of forebrain, diencephalon, and/or midbrain. Caudal half or entire tegmentum invaded.	10	1.1	1	10.0	311	313, 411, 424, 443, 521, 523, 532, 712, 731

[1] For descriptions of the individual operations and summaries of the behavioral data, see page 117.

female oviposition movements, and if the pattern of these spawning responses resembled that of the control males, the operate was classified as exhibiting normal spawning behavior. Among the operates, the distinction between normal and abnormal responses was very sharp. There was seldom any question as to the classification of the spawning reactions of operated individuals.

The abnormal responses of the operates were of three types. In the first group the operated males did not respond to all the oviposition movements of the female, but when they did react, the ejaculatory movements were comparable to those of the intact animal. Some of these males started spawning in quite a normal manner, but in the midst of the egg laying they ceased to react. Others did not exhibit any spawning movements until the spawning was well under way, and then completed the oviposition with typical ejaculatory motions. In the second group the operated males exhibited short, irregular spawning movements which gave the impresion that only a fraction of the muscular complex was being activated. A third group of brain-operated males exhibited no response whatever to the oviposition movements of the female.

The results of this experiment are summarized in table 3. The data for the controls are taken from a previous study on the normal behavior (Noble and Aronson, 1942). It is shown that extirpation of the forebrain, diencephalon, tecta, inferior colliculi, cerebellum, and anterior half of tegmentum (to the caudal end of oculomotor nuclei) did not interrupt the spawning responses. On the other hand, when the posterior tegmentum was invaded, the reaction was significantly altered or completely abolished in all cases except one; and in this particular operate, male 311, the tegmental injury caudal to the posterior end of the oculomotor nuclei was unilateral.

Many of the operates participated in two or more egg layings on successive days. The results were entirely consistent. All the operates showing normal spawning behavior during the first ovipositions showed similarly typical behavior in the succeeding spawnings. Column 3 in table 3 indicates the average number of spawnings per male for the various groups of operates. It will be noted that the males sustaining midbrain lesions were seldom tested more than once, because following midbrain injury the operates seldom remained in a satisfactory physical condition long enough to warrant successive tests.

From these data it can be concluded that neither the forebrain, diencephalon, tecta, inferior colliculi, nor cerebellum is essential for the mediation of normal spawning movements. Likewise, the anterior half of the tegmentum to approximately the caudal end of the oculomotor nuclei is not a necessary region for this reaction. On the other hand, the posterior tegmentum extending caudally from the region of the trochlear nucleus must be intact if normal spawning behavior is to be obtained.

In table 4 it is seen that those groups of operates in which the male spawning behavior was mostly normal (compare with table 3) the per cent of fertile egg masses, as determined by the development of normal embryos, varied from 60 to 100 with a mean of 77.5. In those groups of operates in which the spawning activity of the male was mostly abnormal (last three categories of operates in table 4), the per cent of fertile egg clusters varied from 0 to 44.4 with a mean of 21.5.

The procedure of storing ovulated females in the refrigerator for one or more days while awaiting operated males resulted in an increased incidence of partially egg-bound frogs (Noble and Aronson, 1942) and undoubtedly caused a lowered fertility of the egg masses.

It was a matter of some interest that even brain-operated males that exhibited extremely abnormal mating behavior were still apparently able to emit some sperm. For example, male 714 with an extensive tegmental lesion showed no spawning responses at all, but some of the resulting eggs were fertilized. In all the other spawnings with males that exhibited abnormal spawning behavior and that yielded some fertile eggs, one or more ejaculatory movements were recorded.

The data on male 603 (fig. 24) illustrate a relatively small lesion in the caudal part of the tegmentum which abolished the mating response. On the other hand, the records of male 414 (fig. 22) illustrate a very extensive brain injury which did not modify the spawn-

TABLE 4

EFFECTS OF BRAIN INJURY TO THE MALE UPON THE EFFICACY OF SPERM EMISSION AS DETERMINED BY THE FERTILITY OF THE EGGS AFTER SPAWNING

Operation	No. of Males Tested	Operates Fertilizing Egg Clusters[1]		Record Numbers of Operated Males[2]	
		No.	Percentage of Total Tested	Fertilizing Egg Clusters	Not Fertilizing Egg Clusters
Intact males (controls)	7	5	71.4		
Forebrain completely ablated (including from 75% to 100% of the preoptic area)	8	7	87.5	34, 47, 49, 127, 128, 133, 143	34, 46
Hypothalamus mostly ablated	10	6	60.0	85, 86, 88, 343, 344, 403	93, 845, 924, 941
Forebrain and/or thalamus ablated or extensively injured	5	4	80.0	413, 801, 802, 815	823
Extensive lesions in tecta and inferior colliculi. Three included ablation of cerebellum	4	4	100.0	233, 312, 333, 903	
Extensive lesion in forebrain, diencephalon, and/or midbrain, involving anterior half of tegmentum only	5	3	60.0	36, 414, 832	433, 734
Hypothalamus ablated or extensively injured. Extensive invasion of tegmentum. Two included damage to inferior colliculi, tecta, and thalamus	5	1	20.0	714	542, 702, 713, 724
Lesion in caudal half of tegmentum	1	0	0.0		603
Extensive injury or ablation of forebrain, diencephalon, and/or midbrain. Caudal half or entire tegmentum invaded	9	4	44.4	311, 411, 424, 731	313, 443, 521, 532, 712, 731

[1] As determined by the development of at least one normal embryo.
[2] For descriptions of the individual operations and summaries of the behavioral data, see page 117.

ing response. The operation consisted of a transection slightly anterior to the caudal level of the oculomotor nuclei with the tecta mostly ablated and the inferior colliculi severely damaged.

Figure 15 is a diagrammatic sagittal section through the frog brain. The areas that were explored in testing for the spawning reaction are situated anterior to the heavy broken line, and the region in the tegmentum that appears to be essential for the mediation of the response is delimited by crosshatching.

The lesions in the tegmentum generally caused severe systemic effects, and frogs with such injury were often in poor physiological condition. This was due, no doubt, to the proximity of the injury to the cardiac and respiratory centers in the medulla oblongata. Because of this effect, it was not considered practical to try to test the spawning responses in males with medulla lesions. This raises the question as to whether the operates with posterior tegmental lesions failed to respond to the female's oviposition movements because of a general physiological failure in-

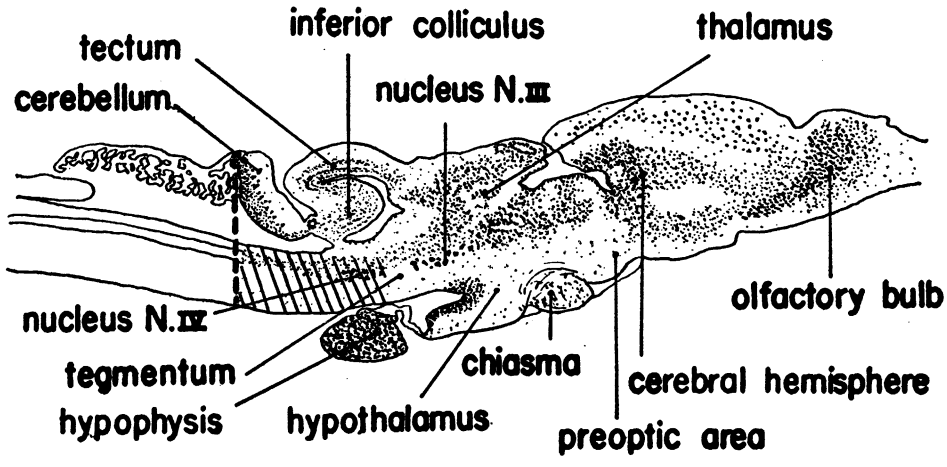

FIG. 15. Diagrammatic sagittal section through the brain of *Rana pipiens*. The regions anterior to the heavy broken line were explored while studying the ejaculatory movements of the male. Lesions in the caudal half of the tegmentum (indicated by cross hatching) alter or abolish this behavior.

stead of the disruption of a specific nervous mechanism directly essential to the behavior in question.

Two facts tend to show that the nonresponsiveness of males with posterior tegmental lesions was not due to excessive debility. First, a number of operates sustaining extensive brain injury not involving the posterior tegmentum were so weak that they died within a few minutes after spawning in a totally normal manner. This can be taken to indicate that if the spawning mechanism is not interrupted, a physiologically weakened animal will still respond normally.

The second answer to this problem was arrived at by transecting the brain at the caudal end of the medulla (caudal to the cardiac and respiratory centers) and anterior to the origin of the brachial plexus (first spinal segment). When the lesion was properly placed above the nuclei of origin of the first cervical nerves, the clasping reflex was not interrupted. The transections were accomplished with a small sharp knife after exposing the spinal cord. The results follow.

The first successful operation was male 43. He remained in good physiological condition for many days. He clasped well, but when the female began to spawn, male 43 did not exhibit any response to the egg-laying movements. Post-mortem examination revealed a complete transection just anterior to the first cervical segment, except for some fine fragments on one side.

Male 38 was also in good condition after the operation and survived two tests. He too clasped well, but he did not respond in either test to the oviposition activities of the females. This operation proved to be a complete unilateral transection at the anterior edge of the first cervical segment.

These data suggest that the failure of the operates with posterior tegmental lesions to exhibit normal spawning movements is due to a direct disruption of a specific nervous mechanism controlling spawning behavior and is not an aftermath of general physiological weakness resulting from respiratory and cardiac failure.

RELEASE AFTER OVIPOSITION

The "release time" was defined as the interval between the time that the female moved out of the oviposition posture and the male's relaxation of the clasp. When this release time was less than six minutes, the release was considered normal. Those males that failed to relax their grip in six minutes were classified as "not releasing." This six-minute interval is a somewhat arbitrary value. It is based upon earlier studies (Noble and Aronson, 1942) in which the average re-

TABLE 5

EFFECTS OF BRAIN INJURY UPON THE RELEASE OF THE FEMALE BY THE EXPERIMENTAL MALE AT THE TERMINATION OF OVIPOSITION

Operation	No. of Males	Av. No. of Trials per Male[1]	Males Releasing[2]		Record Numbers of Operated Males[3]	
			No.	Per cent	Releasing	Not Releasing
Intact males (controls)	37	1.1	32	86.5		
Extensive injury to the forebrain, preoptic area intact	3	1.3	3	100.0	63, 813, 910	
Forebrain ablated excepting the preoptic area	5	2.2	5	100.0	3, 8, 26, 68, 125	
Forebrain ablated excepting caudal three-fourths to one-quarter of the preoptic area	8	1.5	4	50.0	27, 29, 39, 41	40, 48, 60, 99
Forebrain completely ablated (including from 75% to 100% of the preoptic area)	12	1.5	3	25.0	50, 123, 133	34, 47, 49, 126–128, 134, 135, 143
Preoptic area mostly ablated (rest of forebrain intact)	12	1.6	0	0.0		11, 18, 19, 62, 213, 215, 223, 301, 302, 920, 922, 923
Bilateral injury to preoptic nuclei	4	4.5	0	0.0		6, 16, 17, 921
Hypothalamus mostly ablated	12	1.4	2	16.7	845, 924	85, 86, 88, 93, 97, 343, 344, 832, 941, 945
Pars ventralis hypothalami mostly ablated, pars dorsalis hypothalami mostly intact	6	1.2	6	100.0	604, 841, 842, 844, 907, 930	

[1] A trial equals an oviposition with a gravid female.
[2] Within six minutes after the termination of the oviposition.
[3] For descriptions of the individual operations and summaries of the behavioral data, see page 117.

lease time for a group of intact males was determined. Since the vast majority of males in the present experiment either released within one minute or remained clasping for an hour or more, the rather arbitrary six-minute dividing line could not seriously influence the data.

As already noted the method of storing ovulated females in the refrigerator until they were needed sometimes resulted in partial egg-boundness. Since one of the factors causing the male to release at the termination of the oviposition is the reduction in the female's girth resulting from the expulsion of the eggs, the release data on spawnings with partially egg-bound females have been discarded. The only cases considered are those in which the female laid a full complement of eggs in a normal manner.

Table 5 summarizes the results of brain injury upon the male's tendency to release the female at the termination of the oviposition. It will be seen that extensive injury or extirpation of the forebrain leaving the preoptic area intact did not affect the release. Because of the smallness of the samples the increase is probably not significant. The 13.5 per cent difference between these groups of operated males and the controls is less than two and one-half times the standard error.

When the caudal three-quarters to one-quarter of the preoptic area was ablated to-

gether with the forebrain, the number of males releasing was reduced to 50 per cent. When the forebrain was completely ablated (leaving intact less than a quarter of the preoptic area), the number releasing was still further reduced. When the preoptic area alone was ablated, leaving the rest of the forebrain intact, none of the males released. Finally, after lesions in the preoptic nucleus which spared the remainder of the preoptic area, four males failed to release.

When the pars ventralis hypothalami was largely extirpated, leaving the pars dorsalis intact, release was not affected, but complete hypothalamic extirpation interrupted the release behavior in most of the cases.

Column 3 of table 5 indicates the average number of trials for the various groups of operates. Each trial represents the observation of the release time following one spawning with a different gravid female. With only a few exceptions, the operated males were consistent in their release behavior when they were subjected to multiple trials.

Just as in the experiment on the swimming response, complete forebrain extirpation often resulted in slight invasions of the diencephalon, while the cerebral extirpations sparing the preoptic area sometimes left remnants of the hemispheres and varying amounts of the anterior commissure. There was no indication that the survival of cerebral remnants or the invasion of the diencephalon had any significant influence on the release behavior.

The record of male 34 (fig. 18) illustrates a completely decerebrate male which did not release. Note particularly in figure 18c the destruction of the epithalamus. In male 8 (fig. 18), the cerebral extirpation left the preoptic area untouched. Slight remnants of the hemispheres remained. This male released immediately (release times less than one minute) in three separate spawnings. The results obtained with male 213 (fig. 19) illustrate the ablation of the preoptic area, leaving the rest of the brain intact. This male did not release.

The records of males 15 and 918 were not included in the tables but are of some interest. The operation on male 15 was a small unilateral lesion in the left lateral preoptic area and extending into the tip of the left preoptic nucleus. In six ovipositions the male released three times and remained clasping on the other three occasions. In male 918 (fig. 25) the preoptic area of one side was largely ablated. In four egg layings this male released twice and remained clasping on the other two occasions. These two records suggest that unilateral invasion of the preoptic

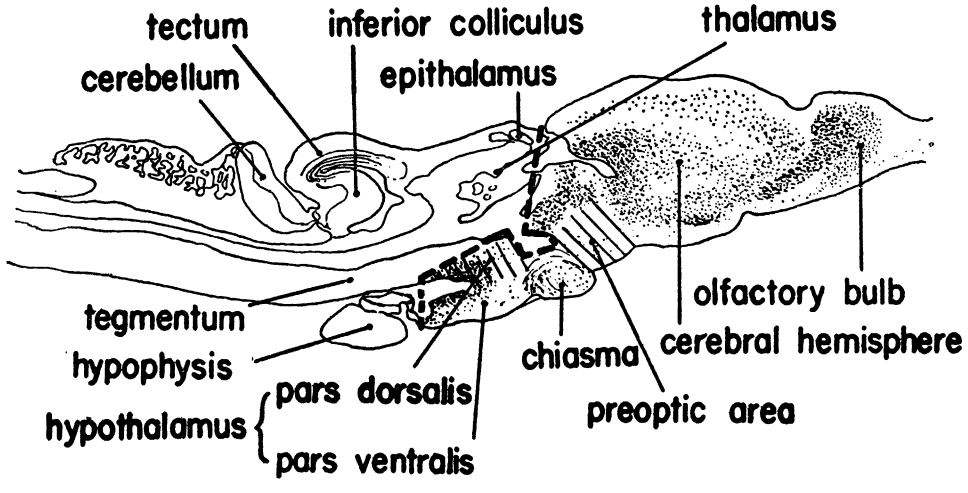

FIG. 16. Diagrammatic sagittal section through the brain of *Rana pipiens*. The regions anterior to the heavy broken line were explored while studying the release of the female by the male at the termination of oviposition. Following lesions in the preoptic area or pars dorsalis hypothalami (indicated by cross hatching), males fail to release the female when the spawnings terminate.

area will reduce the probability of the male's releasing the female after oviposition to about 50 per cent. This is in contrast with unilateral destruction of the inferior colliculus which abolished the warning croak, and unilateral transection through the cervical cord which abolished the ejaculatory movements.

It is concluded from these data that the preoptic area, and the pars dorsalis hypothalami which appears morphologically as a caudal extension of the preoptic area, contain an essential link in the neural mechanism which mediates the male's release of the female at the termination of the oviposition. It is highly probable that some part of this mechanism is located in the rostral part of the preoptic nucleus.

Figure 16 is a diagrammatic sagittal section through the frog brain illustrating the regions explored in this experiment (located anterior to the heavy broken line) and the areas which must remain intact (delimited by cross-hatching) for the proper functioning of the neural circuits controlling the release behavior of the male at the termination of the oviposition.

DESCRIPTION OF THE OPERATED BRAINS AND SUMMARY OF BEHAVIORAL DATA

IN THIS SECTION all the operated brains referred to in the foregoing tables and text are described briefly. A summary of the pertinent behavioral data follows, and in some instances items of behavior not previously mentioned are included. The designation of the ovipositions as "normal" or "abnormal" refer to the male's behavior only. The release time indicates the interval between the termination of the oviposition and the release of the spent female by the male. For those cases in which the male continued to clasp for several hours after the egg laying, the post-spawning observation interval is given. The eggs were counted as fertile if at least one normal embryo developed.

Cross-section drawings at critical levels were made from 10 representative operated brains. These may be compared with the drawings of the intact *pipiens* brain (figs. 1 to 12).

MALE 1: Forebrain ablated except caudal half of preoptic area. Rostral edge of dorsal and ventral thalami invaded. Warning croak elicited. Sex call heard.

MALE 2: Forebrain ablated except preoptic area. Anterior commissure and related bed nuclei removed. Oviposition normal. Warning croak elicited. Sex call heard. Swimming reaction to estrous female observed.

MALE 3: Lesion same as male 2. Oviposition normal. Release immediate. Eggs fertile. Warning croak elicited.

MALE 6: Bilateral lesion in preoptic nuclei. Ventral edges of right corpus striatum and right septal nuclei slightly damaged. Oviposition I normal. No release in 9 hours. Eggs not fertile. Oviposition II normal. No release in 12 hours. Eggs fertile. Warning croak elicited.

MALE 8 (FIG. 17): Cerebral hemispheres ablated except ventral remnants at preoptic level. Preoptic area intact. Anterior commissure and related bed nuclei mostly intact. Pars fimbrialis septi largely ablated. Amygdala partly ablated. Oviposition I normal. Release immediate. Eggs fertile. Oviposition II normal. Release immediate. Eggs fertile. Oviposition III normal. Release immediate. Eggs fertile. Warning croak elicited. Swimming response to estrous female observed.

MALE 11: Preoptic area ablated except caudal remnants of preoptic nuclei dorsal to chiasma. Ventral edges of corpora striata and septal nuclei just rostral to preoptic area invaded. Anterior commissure mostly interrupted. Oviposition I normal. No release in 4 hours. Eggs fertile. Oviposition II normal. No release in 5 hours. Eggs fertile. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 12: Lesion same as male 2. Oviposition I normal. Oviposition II normal. Oviposition III normal. Oviposition IV normal. Eggs fertile. Warning croak elicited. Sex call heard. Swimming response to estrous female demonstrated.

MALE 14: Cerebral hemispheres ablated. Preoptic area intact, except slight invasion of anterior edge. Epithalamus ablated. Dorsal and ventral thalami ablated except caudal remnants of dorsal and ventral thalamic nuclei. Oviposition normal.

MALE 15: Small lesion in left lateral preoptic area which extended into tip of left preoptic nucleus. Oviposition I normal. Released after 2 minutes. Eggs fertile. Oviposition II normal. Release delayed 4 minutes. Eggs not fertile. Oviposition III normal. Release delayed 7 minutes. Eggs not fertile. Oviposition IV normal. Released in 1 minute. Eggs not fertile. Oviposition V normal. Release delayed ½ hour. Eggs not fertile. Oviposition VI normal. Release delayed 9 minutes. Eggs not fertile.

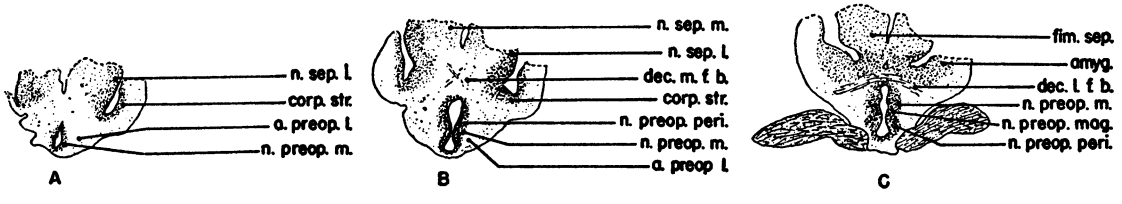

FIG. 17. Three transverse sections of the brain of operated male 8; ×9. A. Through the anterior end of the preoptic area. Intact brain tissue extended only 75μ anterior to this level. B. 330μ farther caudally through the middle of the preoptic area. C. 345μ caudally through the posterior part of the preoptic area.

MALE 16: Large lesion in preoptic area. Anterior commissure intact. Oviposition I normal. Eggs fertile. Oviposition II normal. Release delayed 120 minutes. Eggs fertile. Oviposition III normal. No release in 6 hours. Eggs fertile. Oviposition IV normal. No release in 7 hours. Eggs fertile. Oviposition V normal. No release in 5 hours. Eggs fertile. Oviposition VI normal. Release delayed 1 hour and 48 minutes. Eggs not fertile. Oviposition VII normal. No release in 5 hours. Eggs not fertile. Oviposition VIII normal. Release delayed 10 minutes. Eggs not fertile. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 17: Large lesion in preoptic area. Anterior commissure and related bed nuclei invaded. Oviposition I normal. No release in 3 hours. Eggs fertile. Oviposition II normal. No release in 6 hours. Eggs fertile. Oviposition III normal. Release delayed 3 hours. Eggs not fertile. Oviposition IV normal. No release in 7 hours. Eggs not fertile. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 18: Preoptic area ablated. Nucleus suprachiasmaticus hypothalami invaded. Anterior commissure and related bed nuclei mostly removed. Oviposition I normal. No release after 5 hours. Eggs fertile. Oviposition II normal. No release after 6 hours. Eggs fertile. Oviposition III normal. No release after 4 hours. Eggs not fertile. Warning croak elicited.

MALE 19: Preoptic area ablated excepting the dorsal edge, and the preoptic nuclei dorsal to the chiasma. Oviposition I normal. No release after 4 hours. Eggs fertile. Oviposition II normal. No release after 6 hours. Eggs fertile. Oviposition III normal. Eggs not fertile. Oviposition IV normal. Released after 25 minutes. Eggs not fertile. Warning croak elicited.

MALE 20: Forebrain ablated except caudal end of preoptic nuclei dorsal to chiasma. Emenentia thalami invaded. Warning croak elicited.

MALE 24: Forebrain completely ablated. Emenentia thalami, anterior edges of dorsal and ventral thalami, nuclei of Bellonci, and habenular nuclei ablated. Warning croak elicited.

MALE 26: Cerebral hemispheres ablated. Preoptic area intact except rostral tip. Anterior commissure and related bed nuclei ablated. Oviposition I normal. Release immediate. Eggs fertile. Oviposition II normal. Release immediate. Eggs not fertile. Warning croak elicited.

MALE 27: Cerebral hemispheres ablated. Caudal third of preoptic area intact. Anterior commissure and related bed nuclei extirpated. Habenular nuclei invaded. Oviposition normal. Release immediate. Eggs not fertile. Warning croak elicited.

MALE 29: Cerebral hemispheres ablated. Caudal half of preoptic area intact. Anterior commissure and related bed nuclei extirpated. Emenentia thalami, rostral edges of dorsal and ventral thalamic nuclei, nuclei of Bellonci, and habenular nuclei invaded. Oviposition normal. Release immediate. Eggs fertile.

MALE 32: Cerebral hemispheres ablated. Caudal third of preoptic area intact. Anterior commissure and related bed nuclei extirpated. Warning croak elicited. Swimming reaction to estrous female demonstrated.

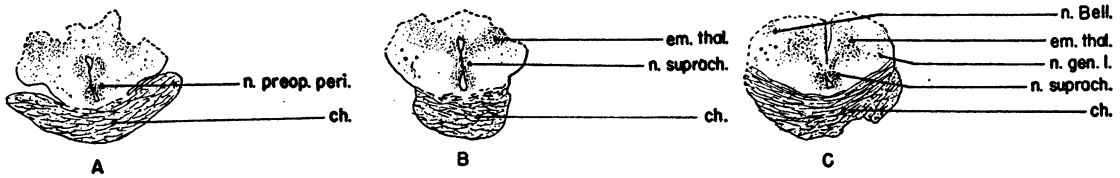

FIG. 18. Three transverse sections of the brain of operated male 34; ×9. A. Through the caudal edge of the preoptic nuclei. Intact brain tissue extended less than 150μ anterior to this level. B. 255μ farther caudally through the emenentia thalami. C. 240μ caudally through the anterior part of the thalamus.

MALE 34 (FIG. 18): Forebrain ablated except caudal remnants of preoptic nuclei dorsal to the chiasma. Emenentia thalami mostly ablated. Nuclei of Bellonci and lateral geniculate nuclei extensively injured. Anterior edges of habenular nuclei invaded. Oviposition I normal. Release delayed 3 hours. Eggs not fertile. Oviposition II normal. Eggs fertile. Warning croak elicited.

MALE 36: Transection at caudal end of oculomotor nucleus. Tecta and inferior colliculi completely ablated. Oviposition normal. No release in 6 hours. Eggs fertile.

MALE 39: Cerebral hemispheres ablated. Caudal two-thirds of preoptic area intact. Anterior commissure and related bed nuclei extirpated. Emenentia thalami, anterior edges of the dorsal and ventral thalamic nuclei, the nuclei of Bellonci, and the habenular nuclei invaded. Oviposition I normal. Release immediate. Eggs not fer-

tile. Oviposition II normal. Release immediate. Warning croak elicited.

MALE 40: Cerebral hemispheres ablated. Caudal third of preoptic area intact. Anterior commissure and related bed nuclei invaded. Oviposition normal. Release delayed 1 hour. Eggs fertile. Warning croak elicited.

MALE 41: Cerebral hemispheres ablated. Caudal two-thirds of the preoptic area intact. Anterior commissure and related bed nuclei invaded. Oviposition I normal. Release immediate. Eggs fertile. Oviposition II normal. Release delayed 4 minutes. Eggs fertile. Oviposition III normal. Release immediate. Eggs fertile. Warning croak elicited. Sex call heard.

MALE 45: Tecta ablated except ventro-lateral edges. Inferior colliculi ablated except along ventral edge. Extensive damage to dorsal and ventral thalami. Slight invasion of cerebral hemispheres. Warning croak could not be elicited.

MALE 46: Forebrain ablated except caudal remnants of preoptic nuclei dorsal to chiasma. Emenentia thalami, nuclei of Bellonci, and habenular nuclei ablated. Oviposition I normal. Eggs not fertile. Oviposition II normal. Eggs not fertile. Warning croak elicited. Male did not exhibit swimming response to estrous female.

MALE 47: Forebrain completely ablated except caudal end of nucleus preopticus dorsal to chiasma. Emenentia thalami, nuclei of Bellonci, lateral geniculate nuclei, and rostral edge of dorsal thalamic nuclei ablated. Habenular nuclei mostly ablated. Oviposition normal. No release after 4 hours. Eggs fertile. Warning croak elicited.

MALE 48: Cerebral hemispheres ablated except remnants of posterior poles. Caudal half of preoptic area intact. Anterior commissure and related bed nuclei intact. Slight invasion of anterior edge of thalamus. Oviposition I normal. Released in 20 minutes. Eggs not fertile. Oviposition II normal. Released after 3 hours. Eggs not fertile. Warning croak elicited.

MALE 49: Forebrain completely ablated except caudal edge of preoptic nuclei dorsal to chiasma and slight remnant of posterior pole on right side. Oviposition I normal. Released after 1 hour. Eggs fertile. Oviposition II normal. No release in 4 hours. Eggs fertile. Oviposition III normal. No release in 4 hours. Eggs fertile. Oviposition IV normal. Oviposition V normal. No release in 5 hours. Warning croak elicited. No response to estrous female observed.

MALE 50: Forebrain completely ablated. Slight damage to anterior edge of dorsal and ventral thalami. Release immediate. Warning croak elicited.

MALE 51: Lesion same as male 2. Warning croak elicited. Sex call heard. Swimming reaction to estrous female observed.

MALE 52: Forebrain and epithalamus ablated. Considerable injury to dorsal and ventral thalami. Small lesion extended to caudal end of thalamus. Warning croak elicited. Swimming reaction to estrous female observed.

MALE 53: Forebrain completely ablated. Epithalamus ablated. Dorsal and ventral thalami ablated except caudal edges of dorsal and ventral thalamic nuclei. Pretectal nuclei and nuclei of the posterior commissure intact. Oviposition normal. Warning croak elicited.

MALE 54: Forebrain ablated except caudal end of preoptic nuclei dorsal to chiasma. Dorsal thalamus, ventral thalamus, and epithalamus ablated. Slight invasion of anterior edges of the tecta. Warning croak elicited. Swimming response to estrous female observed.

MALE 55: Lesion same as male 24. Swimming reaction to estrous female not demonstrated.

MALE 58: Lesion same as male 2. Warning croak elicited. Swimming reaction to estrous female noted.

MALE 60: Cerebral hemispheres ablated. Caudal half of the preoptic area intact. Anterior commissure and related bed nuclei extirpated. Oviposition normal. Release delayed ½ hour. Eggs fertile. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 62: Preoptic area completely ablated. Nucleus suprachiasmaticus hypothalami invaded. Slight damage to ventral edges of septal nuclei and corpora striata just rostral to preoptic area. Anterior commissure and related bed nuclei mostly ablated. Oviposition I normal. No release after 2 hours. Eggs fertile. Oviposition II normal. No release after 1 hour. Eggs not fertile. Warning croak elicited. Swimming reaction to estrous female observed.

MALE 63: Cerebral hemispheres removed from posterior poles to a level just rostral to the anterior commissure. Anterior commissure and related bed nuclei invaded. Preoptic area intact. Epithalamus ablated. Oviposition I normal. Release immediate. Oviposition II normal. Release immediate. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 65: Posterior poles of both cerebral hemispheres ablated. Extensive lesion throughout hippocampi and piriform areas. Anterior commissure and preoptic area intact. Swimming reaction to estrous female demonstrated.

MALE 67: Epithalamus ablated. Extensive lesion in dorsal part of dorsal thalamus. Emenentia thalami invaded. Cerebral hemispheres ablated from level of the anterior commissure to the posterior poles. Sex call heard. Swimming reaction to estrous female demonstrated.

MALE 68: Lesion same as male 8. Oviposition

I normal. Release delayed 4 minutes. Eggs not fertile. Oviposition II normal. Release delayed 3 minutes. Eggs not fertile. Oviposition III normal. Release immediate. Eggs not fertile. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 70: Forebrain ablated except preoptic area and ventral remnants of the corpora striata and septal nuclei. Anterior commissure and related bed nuclei intact. Warning croak elicited. Sex call heard. Swimming reaction to estrous female demonstrated.

MALE 72: Lesion same as male 24. Swimming reaction to estrous female not demonstrated.

MALE 73: Forebrain ablated except slight remnant of left posterior pole. Right habenular nuclei ablated and right emenentia thalami invaded. Swimming reaction to estrous female not demonstrated.

MALE 74: Lesion same as male 16. Swimming reaction to estrous female not demonstrated.

MALE 75: Anterior third of cerebral hemispheres ablated. Swimming reaction to estrous female not demonstrated.

MALE 76: Forebrain ablated except caudal remnants of preoptic nuclei dorsal to the chiasma. Emenentia thalami, nuclei of Bellonci, habenular nuclei, and anterior edges of dorsal and ventral thalamic nuclei extirpated. Swimming reaction to estrous female not demonstrated.

MALE 77: Forebrain ablated except caudal edge of preoptic area. Emenentia thalami ablated. Habenular nuclei invaded. Swimming reaction to estrous female not demonstrated.

MALE 78: Cerebral hemispheres ablated. Caudal two-thirds of preoptic area intact. Anterior commissure and related bed nuclei intact. Swimming reaction to estrous female not demonstrated.

MALE 79: Lesion same as male 8. Warning croak elicited. Swimming reaction to estrous female noted.

MALE 80: Preoptic area completely ablated. Anterior commissure and related bed nuclei mostly removed. Slight damage to ventral edges of septal nuclei and corpora striata rostral to the preoptic area. Swimming reaction to estrous female not demonstrated.

MALE 82: Lesion same as male 32. Swimming reaction to estrous female not demonstrated.

MALE 83: Lesion same as male 46. Swimming reaction to estrous female not demonstrated.

MALE 84: Lesion same as male 24. Swimming reaction to estrous female not demonstrated.

MALE 85: Hypothalamus ablated except the suprachiasmatic nuclei and remnants of the pars dorsalis. Oviposition I normal. Released after 1 hour. Eggs fertile. Oviposition II normal. No release in 4 hours. Oviposition III normal. No release in 3 hours. Eggs fertile. Warning croak elicited. Swimming response to estrous female observed.

MALE 86: Hypothalamus ablated except nucleus suprachiasmaticus. Oviposition normal. No release in 1 hour. Eggs fertile. No swimming response to estrous female observed.

MALE 88: Lesion same as male 85. Oviposition I normal. No release after 1 hour. Oviposition II normal. No release after 3 hours. Eggs fertile. Swimming response to estrous female demonstrated.

MALE 89: Lesion same as male 40. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 91: Lesion same as male 86. Swimming reaction to estrous female not demonstrated.

MALE 92: Lesion same as male 8. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 93: Hypothalamus ablated except nucleus suprachiasmaticus. Nucleus ventralis thalami pars externa invaded. Oviposition I normal. Oviposition II normal. Oviposition III normal. Oviposition IV normal. No release after 1 hour. Eggs not fertile. Swimming response to estrous female demonstrated.

MALE 94: Forebrain ablated. Epithalamus ablated. Dorsal edge of dorsal thalamic nuclei extirpated. Anterior edge of ventral thalamus invaded. Warning croak elicited. Swimming reaction to estrous female not demonstrated.

MALE 95: Cerebral hemispheres ablated. Caudal half of preoptic area intact. Anterior commissure and related bed nuclei intact. Warning croak elicited. Swimming reaction to estrous female not demonstrated.

MALE 96: Lesion same as male 40. Warning croak elicited. Swimming reaction to estrous female not demonstrated.

MALE 97: Lesion same as male 86. Oviposition I normal. Oviposition II normal. Released after 2 hours. Warning croak elicited. Swimming reaction to estrous female observed.

MALE 98: Lesion same as male 32. Swimming reaction to estrous female not demonstrated.

MALE 99: Lesion same as male 40. Oviposition normal. Release delayed ½ hour.

MALE 100: Dorsal thalamus, ventral thalamus, and epithalamus completely ablated. Extensive injury to caudal third of forebrain. Slight invasion of hypothalamus. Warning croak elicited.

MALE 101: Tecta, inferior colliculi, ganglia isthmi, and cerebellum ablated. Slight invasion of caudal end of dorsal thalamus. Warning croak could not be elicited.

MALE 103: Lesion same as male 46. Swimming reaction to estrous female not demonstrated.

MALE 104: Lesion same as male 32. Preoptic area intact. Anterior commissure and related bed nuclei extirpated. Swimming reaction to estrous female demonstrated.

MALE 105: Lesion same as male 95. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 106: Forebrain, emenentia thalami, and nuclei of Bellonci completely ablated. Warning croak elicited. Swimming reaction to estrous female not demonstrated.

MALE 108: Forebrain ablated except caudal third of preoptic nuclei. Dorsal thalamus, ventral thalamus, and epithalamus ablated. Anterior edges of tecta slightly invaded. Warning croak elicited.

MALE 109: Lesion in dorsal thalamic nuclei. Pretectal nuclei and nuclei of posterior commissure extensively damaged. Slight invasion of tecta and inferior colliculi. Warning croak elicited. Sex call heard.

MALE 112: Dorsal thalamus, ventral thalamus, and epithalamus ablated. Caudal third of forebrain extensively invaded. Warning croak elicited. Slight invasion of anterior edge of hypothalamus. Warning croak elicited.

MALE 114: Epithalamus ablated. Dorsal and ventral thalami ablated except caudal remnants. Extensive invasion of posterior third of forebrain. Warning croak elicited. Swimming response to estrous female observed.

MALE 115: Forebrain completely ablated. Emenentia thalami, anterior edges of dorsal and ventral thalami, nuclei of Bellonci, lateral geniculate nuclei, and habenular nuclei ablated. Warning croak elicited. Sex call heard. Swimming reaction to estrous female not demonstrated.

MALE 116: Epithalamus ablated. Dorsal and ventral thalami largely ablated except at caudal end. Caudal third of forebrain extensively invaded. Slight damage to hypothalamus. Warning croak elicited. Swimming response to estrous female observed.

MALE 117: Lesion same as male 116. Warning croak elicited.

MALE 118: Lesion same as male 114. Warning croak elicited. Swimming response to estrous female observed.

MALE 119: Forebrain ablated except caudal remnants of the preoptic nuclei dorsal to chiasma. Swimming reaction to estrous female demonstrated.

MALE 120: Lesion same as male 32. Swimming reaction to estrous female not demonstrated.

MALE 121: Forebrain ablated except preoptic nuclei dorsal to chiasma. Right nucleus of Bellonci and right emenentia thalami invaded. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 123: Lesion same as male 24. Oviposition normal. Release immediate. Warning croak elicited.

MALE 125: Forebrain ablated except preoptic area. Anterior commissure and related bed nuclei ablated. Epithalamus ablated. Emenentia thalami and nuclei of Bellonci invaded. Oviposition I normal. Release immediate. Oviposition II normal. Release immediate. Eggs not fertile. Warning croak elicited. Sex call heard. Swimming reaction to estrous female demonstrated.

MALE 126: Lesion same as male 76. Oviposition I normal. Release delayed 2 hours. Oviposition II normal. Released in 15 minutes. Warning croak elicited.

MALE 127: Lesion same as male 24. Oviposition I normal. No release in 1 hour. Oviposition II normal. Released in 1 hour. Eggs fertile. Warning croak elicited. Swimming response to estrous female observed.

MALE 128: Forebrain completely ablated. Emenentia thalami and nuclei of Bellonci ablated. Anterior edges of dorsal and ventral thalamic nuclei and rostral end of habenular nuclei damaged. Oviposition I normal. No release in 1 hour. Eggs fertile. Oviposition II normal. No release in 2 hours. Eggs fertile. Warning croak elicited.

MALE 133: Lesion same as male 76. Oviposition normal. Release immediate. Eggs fertile.

MALE 134: Cerebral hemispheres ablated except caudal quarter of preoptic area which is slightly invaded. Emenentia thalami and nuclei of Bellonci invaded. Habenular nuclei ablated. Oviposition normal. Release delayed ½ hour.

MALE 135: Lesion same as male 46. Oviposition normal. Released after 1 hour. Warning croak elicited.

MALE 140: Forebrain, epithalamus, and emenentia thalami ablated. Anterior edges of nuclei of Bellonci, dorsal thalamic nuclei, and ventral thalamic nuclei invaded. Warning croak elicited. Swimming reaction to estrous female observed.

MALE 143: Forebrain ablated except caudal remnants of preoptic nuclei dorsal to chiasma. On right side, the emenentia thalami, the nucleus of Bellonci, and the lateral geniculate nucleus were ablated. The right dorsal and ventral thalamic nuclei were extensively invaded. Oviposition normal. No release after 40 minutes. Eggs fertile. Warning croak elicited.

MALE 155: Forebrain completely ablated. Emenentia thalami, anterior edges of dorsal and ventral thalami, nuclei of Bellonci, and left habenular nucleus extirpated. Swimming reaction to estrous female not demonstrated.

MALE 212: Extensive lesion in left tectum. Ovi-

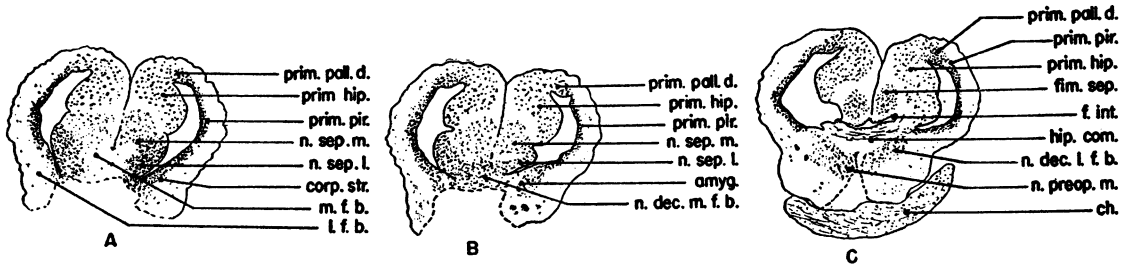

FIG. 19. Three transverse sections of the brain of operated male 213; ×9. A. Through the anterior end of the preoptic area. The lesion extended along the ventral surface of the forebrain for almost 1 mm. anterior to this level. B. 165μ farther caudally through the middle of the preoptic area. C. 210μ farther caudally through the caudal end of the preoptic nucleus. The lesion extended about 90μ caudal to this level.

position normal. Release immediate. Eggs fertile. Warning croak elicited. Sex call heard.

MALE 213 (FIG. 19): Preoptic area ablated except caudal remnants of preoptic nuclei dorsal to chiasma. Ventral edges of septal nuclei and corpora striata just anterior to the preoptic area invaded. Decussations of medial and lateral forebrain bundles and related bed nuclei invaded. Right amygdala injured. Oviposition normal. Release delayed over 2 hours. Eggs fertile.

MALE 214: Tecta and inferior colliculi completely ablated. Slight damage to caudal end of thalamus. Warning croak could not be elicited.

MALE 215: Preoptic nuclei completely ablated. Nuclei suprachiasmatici hypothalami extensively damaged. Slight injury to decussations of medial and lateral forebrain bundles and associated bed nuclei. Slight damage to ventral edges of septal nuclei and corpora striata. Oviposition normal. No release after 5 hours. Swimming reaction to estrous female demonstrated.

MALE 223: Preoptic area ablated except caudal remnant of preoptic nuclei dorsal to chiasma. Decussations of medial and lateral forebrain bundles and related bed nuclei extensively invaded. Slight damage to ventral edge of corpora striata, particularly on the right side. Slight injury to ventral edge of medial and lateral septal nuclei. Slight invasion of anterior edge of thalamus on right side. Ovipositon normal. Release delayed 1 hour. Eggs fertile.

MALE 233 (FIG. 20): Tecta ablated. Inferior colliculi ablated except remnants along ventral edges. Nuclei of the posterior commissure and

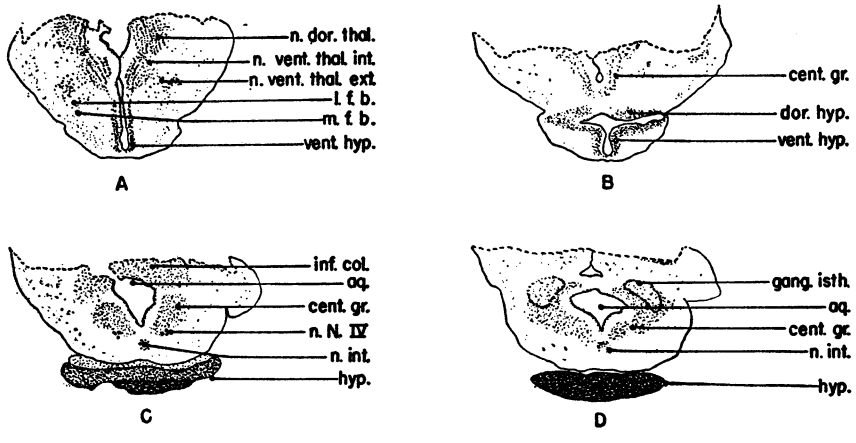

FIG. 20. Four transverse sections of the brain of operated male 233; ×9. A. Through the caudal part of the thalamus. The lesion extended anteriorly along the dorsal surface of the diencephalon for about 300μ from this level. B. 600μ farther caudally through the anterior part of the tectum. C. 1 mm. farther caudally through the middle of the inferior colliculi. D. 345μ caudally through the ganglia isthmi.

pretectal nuclei ablated. Dorsal half of posterior portion of dorsal thalamic nuclei ablated. Dorsal half of central gray surrounding aqueduct extensively injured. Oviposition normal. Release immediate. Eggs fertile. Warning croak could not be elicited. Sex call heard repeatedly.

MALE 241: Tecta, inferior colliculi, cerebellum, pretectal nuclei, and nuclei of the posterior commissure completely extirpated. Extensive invasion of ganglia isthmi. Slight invasion of caudal edge of dorsal thalamus. Oviposition normal. No release in 5 hours. Warning croak elicited.

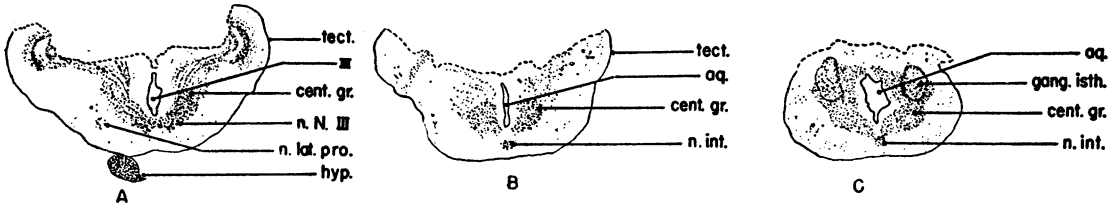

FIG. 21. Three transverse sections through the brain of operated male 314; ×9. A. Through the anterior part of the tectum. Lesion extended anteriorly from this level for about 450μ along the dorsal surface of midbrain. B. 390μ farther caudally through the middle of the tectum. C. 375μ farther caudally through the ganglia isthmi.

MALE 242: Transection at anterior edge of cerebellum. Warning croak could not be elicited.

MALE 301: Preoptic area ablated. Septal nuclei mostly destroyed. Extensive lesion in corpora striata. Anterior commissure and related bed nuclei extirpated. Nuclei suprachiasmatici hypothalami invaded. Oviposition normal. Release delayed 10 minutes.

MALE 302: Preoptic area ablated except caudal remnants of preoptic nuclei dorsal to the chiasma. Decussations of medial and lateral forebrain bundles partially interrupted. Septal nuclei invaded. Oviposition normal. No release in 8 hours. Sex call heard.

MALE 304: Cerebellum ablated. Caudal third of left tectum and caudal two-thirds of right tectum ablated. Warning croak elicited.

MALE 311: Tecta completely ablated except lateral remnant on right side. Inferior colliculi extensively invaded. Posterior part of dorsal thalamus mostly ablated. Tegmentum invaded on left side only at level of interpeduncular nucleus. Trochlear and oculomotor nuclei ablated. Nucleus opticus tegmenti and nucleus lateralis profundi ablated on left side. Pretectal nuclei and nuclei of the posterior commissure ablated. Oviposition normal. Release delayed 5 hours. Eggs fertile. Warning croak could not be elicited.

MALE 312: Tectum ablated except lateral edges at anterior end. Inferior colliculi ablated except anterior edge. Pretectal nuclei and nuclei of posterior commissure invaded. Oviposition normal. No release in 7 hours. Eggs fertile. Warning croak could not be elicited.

MALE 313: Tecta completely ablated. Dorsal half of dorsal thalamus ablated. Inferior colliculi, ganglia isthmi, and mesencephalic central gray ablated. Lesion invaded tegmentum along midline at level of interpeduncular nucleus injuring the latter. Oviposition abnormal. Release immediate. Eggs not fertile. Warning croak could not be elicited.

MALE 314 (FIG. 21): Tecta ablated except lateral remnants at anterior end. Pretectal nuclei, nuclei of the posterior commissure, and inferior colliculi bilaterally ablated. Warning croak could not be elicited.

MALE 321: Tecta and inferior colliculi completely ablated. Caudal half of thalamus invaded. Warning croak could not be elicited.

MALE 322: Lesion same as male 24. Oviposition normal. Warning croak elicited.

MALE 324: Epithalamus ablated. Dorsal and ventral thalami ablated except near rostral and caudal ends. Caudal third of forebrain extensively invaded. Some damage to right tectum. Warning croak elicited. Swimming response to estrous female observed.

MALE 331: Epithalamus ablated. Dorsal and ventral thalami ablated except caudal remnants. Caudal third of forebrain extensively invaded. Anterior part of tecta damaged. Warning croak elicited. Swimming response to estrous female demonstrated.

MALE 333: Caudal half of tectum ablated. Caudal end of inferior colliculi invaded. Cerebellum ablated. Oviposition normal. Release delayed 1 hour. Eggs fertile. Warning croak elicited.

MALE 341: Forebrain completely ablated. Epithalamus ablated. Dorsal and ventral thalami ablated except caudal remnants. Warning croak elicited.

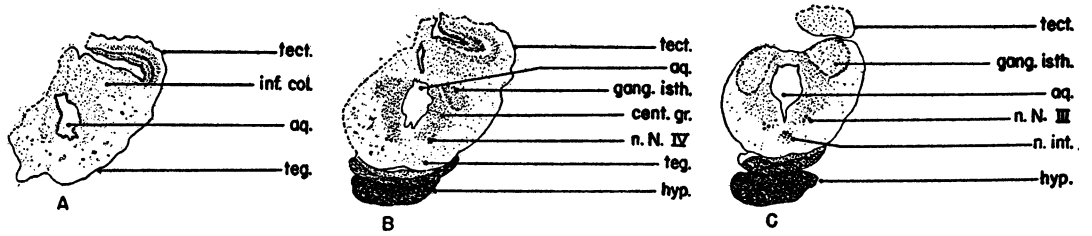

FIG. 22. Three transverse sections through the brain of operated male 414; ×9. A. Through the middle of the tectum. Intact brain tissue extended about 300μ anterior to this level. B. 540μ farther caudally through the caudal part of the tectum. C. 195μ farther caudally through the ganglia isthmi.

MALE 342: Transection a little rostral to the caudal end of the oculomotor nuclei. Tecta ablated except caudal lateral edges. Oviposition normal.

MALE 343: Hypothalamus ablated except remnants of nuclei suprachiasmatici. Slight invasion of ventral thalamic nuclei pars externa and the preoptic nuclei. Oviposition normal. No release after 5 hours. Eggs fertile.

MALE 344: Hypothalamus completely ablated. Slight invasion of ventral edges of preoptic nuclei. Optic chiasma mostly ablated. Oviposition normal. No release in 1 hour. Eggs fertile. Warning croak elicited.

MALE 402: Forebrain completely ablated. Epithalamus ablated. Dorsal and ventral thalami ablated except caudal remnants. Extensive lesion in roof of tecta and slight invasion of caudal end of inferior colliculi. Warning croak could not be elicited.

MALE 403: Lesion same as male 85. Oviposition normal. Release immediate. Eggs fertile. Warning croak elicited. Sex call heard.

MALE 411: Transection at caudal level of oculomotor nuclei. Anterior half of tectum ablated. Anterior edge of inferior colliculus invaded on left side. Tegmentum ablated to caudal end of oculomotor nuclei. Oviposition abnormal. Release immediate. Eggs fertile. Warning croak elicited.

MALE 412: Forebrain completely ablated. Rostral half of dorsal and ventral thalami ablated. Lesion extended caudally into dorsal thalamic nuclei. Epithalamus extirpated. Warning croak elicited.

MALE 413: Forebrain completely ablated. Epthalamus ablated. Dorsal and ventral thalamus ablated except caudal edges of dorsal and ventral thalamic nuclei. Pretectal nuclei and nuclei of the posterior commissure intact. Oviposition normal. Eggs fertile. Warning croak elicited.

MALE 414 (FIG. 22): Transection just anterior to caudal end of oculomotor nuclei. Tecta extirpated except caudal remnant on right side. Left inferior colliculus ablated. Right inferior colliculus damaged. Oviposition normal. Release immediate. Eggs fertile. Warning croak could not be elicited.

MALE 422: Forebrain completely ablated. Dorsal and ventral thalami ablated except at caudal end. Epithalamus removed. Warning croak present.

MALE 424: Transection at anterior end of tegmentum. Tecta and inferior colliculi completely ablated. Small lesion extended caudally near midline of tegmentum to level of trochlear nucleus. Oviposition abnormal. Eggs fertile. Warning croak could not be elicited.

MALE 431: Transection at caudal end of cerebellum. Warning croak could not be elicited.

MALE 433 (FIG. 23): Transection at level just anterior to oculomotor nucleus. Tecta ablated except lateral remnants. Anterior edge of inferior colliculi damaged, rest intact. Oviposition normal. Eggs not fertile. Warning croak elicited.

MALE 441: Complete transection through ganglia isthmi. Warning croak could not be elicited.

MALE 443: Transection at caudal end of inter-

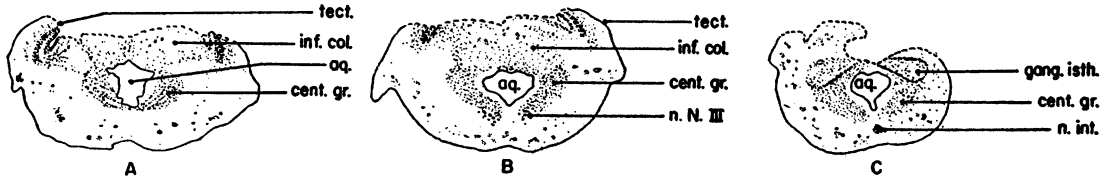

FIG. 23. Three transverse sections through the brain of operated male 433; ×9. A. Through middle of tectum. Intact brain tissue extended about 225μ anterior to this level. B. 290μ farther caudally through the posterior part of the tectum. C. 260μ farther caudally through the ganglia isthmi.

peduncular nucleus. Tecta, inferior colliculi, and ganglia isthmi completely ablated. Oviposition abnormal. Eggs not fertile. Warning croak could not be elicited.

MALE 521: Transection at caudal end of oculomotor nuclei. Tecta and inferior colliculi completely ablated. Injury to tegmentum extended caudally to trochlear nuclei. Oviposition abnormal. Release immediate. Eggs not fertile. Warning croak could not be elicited.

MALE 523: Transection through interpeduncular nucleus. Tecta and inferior colliculi ablated. Oviposition abnormal.

MALE 531: Transection just caudal to cerebellum. Warning croak could not be elicited.

MALE 532: Transection at caudal end of cerebellum. Oviposition abnormal. Released in 3 minutes. Eggs not fertile. Warning croak could not be elicited.

MALE 542: Shallow ablation of entire rostro-caudal extent of tegmentum. Lesion deeper at caudal end to include interpeduncular nucleus and right ganglion isthmi. Pars ventralis hypothalami mostly ablated on right side and invaded on left side. Lateral edge of thalamus injured on right side. Oviposition abnormal. No release for 45 minutes when male died. Eggs not fertile.

MALE 702: Deep midline lesion along entire rostro-caudal extent of tegmentum ablating the nucleus interpeduncularis. Pars ventralis hypothalami ablated except the nuclei suprachiasmatici. Pars dorsalis hypothalami injured. Oviposition abnormal. Eggs not fertile.

MALE 704: Hypothalamus ablated except nuclei suprachiasmatici. Deep midline lesion extending from the anterior to the posterior end of the tegmentum and invading the rostral part of the medulla oblongata. Oculomotor nuclei, trochlear nuclei, nuclei optici tegmenti, and interpeduncular nucleus invaded. Warning croak could not be elicited.

MALE 711: Hypothalamus ablated. Anterior tegmentum ablated to level of trochlear nuclei. Right inferior colliculus mostly destroyed; left inferior colliculus intact. Ventral thalamic nuclei invaded on right side. Slight injury to right preoptic nucleus. Warning croak could not be elicited.

MALE 712: Transection just anterior to cerebellum. Oviposition abnormal. Released in 4 minutes. Eggs not fertile. Warning croak could not be elicited.

MALE 713: Large midline lesion along entire rostro-caudal extent of tegmentum ablating the nucleus interpeduncularis, the oculomotor and

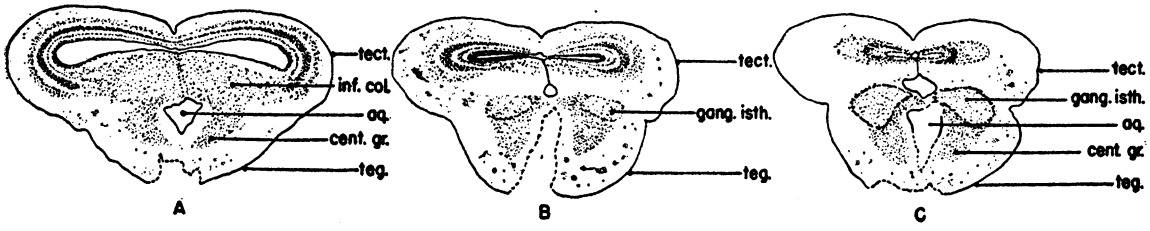

FIG. 24. Three transverse sections through the brain of operated male 603; ×9. A. Through the middle of the tegmentum. Lesion extended along the ventral surface of the tegmentum for about 300μ anterior to this level. B. 495μ farther caudally through the posterior part of the tegmentum. C. 165μ farther caudally through the posterior part of the tegmentum.

MALE 603 (FIG. 24): Lesion in tegmentum along midline starting at caudal end of oculomotor nuclei and extending to medulla. Left trochlear nucleus ablated; right mostly intact. Interpeduncular nucleus ablated. At caudal end of midbrain, the central gray surrounding the aqueduct was invaded. Oviposition abnormal. Release immediate. Eggs not fertile. Oviposition II abnormal. Release immediate. Eggs not fertile. Warning croak elicited.

MALE 604: Pars ventralis hypothalami ablated except suprachiasmatic nuclei. A shallow midline tegmental lesion extended caudally to rostral level of interpeduncular nucleus. Oviposition normal. Release immediate. Eggs not fertile. Warning croak elicited.

trochlear nuclei, the nuclei profundi laterale, the nuclei optici tegmenti, and the ventral half of the mesencephalic central gray. Hypothalamus completely ablated except remnants of suprachiasmatic nuclei. Both ganglia isthmi invaded. Oviposition abnormal. Release immediate.

MALE 714: Broad deep extirpation along entire rostro-caudal extent of tegmentum ablating the interpeduncular nucleus, the oculomotor and trochlear nuclei, the nuclei profundi laterale, the nuclei optici tegmenti, and the ventral half of the mesencephalic central gray. Hypothalamus ablated except remnants of suprachiasmatic nuclei. Caudal two-thirds of tecta ablated except lateral edges. Inferior colliculi ablated except ventral remnants. Cerebellum ablated. Oviposition

abnormal. Release delayed 25 minutes. Eggs fertile. Warning croak could not be elicited.

MALE 721: Hypothalamus extirpated except nuclei suprachiasmatici. Tegmentum ablated except lateral edges. Inferior colliculi extensively invaded with some damage to the caudal part of the tecta. Warning croak could not be elicited.

MALE 722: Transection at the anterior end of the oculomotor nuclei. Tecta and inferior colliculi extensively damaged. Warning croak could not be elicited.

MALE 723: Transection near caudal end of cerebellum. Warning croak could not be elicited.

MALE 724: Broad deep lesion along entire rostro-caudal extent of tegmentum ablating the nucleus interpeduncularis, the oculomotor and trochlear nuclei, the nuclei profundi laterale, the nuclei optici tegmenti, and the ventral edges of the mesencephalic central gray. Hypothalamus ablated except suprachiasmatic nucleus. Oviposition abnormal. Release immediate. Eggs not fertile. Warning croak elicited.

MALE 731: Transection through anterior end of tegmentum. Tecta ablated except dorsal and lateral remnants. Inferior colliculi ablated except caudal remnants. Tegmentum invaded to level of trochlear nucleus. Oviposition I abnormal. Release delayed 11 minutes. Eggs not fertile. Oviposition II abnormal. Release delayed 5 minutes. Eggs fertile. Warning croak could not be elicited.

MALE 733: Anterior half of tegmentum mostly ablated. Lesion extended caudally to interpeduncular nucleus which was slightly damaged. Hypothalamus completely ablated. Inferior colliculi extensively injured. Ventral thalamus on left side extensively injured and lesion extended to dorsal thalamus. Oviposition abnormal. Release immediate. Warning croak could not be elicited.

MALE 734: Hypothalamus ablated. Tegmentum extirpated to middle level of oculomotor nucleus. Inferior colliculi and ventral edge of dorsal thalamus invaded. Preoptic area and optic chiasma destroyed. Lesion extended into right cerebral hemisphere at level of anterior commissure, particularly the right primordium hippocampus. Oviposition normal. No release in 3 hours. Eggs not fertile.

MALE 741: Forebrain, dorsal thalamus, ventral thalamus, and epithalamus ablated. Anterior ends of hypothalamus and tecta invaded. Warning croak elicited.

MALE 742: Posterior poles of cerebral hemispheres ablated from level of interventricular foramen. Anterior commissure and related bed nuclei ablated. Preoptic area intact. Anterior end of epithalamus, dorsal thalamus, and ventral thalamus invaded. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 743: Epithalamus ablated. Dorsal and ventral thalami ablated except caudal remnants. Left cerebral hemisphere largely ablated. Caudal third of right hemisphere extensively invaded. Warning croak elicited.

MALE 801: Extensive invasion of dorsal and ventral thalamic nuclei and the habenular nuclei. Slight invasion of the posterior poles of the hemispheres and the nuclei suprachiasmatici hypothalami. Oviposition normal. No release after 15 hours. Eggs fertile. Warning croak elicited. Sex call heard.

MALE 802: Dorsal and ventral thalami mostly ablated, except caudal edges and lateral edge on right side. Epithalamus removed. Posterior pole of left cerebral hemisphere destroyed to level of anterior commissure. Oviposition I normal. Oviposition II normal. Release delayed 2 hours. Eggs fertile.

MALE 805: Epithalamus ablated. Dorsal and ventral thalami ablated, except caudal and ventral edges. Extensive injury to posterior third of forebrain. Warning croak elicited.

MALE 813: Hippocampi and piriform areas ablated except ventral remnants. Oviposition normal. Release immediate. Warning croak elicited. Sex call heard. Swimming reaction to estrous female observed.

MALE 815: Dorsal thalamus destroyed except remnants along the ventral edge. Slight invasion of ventral thalamus. Epithalamus ablated. Extensive invasion of the primordia hippocampi. Oviposition normal. Release delayed 4 minutes. Eggs fertile. Warning croak elicited. Sex call heard.

MALE 816: Epithalamus ablated. Small lesion in primordia hippocampi. Warning croak elicited. Sex call heard.

MALE 822: Epithalamus ablated. Dorsal edge of dorsal thalamic nuclei invaded. Warning croak elicited. Sex call heard.

MALE 823: Epithalamus extirpated. Extensive lesion in dorsal half of dorsal thalamus. Oviposition normal. Release immediate. Eggs not fertile.

MALE 831: Hypothalamus ablated except nuclei suprachiasmatici. Anterior third of tegmentum mostly ablated. Warning croak elicited. Swimming reaction to estrous female observed.

MALE 832: Hypothalamus ablated except remnants of suprachiasmatic nuclei. Ventral thalamic nuclei, pars externa invaded. Rostral end of tegmentum ablated to anterior level of oculomotor nuclei. Nucleus opticus tegmenti ablated. Oviposition normal. Release delayed 4 hours. Eggs fertile. Warning croak elicited.

MALE 841: Pars ventralis hypothalami mostly ablated except the suprachiasmatic nuclei. Oviposition normal. Release immediate. Eggs fertile.

MALE 842: Large lesion in rostral half of pars

ventralis hypothalami. Oviposition normal. Release immediate. Eggs fertile. Warning croak elicited.

MALE 844: Large lesion in caudal half of pars ventralis hypothalami. Oviposition normal. Release immediate. Eggs fertile. Warning croak elicited.

MALE 845: Lesion same as male 86. Oviposition normal. Release immediate. Eggs not fertile. Warning croak elicited.

MALE 911: Forebrain and epithalamus completely ablated. Dorsal thalamus, ventral thalamus, and hypothalamus ablated except at caudal end. Warning croak elicited.

MALE 915: Extensive bilateral lesions in hippocampi and piriform areas. Anterior commissure and preoptic area intact. Left habenular nuclei ablated, and edges of left dorsal and ventral thalami invaded. Warning croak elicited. Swimming reaction to estrous female demonstrated.

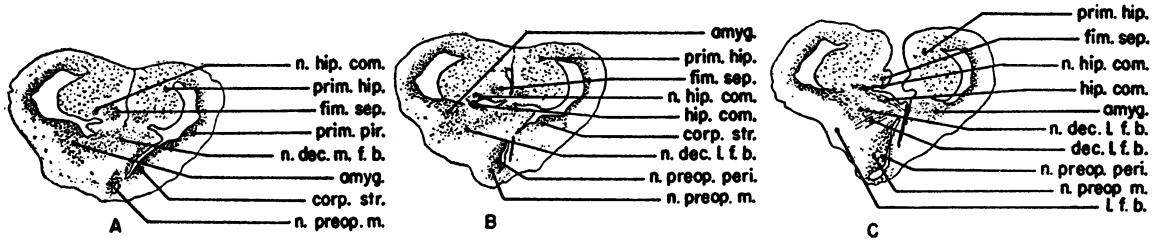

FIG. 25. Three transverse sections through the brain of operated male 918; ×9. A. Through the anterior end of the preoptic area. Lesion extended about 375μ anterior to this level. B. 90μ farther caudally through the middle of the preoptic area. C. 135μ farther caudally through the posterior part of the preoptic area. The lesion extended about 45μ caudad from this level.

MALE 901: Midsagittal cut through midbrain. At anterior end, incision extended from third ventricle to dorsal surface cutting posterior and tectal commissures. At level of inferior colliculi cut extended into the aqueduct, and from interpeduncular nucleus to medulla cut extended from dorsal to ventral surfaces. Warning croak elicited.

MALE 903: The following areas were completely ablated: tecta, inferior colliculi, cerebellum, ganglia isthmi, nucleus pretectalis, and nucleus of the posterior commissure. The caudal portions of the nuclei dorsalis thalami pars interna and externa were ablated, and the central gray around the aqueduct was severely damaged, particularly the dorsal half. Oviposition normal. Release immediate. Eggs fertile. Warning croak could not be elicited.

MALE 906: Pars ventralis hypothalami ablated except the nuclei suprachiasmatici. Some injury to ventral edge of the pars dorsalis hypothalami. Oviposition I normal. Release delayed ½ hour. Eggs fertile. Oviposition II normal. Release immediate. Warning croak elicited. Sex call heard. Swimming response to estrous female observed.

MALE 907: Lesion same as male 841. Oviposition normal. Release immediate. Eggs fertile.

MALE 910: Bilateral lesion along entire length of hippocampi slightly injuring the piriform areas. Oviposition normal. Release immediate. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 918 (FIG. 25): Left half of preoptic area largely extirpated. Anterior commissure and related bed nuclei extensively invaded. Oviposition I normal. Oviposition II normal. Release delayed 10 minutes. Eggs not fertile. Oviposition III normal. Release immediate. Eggs not fertile. Oviposition IV normal. Eggs not fertile. Oviposition V normal. No release in 15 minutes. Eggs fertile. Oviposition VI normal. Released immediately. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 920: Lesion same as male 11. Oviposition I normal. Oviposition II normal. No release in 5 hours. Eggs not fertile. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 921: Anterior third of preoptic area ablated. Slight invasion of ventral edges of septal nuclei and corpora striata. Anterior commissure intact. Oviposition I normal. No release in 5 hours. Eggs not fertile. Oviposition II normal. No release in 4 hours. Eggs not fertile. Oviposition III normal. No release in 4 hours. Oviposition IV normal. No release in 4 hours. Oviposition V normal. No release in 3 hours. Eggs fertile. Swimming reaction to estrous female noted.

MALE 922: Lesion same as male 11. Oviposition I normal. Release delayed 41 minutes. Eggs fertile. Oviposition II normal. Release delayed 9 minutes. Eggs not fertile. Swimming reaction to estrous female demonstrated. Sex call heard.

MALE 923: Lesion same as male 62. Oviposition normal. Release delayed over 2 hours. Eggs not fertile. Warning croak elicited. Sex call heard. Swimming reaction to estrous female demonstrated.

MALE 924: Lesion same as male 85. Oviposition I normal. Release immediate. Eggs not fertile. Oviposition II normal. Release immediate. Eggs not fertile. Warning croak elicited. Sex call heard. Swimming reaction to estrous female noted.

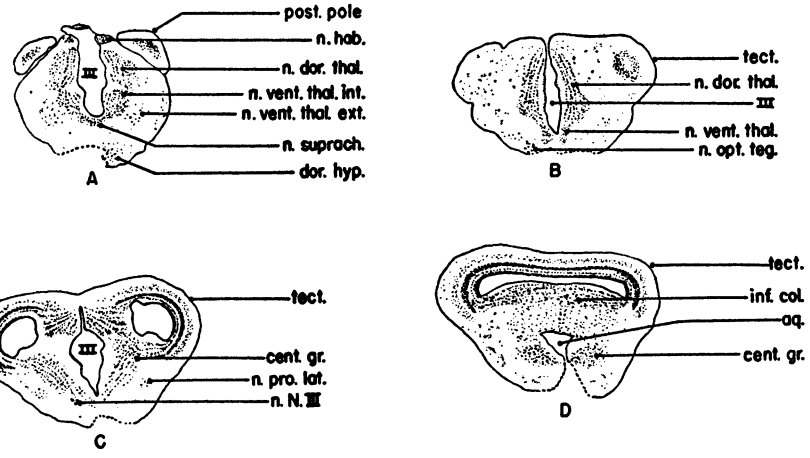

FIG. 26. Four transverse sections through the brain of operated male 928; ×9. A. Through the anterior part of the hypothalamus. Lesion extended about 150μ anterior to this level. B. 495μ farther caudally through the posterior end of the hypothalamus. C. 495μ farther caudally through the middle of the tegmentum. D. 480μ farther caudally through the posterior part of the tegmentum.

MALE 928 (FIG. 26): Hypothalamus ablated except nuclei suprachiasmatici. Midventral lesion along entire rostro-caudal extent of tegmentum. Nucleus interpeduncularis ablated and nuclei optici tegmenti damaged. At level of cerebellum, lesion extended dorsally and damaged central gray surrounding the aqueduct. Warning croak elicited.

MALE 929: Extensive lesion in primordia hippocampi at level of interventricular foramen. On left side the piriform area was invaded. Swimming reaction to estrous female demonstrated.

MALE 930: Caudal two-thirds of pars ventralis hypothalami ablated. Slight invasion of pars dorsalis hypothalami. Oviposition I normal. Release immediate. Eggs not fertile. Oviposition II normal. Release immediate. Eggs not fertile. Sex call heard. Swimming reaction to estrous female demonstrated.

MALE 936: Left cerebral hemisphere ablated. Extensive injury to primordium hippocampus and septum on right side. Anterior commissure and related bed nuclei invaded. Sex call heard. Swimming response to estrous female demonstrated.

MALE 939: Cerebral hemispheres ablated. Caudal two-thirds of preoptic area intact. Anterior commissure and related bed nuclei mostly interrupted. Epithalamus ablated. Emenentia thalami and nuclei of Bellonci invaded. Warning croak elicited. Swimming reaction to estrous female demonstrated.

MALE 941: Hypothalamus ablated. Optic chiasma destroyed. Slight invasion of caudal end of preoptic nuclei. Oviposition I normal. Release delayed 8 minutes. Eggs not fertile. Oviposition II normal. Eggs not fertile. Oviposition III normal. No release in 3 hours. Sex call heard.

MALE 942: Rostral quarter of cerebral hemispheres extirpated. Swimming reaction to estrous female demonstrated.

MALE 945: Hypothalamus ablated except remnants of nuclei suprachiasmatici. Slight invasion of anterior edge of tegmentum. Oviposition normal. No release in 4 hours. Warning croak elicited.

MALE 946: Pars ventralis hypothalami ablated except suprachiasmatic nuclei. Pars dorsalis hypothalami intact. Oviposition normal. Warning croak elicited. Sex call heard. Swimming reaction to estrous female demonstrated.

DISCUSSION

IN RECENT YEARS the once popular concept of "strict localization of function" within the brain and spinal cord has been modified by most neurologists and psychologists. Much more importance is now attributed to the functional interrelationships of the various neural systems (Papez, 1937; Bard, 1939) and to the generalized autogenous activity and plasticity of the masses of central nervous tissue, particularly the mammalian cerebral cortex (Lashley, 1930, 1942).

Nevertheless, the precise definition of anatomical and physiological centers and systems still remains a major tool in the hands of the neurological investigator (Bard, 1939), providing one constantly bears in mind that these localized areas and systems do not function independently in the intact animal; that the discrete activity hypothesized for a given region might be superimposed upon a more general function of the same nervous tissue; and that, with the destruction of a given region, the remaining parts of the brain might reorganize some of the activity of the damaged area. While these generalizations were developed mainly from mammalian brain studies, there seems little reason to doubt that they apply to the lower vertebrates as well.

WARNING CROAK

In the present investigation, the warning croak mechanism appears to be the least complex and most easily analyzed. Tactile stimulation of the back of the male is the primary stimulus which elicits the warning vocalization (Noble and Aronson, 1942).

The sensory nerves to the skin of the back carry the tactile impulses to the spinal cord, where they are relayed via the spino-tectal tracts to the inferior colliculi. This latter region appears to be a center of sensori-motor correlation which projects upon the motor centers in the medulla oblongata and spinal cord by means of the tecto-bulbar and tecto-spinal tracts. Motor nerves from the medulla and spinal cord complete the circuit by carrying the impulses to the buccal, hyoid, and abdominal muscular complex. This circuit constitutes the basic mechanism of the warning croak which functions efficiently without the participation of the forebrain, diencephalon, cerebellum, and remaining midbrain areas (superior colliculus and tegmentum) not included in this system. While this apparatus appears to be relatively independent of large areas of the brain, it is nevertheless conceivable that these regions do exert a slight regulatory influence on the warning croak as suggested by the work of Goltz (1869), Bechterew (1884), and Blankenagel (1931).

Glass and Rugh (1944) have demonstrated a sharp decrease in the amount of interstitial tissue of the testes of *Rana pipiens* during the summer months, and this in turn probably results in a reduced output of testicular hormone, although there is still some question as to the exact origin of this secretion. It has previously been noted that it is more difficult to elicit the warning croak during the summer months after the breeding season (Noble and Aronson, 1942), and hence this reduced vocality may be correlated with a low production of testis hormone. If we accept the prevalent hypothesis that the gonadal hormones sensitize certain central nervous mechanisms (Lashley, 1938) and reduce the thresholds of the motor circuits mediating or facilitating sexual responses (Beach, 1942), it must then be concluded that the testicular hormone acts either upon the spinal cord, medulla oblongata, caudal tegmentum, or inferior colliculus for the facilitation of the warning croak.

An alternative explanation of the increased difficulty of eliciting the warning croak after the breeding season cannot be excluded. It has been known for many years that after the spawning period, males of many species of Anura undergo pronounced somatic changes. The most striking of the external changes in *Rana pipiens* are the regressions of the thumb pad and the brachial and antibrachial musculatures associated with the clasp reflex. Glass and Rugh (1944) cite evidence showing that the secondary sex characters of Anura are controlled by gonadal secretions, although the secretions of the anterior pituitary gland may stimulate these sex characters directly. Blair (in MS) has shown that in juvenile male and female *Bufo*

fowleri the size of the laryngeal vocal apparatus is strongly influenced by mammalian sex hormones, particularly testosterone propionate. It is conceivable that in *Rana pipiens* the waning of the warning croak response after the breeding season is due in part to a regression of the laryngeal vocal apparatus.

SEX CALL

The stimuli which call forth the sex croak are not well known, nor have we been able to define clearly the central nervous mechanisms responsible for this behavior. As for the stimuli, it can be said that the voice of one calling male appears to stimulate other males to call, and if the experimenter imitates the sex call, the frog will sometimes give the response. Males often call as they swim after a female, and the sex call and warning croak are sometimes combined into what we have termed the sex-warning croak (Noble and Aronson, 1942). Thus, optic and auditory stimuli appear to arouse the sex call, and other sensory stimuli may also be effective (Noble, 1931).

With this meager information we can outline the sex call mechanism only in a very tentative fashion. Optic impulses to the tectum are relayed to the inferior colliculi, as are auditory impulses from the cochlear nuclei in the medulla oblongata (nucleus dorsalis magnocellularis). Since in one of our operates (male 233) the sex call was heard following ablation of the tectum and inferior colliculi, the sex call mechanism probably includes other midbrain areas, possibly the ganglion isthmi, as we have already suggested. This region would then correlate the optic and auditory impulses with the other sensory stimuli which arouse the sex call, and would project on the lower motor centers.

CLASP REFLEX

Our observations on the effects of brain injury on the clasp reflex confirm the findings of many previous investigators (Tarchanoff, 1887; Albertoni, 1887; Schrader, 1887; Steinach, 1910; Busquet, 1910, 1910a), indicating that midbrain lesions affect the clasp reflex. Most of these authors hypothesized clasp inhibitory centers located in the diencephalon, midbrain, cerebellum, and medulla oblongata, since with the removal of these areas, chronic clasping would result. We are more inclined to agree with Baglioni (1913) who noted that the clasping behavior after midbrain injury is qualitatively different from the sexual clasp of the intact animal. Hence it is better to consider the midbrain mechanism as a modifier rather than an inhibitor of the spinal clasp reflex.

Starting with extensive thalamic invasions and transections, we noted mild changes in the clasping behavior. As our invasions and transections were placed farther and farther caudally, the clasp became modified to a greater and greater extent with the greatest modification occurring after transections through the medulla.

In view of these observations, together with the fact that no specific loci for the modification of the clasp were located, and considering that previous investigators had postulated various inhibitory centers in the diencephalon, midbrain, cerebellum, and medulla, it is probably better to consider the total structure of these regions as normally contributing to the modification or regulation of the spinal clasp reflex.

SPAWNING MOVEMENTS

In our previous communication (Noble and Aronson, 1942) it was hypothesized that the stimuli for the male's ejaculatory responses derived from the oviposition movements of the female, and that these stimuli impinged upon the sensory receptors in the skin of the ventral pectoral region and ventral surfaces of the forelimbs. Sensory nerves from the receptors convey impulses to the spinal cord whence they possibly are transmitted to the tegmentum by the component of the spinotectal tract (Papez, 1929) which terminates in this region.

Herrick (1936) notes that in the motor tegmentum many of the most fundamental patterns of behavior are organized. In *Amblystoma*, according to Herrick, the tegmentum "receives no sensory fibers directly from the periphery except the nervus terminalis and the basal optic tract (tractus opticus accessorius posterior), most of its excitations coming from the centers of adjustment—cerebral hemispheres, all parts of the diencephalon, tectum mesencephali, cerebellum, and the sensory field of the medulla oblongata."

If Herrick's analysis of the tegmentum of *Amblystoma* can be applied to *Rana*, it would appear that the sensory excitations for the ejaculatory movements must come from the sensory field of the medulla oblongata, since the forebrain, diencephalon, tecta, inferior colliculi, and cerebellum may be removed in their entirety without any noticeable disturbances in this behavior.

It is of interest to recall that Spallanzani (1786) reported normal spawnings of decapitated males. However, it is not clear whether Spallanzani actually witnessed the oviposition, or whether he judged their normality by the resulting embryos. Since one of our males with a large tegmental lesion fertilized some eggs without showing any spawning or ejaculatory motions during an oviposition with a normal female, it appears that some sperm may be discharged without the customary movements that accompany sperm emission.

RELEASE

In our study of the normal behavior of *Rana pipiens*, we postulated on the basis of various bits of experimental evidence that the release of the female by the male at the termination of the oviposition was due to a complex of factors which included (1) the ejaculation of the male, (2) the reduction in girth of the female, (3) the cessation of the female's oviposition movements, and (4) the movement of the female from the egg-laying posture. It is believed that if the sum of the sensory stimuli resulting from these actions surpasses a certain minimum, release will occur.

In mammals the genital organs are innervated in part by the autonomic nervous system (Stone, 1923; Semans and Langworthy, 1938), and the ejaculation is considered by some to include an autonomic discharge. In Anura the genital organs are similarly innervated (Gaupp, 1899), and an autonomic regulation of the ejaculatory response can be hypothesized. The hypothalamus has long been recognized as an autonomic center for the control of visceral responses, and it now appears likely that invasions of the hypothalamus and its rostral extension, the preoptic area, result in a disturbance of the autonomic discharge associated with the ejaculatory response, thus accounting for the failure of many of the males with such brain injury to release the female when the spawning terminates.

From table 5 it is seen that, following extensive hypothalamic and preoptic invasion, a few males still released. These exceptional cases can be accounted for by assuming that the sum of the stimuli received from the other three factors causing a male to release was greater than the necessary minimum. Reduction in girth and movement of the female seemed particularly important in these cases, and some males were actually seen to slide off the thin, actively moving females.

SWIMMING RESPONSE

The swimming reaction of the sexually active male towards the estrous female is primarily a visually directed response (Noble and Aronson, 1942). Since some completely decerebrate males exhibit this behavior, the forebrain cannot be considered a basic link in the neural mechanisms responsible for this behavior. Moreover, a few observations (males 54, 114, 116, 118, 324, 331) indicate that the response can sometimes be elicited after extensive diencephalic injury. It is not until the midbrain is destroyed that the response is completely lost. Thus we should consider the swimming response to the estrous female as being integrated primarily by the midbrain.

The role of the forebrain in the mediation of the swimming response appears to be that of a facilitator or regulator. In this sense, the part played by the forebrain is comparable to that of the cerebral cortex in mammals, where the cortex is held to facilitate or control the "ease of arousal of the copulatory pattern which is mediated by the lower centers of the brain stem and spinal cord" (Beach, 1940, 1942). This interpretation of the function of the mammalian cerebral cortex in relation to mating behavior agrees in part with Herrick's conception of the function of the cerebral hemispheres in Amphibia. He states that in Amphibia "The cerebral hemispheres regulate all diencephalic and mesencephalic functions, with a strong preponderance of olfactory influence. Inhibition, conditioning of reflexes, an intrinsic activity which expresses itself as so-called spontaneity, and a general

facilitation of lower functions are characteristic expressions of the activity of the cerebral hemispheres, and these are all represented at a low level of efficiency in the amphibian brain" (Herrick, 1933a).[1]

A second function of the forebrain, namely, the correlation of the various sensory impulses relayed from the dorsal thalamus, is weakly developed in Amphibia, as reflected in the sparsity of thalamic radiations, and the presence of any correlative activity did not manifest itself in the foregoing experiments.

The medial wall of the amphibian cerebral hemisphere (hippocampus and septum), the preoptic area, and hypothalamus form an integrated system (Herrick, 1933). This system is elaborated in higher vertebrates and humans, and here it is believed to regulate the "conscious" or emotive state (Papez, 1937, 1937a, 1939). It seems probable that in *Rana pipiens* this system is the one which is involved in the facilitation of the swimming reaction of the male towards the clasp object.

Our findings suggest that as far as this generalized facilitative function is concerned, the medial wall of the hemisphere and the preoptic area are not functionally discrete; for as long as either of these regions remains, the response is readily exhibited, but if both are removed the response occurs only rarely. Although our data on this point are incomplete, they can be interpreted as indicating that this facilitative function of the forebrain can be maintained by a relatively small amount of forebrain tissue which remains in contact with the diencephalon. The fact that removal of most of the hypothalamus does not markedly reduce the swimming response seems to indicate that the system is still more diffuse than outlined above.

As already noted in the review of the literature a number of authors have recognized a facilitative function of the forebrain by recording the loss of spontaneity subsequent to forebrain deprivation. While Blankenagel (1931) postulated two discrete centers in the caudo-ventral part of the forebrain, one for the mediation of feeding reactions and the other for the swimming reaction to the female, our observations, agreeing with those of Diebschlag (1934), suggest that the hypothalamic-preoptic-septo-hippocampal mechanism outlined above regulates all mass movements requiring extensive sensori-motor integration. In the frog these mass movements are particularly involved in the reactions to food, response to a clasp object, and by general activity.

It is rather difficult to harmonize our results with the findings of those authors who found no loss of "spontaneity" following forebrain extirpation (Desmoulin, 1825; Schrader, 1887; Burnett, 1912). Schrader attempts to explain this discrepancy by assuming that other investigators inadvertently damaged the diencephalon while removing the forebrain, and we could likewise assume that Schrader and Burnett left the preoptic area intact. However, since none of the previous investigators included detailed descriptions of the lesions which they inflicted, such postulations are fruitless.

There are other explanations which might be advanced to account for these contradictory findings. We have found that forebrain extirpation and extensive diencephalic injury neither alter nor abolish the swimming response; these operations merely reduce the frequency with which this behavior is displayed. Hence the discrepancies noted above might be due to a failure to test carefully a sufficient number of operated animals. Finally, some degree of recovery of function can be expected after every injury to the central nervous system (Lashley, 1938a). The majority of studies have dealt with more or less immediate postoperative changes, whereas Schrader first tested his operated frogs after allowing them to hibernate over the winter.

Males castrated prior to the breeding season do not exhibit the swimming response towards the estrous female (Schrader, 1887), while in the intact animal this behavior is enhanced by anterior pituitary injections (Rugh, 1935a). The effectiveness of the pituitary treatment is believed to be due to in-

[1] A recent experiment by Detwiler (1944, Proc. Soc. Exp. Biol. Med., vol. 56, pp. 195–196) is of interest in this connection. He found that the lateral line system alone was sufficient to elicit feeding reactions in *Amblystoma* larvae whose eyes and nasal organs had been removed. While this activity was carried out in a normal integrated manner in animals that also lacked the forebrain, feeding was reduced in both amount and vigor when compared to those larvae with similar sensory deprivations but having intact cerebral hemispheres.

creased hormone output by the testes resulting from gonadotropic effects of the pituitary substance. In other words, male hormone secreted by the testes somehow modifies the functional condition of the central nervous elements mediating the swimming response. In this respect the hormonal and nervous mechanism is similar to that obtaining in mammals where the injection of testosterone propionate "increases the male's susceptibility to sexual excitement" (Beach, 1942b). It has been suggested (Beach, 1942) that the gonadal hormones may increase the excitability of a hypothetical "central excitatory mechanism" and simultaneously lower the thresholds of the integrative circuits wherein the male copulatory patterns are organized. Moreover, there are indications that the cerebral cortex contains or is the "central excitatory mechanism." Whether, in Amphibia, the male hormone affects some facilitative mechanism in the forebrain, or acts only on the basic sensori-motor apparatus in the midbrain and lower centers has yet to be determined.

COMPARISON WITH MAMMALS

In this discussion six discrete phases of sexual activity of the male have been considered. The clasp response is basically a spinal reflex, but it appears to be regulated and modified by some diffuse generalized activity of the diencephalon, midbrain, cerebellum, and medulla oblongata. The warning croak is integrated by a midbrain mechanism localized in the inferior colliculi, and this response is relatively independent of the higher centers. The sex call is probably mediated by a midbrain mechanism of a more diffuse nature than that responsible for the warning croak. The spawning movements are also controlled by midbrain elements localized in the tegmentum, and the release of the female by the male after spawning is dependent upon circuits in the pars dorsalis hypothalami, and the preoptic area. Although the swimming response towards the clasp object appears to be mediated basically by a neural mechanism situated in the midbrain, it is so strongly dependent on the facilitory action of the forebrain that extirpation of this part of the brain eliminates this behavior to a very large degree.

These six phases of the male frog's sexual behavior are discrete to the extent that each one can be elicited as long as its own neural circuits remain intact. Each of the behavioral items studied appears to function relatively independently of the neural mechanism important for the other phases.

Comparing our findings with the extensive literature on the neural mechanisms controlling sexual behavior in mammals, it appears that some general relationships exist. The comparison between the action of the forebrain in facilitating the swimming response of the male frog towards the estrous female, and the facilitative activity of the cortex in controlling the ease of arousal of the copulatory pattern in the male rat has already been discussed.

Beach (1944b) reports that in the rat "the changes produced by forebrain injury instead of being specifically sexual, affect responsiveness to a wide variety of nonsexual stimuli. . . . The reduced sexual responsiveness of the decorticate male is merely one reflection of a general loss of reactivity to a wide range of environmental situations." A similar situation exists in the male frog. Decerebration not only decreases the frequency of the swimming responses of the male frog towards the clasp object but reduces the male's responsiveness to other environmental stimuli, particularly food.

Investigation of the relationship of subcortical brain centers to mating behavior in male mammals has not been made, and hence direct comparisons with male Anura are not possible. Since the sexual patterns of female mammals are dependent to a much lesser extent than the males upon an intact cortex (Beach, 1944a), numerous investigators have studied the effects of subcortical lesions on female mating behavior. It appears from these studies that in the female guinea pig, rat, and cat, the appearance of the female mating pattern depends upon the integrity of a relatively small discrete region in the hypothalamus or anterior edge of the mesencephalon (reviewed by Bard, 1940; Young, 1941; Beach, 1942a). Here we have a rather striking comparison with male frogs where we have described an area of the tegmentum which must be intact for the proper performance of the spawning movements.

It is conceivable that if one could study certain phases of the sexual behavior of male mammals which were not strongly dependent for their elicitation upon cortical facilitation (or upon previous acts dependent upon cortical facilitation), discrete subcortical areas might be discovered upon whose integrity the behavior in question would depend.

In the foregoing experiments we have surveyed the effects of brain lesions upon the major aspects of the male's sexual behavior. On the basis of our results, together with the observations of previous investigators and particularly the extensive literature on mammalian sexual behavior, we have formulated some hypotheses on amphibian brain function. It remains for subsequent investigations to test these formulations in a much more rigid and quantitative manner.

SUMMARY AND CONCLUSIONS

THESE EXPERIMENTS were designed to survey the effects of brain injury on certain outstanding phases of the mating behavior of the male *Rana pipiens*. The normal mating pattern has been described, and the nuclear pattern and other important landmarks of the *pipiens* brain were reviewed briefly.

Quantitative records of behavior after various types of brain injury revealed the effects of various lesions upon: (1) the warning croak; (2) the swimming response of the male towards the estrous female; (3) the spawning movements of the male which synchronize with the oviposition movements of the female; and (4) the release of the female by the male at the termination of the oviposition. Qualitative observations were also made on the effects of brain injuries upon the sex call and clasp reflex.

Ablation of all the forebrain excepting the preoptic area caused no changes in the behavior studied. Following the removal of the entire forebrain (including the preoptic area), the tendency of males to pursue and attempt to clasp estrous females was markedly reduced, but the swimming reaction was not completely abolished unless the midbrain was also extensively invaded. When forebrainless males were placed upon ovulated females, normal amplexus and oviposition followed. Males with this type of lesion did not release the female at the end of the oviposition. Lesions to, or ablation of, the preoptic area abolished the release behavior but not the male's swimming response to the female.

Removal of the entire forebrain, diencephalon, optic lobes, cerebellum, and anterior tegmentum did not interfere with the male's spawning movements, as evidenced by the recovery in some instances of fertilized eggs. Lesions in the tegmentum at the level of the motor nucleus of the trochlear nerve markedly disturbed or completely abolished these spawning responses.

Complete ablation of the forebrain, diencephalon, tectum (superior colliculus), cerebellum, and anterior tegmentum did not interfere with the mediation of the warning

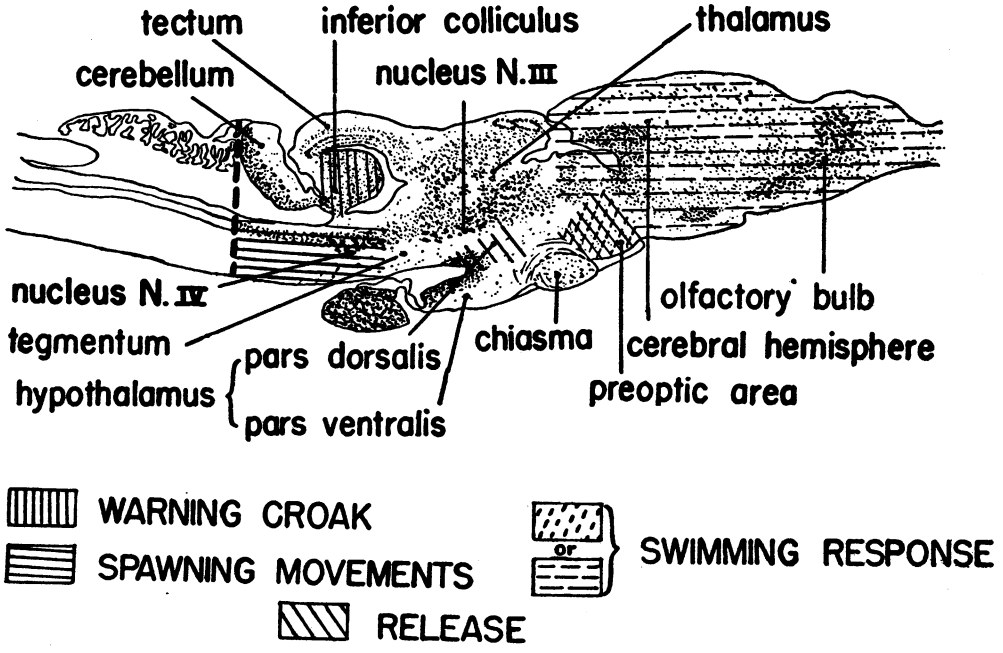

FIG. 27. Diagrammatic sagittal section through the brain of *Rana pipiens* indicating the regions of the brain that were found to be of primary importance for the mediation of each of four phases of sexual behavior.

croak, but extensive invasion of the inferior colliculi abolished this response.

The regions of the brain which we have found to be important for the mediation of each of these four phases of the male's sexual activity are indicated diagrammatically in figure 27.

Qualitative observations indicated that the sex call sometimes survived ablation of the forebrain, diencephalon, optic lobes, cerebellum, and anterior tegmentum. The sexual clasp reflex was modified by a variety of lesions in the diencephalon, midbrain, and anterior edge of the medulla oblongata. It appeared that the more extensive invasions and especially lesions placed farther caudally modified the clasp reflex to a greater extent than equal amounts of destruction to anterior brain areas.

On the basis of our results, together with the findings of other investigators, the following hypotheses have been tentatively formulated:

1. The clasp response is basically a spinal reflex which is modified by a diffuse mechanism situated in the diencephalon, midbrain, cerebellum, and medulla oblongata.

2. The warning croak is integrated in the inferior colliculi and is relatively independent of higher centers.

3. The nervous mechanism mediating the sex call is probably located in the midbrain but is somewhat more diffuse than the warning croak.

4. The spawning movements are integrated in the tegmentum at the level of the motor nucleus of the trochlear nerve.

5. The neural mechanism controlling the release of the female by the male after the oviposition is situated in the pars dorsalis hypothalami and preoptic area of the forebrain and is probably related to the autonomic functions generally attributed to this region.

6. The swimming response of the male towards the estrous female is basically a midbrain response which is strongly facilitated by a forebrain mechanism located for the most part in the preoptic area and medial walls of the cerebral hemispheres.

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ABBREVIATIONS FOR ALL FIGURES

III, third ventricle
a. preop. l. (or lat.), lateral preoptic area
a. pret., area pretectalis
amyg., amygdala
aq., aqueduct
cent. gr., central gray
ch., chiasma opticus
com. tect., tectal commissure
corp. str., corpus striatum
dec. l. f. b., decussation of lateral forebrain bundle
dec. m. f. b., decussation of medial forebrain bundle
dor. hyp., pars dorsalis hypothalami
em. thal., emenentia thalami
epistr., epistriatum
f. int., foramen interventriculare
fim. sep., pars fimbrialis septi
gang. isth., ganglion isthmi
hab. com., habenular commissure
hip. com., hippocampal commissure
hyp., hypophysis
inf. col., inferior colliculus
l. f. b., lateral forebrain bundle
lat., lateral ventricle
m. f. b., medial forebrain bundle
N. II, optic nerve
N. III, oculomotor nerve
n. N. III, nucleus of oculomotor nerve
n. N. IV, nucleus of trochlear nerve
n. Bell., nucleus of Bellonci
n. Bell. neur., nucleus of Bellonci neuropil
n. dec. l. f. b., bed nucleus of the decussation of the lateral forebrain bundle
n. dec. m. f. b., bed nucleus of the decussation of the medial forebrain bundle
n. dor. thal., nucleus dorsalis thalami
n. dor. thal. ext., nucleus dorsalis thalami pars externa
n. dor. thal. int., nucleus dorsalis thalami pars interna
n. gen. l. (or lat.), nucleus geniculatus lateralis
n. gen. l. (or lat.) neur., nucleus geniculatus lateralis neuropil
n. hab., nucleus habenularis
n. hip. com., bed nucleus of hippocampal commissure
n. int., nucleus interpeduncularis
n. lat. pro., nucleus lateralis profundus
n. mes. V, nucleus mesencephalicus trigeminus
n. op. teg., nucleus opticus tegmenti
n. post. com., nucleus of the posterior commissure
n. preop. m., nucleus preopticus pars medialis
n. preop. mag., nucleus preopticus pars magnocellularis
n. preop. peri., nucleus preopticus pars periventricularis
n. sep. l., nucleus septi lateralis
n. sep. m., nucleus septi medialis
n. subhab., nucleus subhabenularis
n. suprach., nucleus suprachiasmaticus
n. vent. thal., nucleus ventralis thalami
n. vent. thal. ext., nucleus ventralis thalami pars externa
n. vent. thal. int., nucleus ventralis thalami pars interna
post. com., posterior commissure
post. pole, posterior pole of cerebral hemisphere
prim. hip., primordium hippocampi
prim. pall. d., primordium pallii dorsalis
prim. pir., primordium piriforme
rec. preop., preoptic recess
s. endorh., sulcus endorhinalis
s. intrahyp., sulcus intrahypothalamicus
s. lim. hip., sulcus limitans hippocampi
s. lim. lat., sulcus limitans lateralis
s. med., sulcus medius
s. sep. pall., sulcus septopallialis
s. subhab., sulcus subhabenularis
s. vent., sulcus ventralis
str. alb. cent., stratum album centrale
str. gr. cent., stratum griseum centrale
str. med., stria medullaris
str. op., stratum opticum
str. peri., stratum griseum et fibrosum periventriculare
str. sup., stratum fibrosum et griseum superficiale
tect., tectum opticum
teg., tegmentum
tr. c. hab., tractus cortico-habenularis
tr. olf. hab. l., tractus olfacto-habenularis lateralis
tr. opt., tractus opticus
tr. opt. lat., tractus opticus lateralis
tr. opt. med., tractus opticus medialis
vent. hyp., pars ventralis hypothalami
vent. opt., optic ventricle
z. lim. l., zona limitans lateralis
z. lim. m., zona limitans medialis