

THE EFFECT OF EXPERIMENTAL EXCISION OF
ONE EYE ON THE DEVELOPMENT OF THE OPTIC
LOBE AND OPTICUS LAYER IN LARVAE OF THE
TREE-FROG (*HYLA REGILLA*)

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TWELVE FIGURES

INTRODUCTION

The experiment of Burr ('20) indicates that the cerebral hemispheres develop normally, at least up to a certain stage, in *Amblystoma*, although deprived of olfactory stimuli, provided the olfactory fibers make their normal growth into the forebrain. Burr concludes that it is the stimulus of the ingrowing nerve fibers, rather than that of any nervous impulses normally carried by them, which activates the development of the forebrain. Whether such growth of the brain, having once begun, would continue if the fibers are caused to degenerate after they have reached their destination in the larval brain, but before the brain has developed far, constitutes a problem of considerable interest in connection with the factors which control the development of the nervous system.

In the present report will be presented the effect on the development of the optic lobe of the frog produced by enucleation of one eye at a larval stage in which the retina is well developed and fibers of the optic nerve have entered the opticus layer of the optic lobe. It is well known that in the frog the optic fibers, which have their origin in the ganglionic layer of the retina, undergo complete decussation in the optic chiasma and terminate in the optic lobe of the opposite side.

Removal of one eye, therefore, gives a clean-cut experiment and control in the same experimental animal, eliminating individual variations of development and other complicating factors.

MATERIAL AND METHODS

The tree-frog, *Hyla regilla* (B. and G.), was used in the experiments herein reported and also in the observations of development of the optic lobes of normal larvae at various stages. In *Hyla* the retina and optic tracts, and with them the optic lobes, have apparently begun to function by the 16-mm.-total-length stage, and probably much earlier, so far as may be judged from the histological differentiation of the retina and from observation of the avoiding reactions to optic stimuli of these larvae in the living state. The layers of the retina are well differentiated at the 16-mm. stage, and the ganglionic cells give off optic fibers which penetrate into the optic lobes, as more fully described below. The living larvae are free-swimming and active, and avoid, as they swim about, obstacles in the water, and, unless approached with the greatest care, they avoid also the net or other instrument for removing them from the water, as well as other moving objects, obviously because of optic stimulation. It seems safe to conclude that by the 16-mm. stage, and probably much earlier, the retina, the optic tracts, and the optic lobes have begun to function, but no attempt has been made to determine the exact stage at which this functioning commences. To allow for any possible individual variation in rate of development, the larvae selected for enucleation of the eye were from 18-mm. to 22-mm. total length. The bulbus oculi, always the left, was removed from fifty larvae, after which they were allowed to develop without further interference. A pair of fine, flattened needles was used to remove the eyeball, the operations being performed under the binocular microscope. After the operative procedure, the larvae were transferred, without further treatment, to a dish of tap-water in which they recovered from the anaesthetic sufficiently in a few minutes to be placed into an aquarium con-

taining algae and small aquatic animals, in which they were raised to metamorphosis. Late stages of metamorphosis were reached twenty-five to thirty days after the stage of operation. This length of time appears to be normal for summer larvae of *Hyla regilla* in this region, as observation for several years of control larvae in captivity and also of larvae in ponds has indicated.

After some preliminary operations in which the customary chloretone was used as an anaesthetic, paraldehyde was resorted to instead. Paraldehyde is said to be entirely oxidized in the tissues, leaving no toxic substances which could be injurious to the developing larvae. Four or five drops of a 10 per cent aqueous solution of paraldehyde in about 15 cc. of water was found sufficient to immobilize the tadpoles in five to ten minutes.

The newly metamorphosed young frogs and larvae at various stages of metamorphosis were fixed in Bouin's fluid, in Carnoy's fluid, or in vom Rath's mixture. Serial paraffin sections of the Bouin and Carnoy material were stained in hematoxylin, with orange G, Congo red, erythrosin, or eosin as counterstains. The vom Rath material was mounted as serial sections without further staining. Sections were cut at 5 μ to 15 μ . A number of operated larvae at various stages were prepared by the method of Ramón y Cajal, with the object in view of a detailed study of the histogenesis of the nerve cells in the affected regions of the brain, but with unsatisfactory results in technique. Six of the fully metamorphosed young frogs or larvae in late stages of metamorphosis were sectioned and showed such uniform results in the effect on the optic lobes that it was thought unnecessary to prepare additional series for study of the general effects of the experiment. A model of the optic lobes and cerebellar region of one specimen was prepared by the blotting-paper method. In addition to a study of the morphological changes, the general histological picture of the parts affected was obtained from the sections. The more detailed study of stages of histogenesis, which it was hoped that the Cajal material

would make possible, must await a repetition of the experiment, with more favorable results from the silver impregnation.

More detailed studies of the normal differentiation of the structural elements of the optic lobes at various stages of development of the normal *Hyla* must also be made before a satisfactory comparison with experimental material is possible, with respect to histogenesis and many details of histological structure. Such studies must be accompanied by physiological controls before and after the operative procedure. This work is in progress, but bids fair to require a long time for its completion. It appears desirable, therefore, to present the morphological and general histological results at the present time and to report the more detailed findings later as another phase of the problem.

DESCRIPTIVE

The more obvious results on the optic lobe of enucleation of the eye at the stages indicated above are apparent at a glance in figure 1. It will be noted that the right optic lobe, to which the tract from the left eye normally passes, is reduced approximately one-third in volume, as compared with

ABBREVIATIONS FOR ALL FIGURES

<i>ax.1</i> , first axis of measurement	<i>str.med.pr.</i> , deep medullary layer (seventh layer of Gaupp)
<i>ax.2</i> , second axis of measurement	<i>tr.op.</i> , optic tract
<i>cb.</i> , cerebellum	<i>tr.op.ax.</i> , axial bundle of optic tract
<i>fi.</i> , nerve fibers	<i>tr.sp.t.</i> , spino-tectal tract
<i>hyp.</i> , hypophysis	<i>vent.III</i> , third ventricle
<i>lo.opt.dex.</i> , right optic lobe	Numerals in figures, based on Gaupp, p. 58:
<i>lo.opt.si.</i> , left optic lobe	1, epithelial layer
<i>med.obl.</i> , medulla oblongata	2, narrow subepithelial granule layer
<i>n.II</i> , optic nerve	3, inner molecular layer
<i>n.II r.po.</i> , posterior root of optic nerve	4, narrow middle granular layer
<i>n.III</i> , oculomotor nerve	5, outer molecular layer
<i>n.IV</i> , trochlear nerve	6, broad outer nuclear layer
<i>n.V</i> , trigeminal nerve	7, deep medullary layer
<i>n.VII, VIII</i> , facial and acoustic nerve roots	8, intermediate granule layer
<i>str.opt.</i> , opticus layer of optic lobe (ninth layer of Gaupp)	9, superficial medullary or opticus layer

the normal left lobe. The right optic nerve and left optic tract remain intact in these tadpoles, but the left nerve and its continuation, the right optic tract, completely degenerated subsequent to removal of the left eyeball. Study of the sections and comparison with sections of normal *Hyla*, both at metamorphosis and in the adult, indicate that the great reduction in volume of the lobe is due directly only in small part to absence of the fibers, as more fully described below.

Other effects in our tadpoles, such as atrophy of the eye muscles, changes in the cranial wall of the operated side, etc., were also noted, confirming in these respects, as in the



Fig. 1 Photograph of model of optic lobes and cerebellum of *Hyla* (III-5, *Exp.*) from which the left eye had been excised at 20-mm. total-length stage.

reduced volume of the optic lobe and atrophy of the optic nerve and tract, the results described by Steinetz ('05) and Dürken ('13). The latter author, however, states that, in addition to the modifications noted above, some of his larvae also showed various malformations of the limbs, which he explains as due to a correlation mechanism of development between the various embryonic organs. In my experience I have never noted any effect on the developing legs of larvae from which the eyeball had been removed.

There appears to be a slight reduction of the cerebellum in my specimens. The model (fig. 1) is merely suggestive in this respect, and the question must be left open. Such an

effect, if any, must involve the tecto-cerebellar tract, which is quite small in *Hyla*, although it is present, as a restudy of my Golgi series shows. It is necessary here to correct an error in a previous publication (Larsell, '25, p. 286) in which it was stated that this tract was not found in larval *Hyla*. In an earlier paper (Larsell, '23), based chiefly on adult *Rana pipiens*, the tract is described and figured. I can now state definitely that it is present in the larvae of *Hyla* also. It passes more ventrally than in *Rana pipiens*, corresponding more closely to the description and figure of Röthig (fig. 362, Kappers, '21) for *Rana temporaria*.

Excision of both eyes instead of one in the young larvae should produce a more pronounced effect on the developing cerebellum, if any results, but Steinetz ('05), who carried out such an experiment with reference to the effect on the brain in general, makes no specific mention of the effect on the cerebellum, but states that no changes from the normal were found in those brain parts not directly related to the eye or the optic tracts. Ramsey ('01) also found no variation from normal size in the cerebellum of the blind-fish, *Amblyopsis*, as compared with the cerebellum in several species of seeing fishes.

In the following description of the optic lobes the subdivisions into layers used by Gaupp ('99) rather than that of Pedro Ramón y Cajal ('90) will be employed. The ninth or optic layer of Gaupp, which Ramón subdivides into seven layers, making fifteen in all, is not sufficiently differentiated into strata in our larvae to make possible the application of Ramón's designations. To avoid confusion, the optic layers will therefore be considered as a unit in all stages, and will be designated as the ninth or the optic layer. The other layers, namely, one to eight, will be designated according to the usage of both Ramón and Gaupp, who adopt the same numbering for them.

The optic layer contains numerous cells, described by Gaupp (p. 60) as constituting neurones of several types. These cells, as indicated by nuclei, are present in increasing

numbers in later stages of our larvae. As shown in table 1, the number tends to become stabilized after metamorphosis. These neurones are not shown in Pedro Ramón's figure, from Golgi preparations, of the optic lobes of the frog (fig.

TABLE 1

Number of nuclei in 0.1 mm. rostro-caudal thickness of the eighth and ninth layers of the optic lobes in Hyla regilla, at various stages of development and in two experimental specimens. The sections in which nuclei were counted began at or near the rostral level of the oculomotor roots and continued toward the caudal pole of the optic lobe

SPECIMEN	THICKNESS OF SECTIONS IN MICRA	NUCLEI IN EIGHTH AND NINTH LAYERS	NUCLEI IN NINTH LAYER	NUCLEI IN OCULAR FIELD 5 MM. LONG AND WIDTH OF EIGHTH AND NINTH LAYERS, USING 10X OC., 8-MM. OBJ.	
				Eighth layer	Ninth layer
16-mm. total length	10	1550	470		
18-mm. total length	10	2263	774		
18-mm. total length	5	2125	760		
22-mm. total length	10	2176	913		
8-mm. tail length	10		1465		
0.5-mm. tail length	10		1504		
II-3 Exp. 0.5-mm. tail length	15	3379 experimental side 5332 normal side	846 experimental side 1557 normal side		
VI-2 Exp. 3-mm. tail length	10	4208 experimental side 5536 normal side	788 experimental side 1622 normal side	945 experimental side 1224 normal side	329 experimental side 420 normal side

143, S. Ramón y Cajal, '11), although in figure 142 of the same work, which figure was prepared from a Weigert-Pal and carmine-stained series, nuclei are shown from the ninth layer of Ramón and outward, corresponding to the distribution in our larvae. In hematoxylin and carmine sections it is not possible to determine if these are the nuclei of neurones

or of neuroglia cells, or of both. This is a point of considerable importance in the interpretation of our results. Examination of about ninety Golgi series of various larval stages of *Hyla* shows that many of the cells of the opticus layer are definitive neurones (fig. 2) by the 42-mm. stage, which in *Hyla regilla* is the largest size usually attained by the larvae just preceding the changes in tail length and body form attending the beginning of metamorphosis. Neurones also were observed in Golgi sections of earlier stages, and

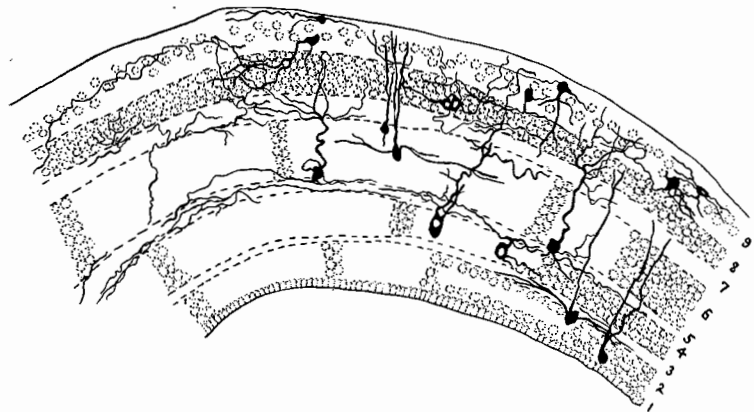


Fig. 2 Composite drawing from several Golgi series of *Hyla* of 42-mm. total length, with small hind legs and no fore legs. The outline of the section and position of layers were drawn from a section of horizontal series L 34 with the camera lucida. Cells were filled in with the help of the camera lucida, chiefly from horizontal sections, but with some from sagittal sections. $\times 155$.

in 18-mm. and 22-mm. larvae a few fairly well differentiated neurones can be seen, in their early stages, in hematoxylin sections, in the opticus layer. These observations are confirmed by a study of a number of Golgi series of adult *Rana pipiens*, in which the same types of neurones, although further differentiated, are found in the opticus layer and in the eighth layer, as are described by Gaupp for *Rana* and found in *Hyla*.

In the other layers of the optic lobe my observations are in accord with those of Gaupp and of Ramón, so far as the latter

are indicated in S. Ramón y Cajal's "Histologie du Système Nerveux." I have not had access to Pedro Ramón's original paper on this subject. In larval *Hyla* the neurones are not as fully differentiated as in *Rana* or as represented in Ramón's figures of the frog (*Rana*?), but the arrangement of the neurones in the several cellular layers, the disposition of cell processes, and the relation of incoming fibers to the various layers are similar in my preparations to those figured by Ramón, with the additions above noted in the eighth and ninth layers.

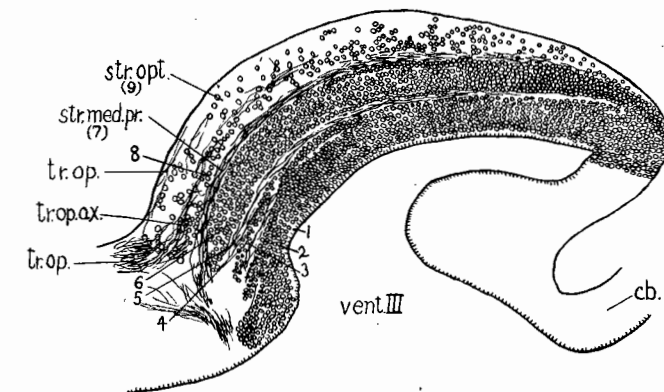


Fig. 3 Longitudinal section through optic lobe of *Hyla* of 16-mm. total length, cut at an angle of about 45° to the sagittal plane. Method of Ramón y Cajal. Cam. luc. $\times 94$.

The reduced size of the right optic lobe in our experimental material of *Hyla* appears due to failure of some of the elements in this region of the brain to develop normally. The degree of general histological differentiation of the midbrain cortex in larval *Hyla* of 16-mm. length, a stage somewhat younger than the larvae operated, as described above, is illustrated in figure 3, which represents a longitudinal section cut at an angle of about 45° with the sagittal plane. The series was made from a normal 16-mm. larva prepared by the method of Ramón y Cajal. Two other series of the same stage and prepared by the same method, but cut in different planes, show the same features. It will be noted that in the

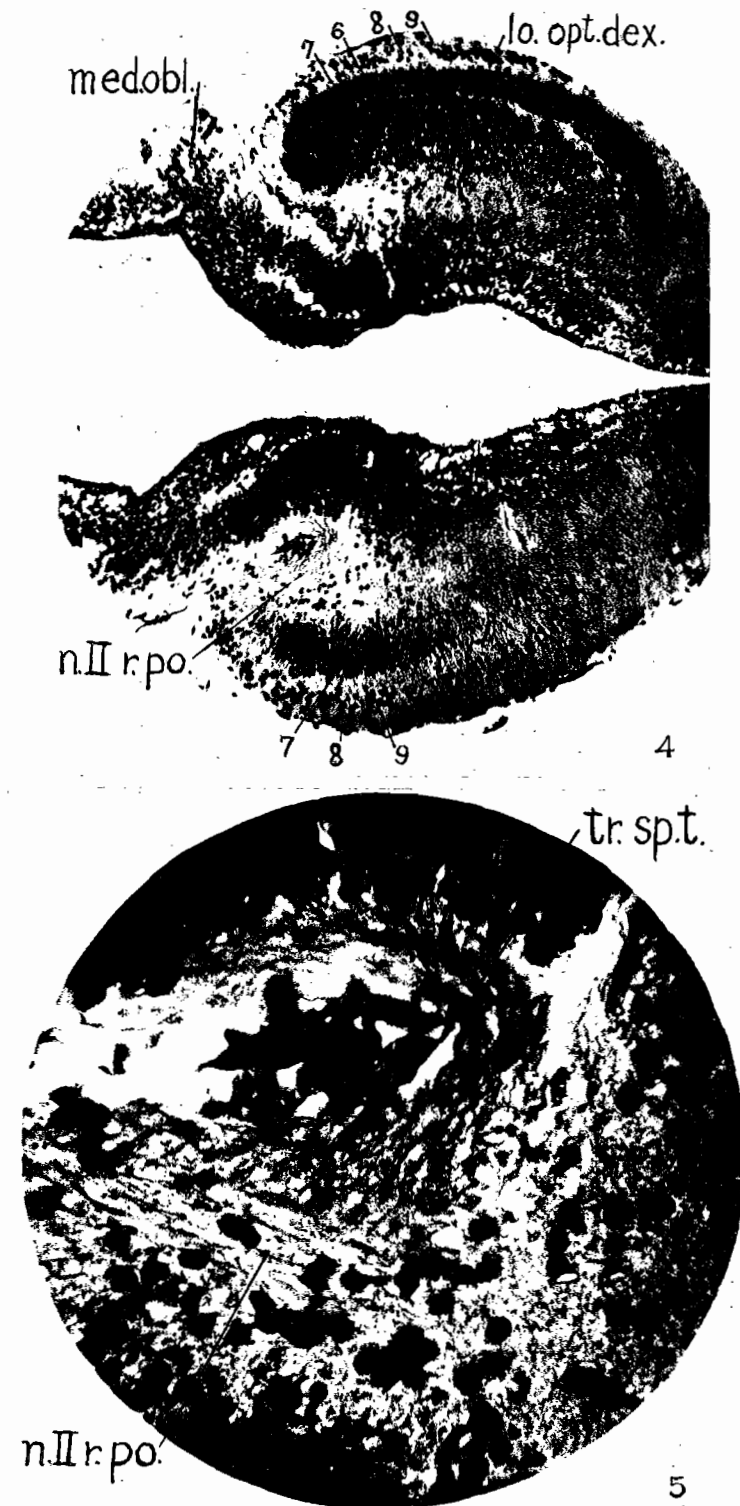
rostral region of the optic lobe the nine layers of Gaupp are already differentiated, but that toward the caudal pole the differentiation is less complete. The point of greatest interest is the presence of optic fibers in the opticus layer. Two bundles may be recognized, which correspond to the axial and marginal bundles described by Wlassak ('93) in adult *Rana*. The basal bundle of Wlassak is also present in the 16-mm. *Hyla*, and corresponds in general to Wlassak's description, as passing caudally to a ventrally situated small nucleus just in front of the oculomotor nucleus. Herrick ('17, '25) describes corresponding tracts in adult *Necturus* and in *Amblystoma*, and states with Wlassak that the marginal bundle is the chief tract. This does not appear to be the case in the 16-mm. *Hyla*, in which the coarser-fibered axial layer seems more important. However, it is not the purpose of the present study to enter into a description of anatomical details of the optic lobes beyond that necessary to identify the features involved in the experiment under consideration.

Coghill ('14, '24) has pointed out that the afferent and efferent systems in *Amblystoma* are structurally ready to function for some time before response to tactile stimulation occurs. If this holds true for the optic fibers of *Hyla*, their presence in the opticus layer, together with the well-developed retina of the 16-mm. *Hyla*, indicates that structurally they are ready for functional activity. That they do function at the stage indicated is apparent, as already stated, from the avoiding reactions to optic stimuli of normal larvae at this stage.

The 18-mm. stage, at which most of the larvae employed were operated, shows some advance in differentiation of the parts under consideration, as compared with the 16-mm. stage. The opticus or ninth layer is somewhat thicker and

Fig. 4 Photomicrograph of optic lobes in *Hyla* of 18-mm. total length. Horizontal series. Hem. and erythrosin. Leitz 4 per. oc., 8 apo. obj.

Fig. 5 Photomicrograph of region in neighborhood of *n.II r.po.* of figure 4, showing posterior bundle of optic tract and spino-tectal tract. Leitz 5 per. oc., 2 mm. apo. oil im. obj. Same section as figure 4.



contains a larger number of cells than in the earlier stage (table 1). It is also well defined at the caudal pole of the optic lobe at this stage (fig. 4). The optic fibers in the layer are also more numerous (fig. 5). These fibers may be traced from the retina (fig. 6), through the optic nerve and tract,



Fig. 6 Photomicrograph of portion of retina at entrance of optic nerve, in *Hyla* of 18-mm. total length. Leitz 5 per. oc., 2 mm. apo. oil im. obj. Hem. and Congo red.

into the opticus layer. It will be noted in figure 5 that, in addition to the optic fibers of the posterior optic bundle, there also enters into the optic lobe another tract, the spino-tectal, which comes from the cord through the medulla oblongata. This tract probably includes bulbo-tectal fibers also. According to Gaupp, the spino-tectal tract in the frog passes into

the deep part of the tectum and into the homologue of the posterior quadrigeminal body. Gaupp states (p. 62) that the posterior bundle of the optic tract, in its caudal part, also sends fibers to the deep part of the tectum. These points are of interest in connection with the relative development of the deep layers of the tectum opticum, as compared with the superficial layers, in the experimental larvae. In larvae of 22-mm. length, the oldest stage in which the eye was excised, the differentiation of the various layers is somewhat sharper than in the 18-mm. stage.

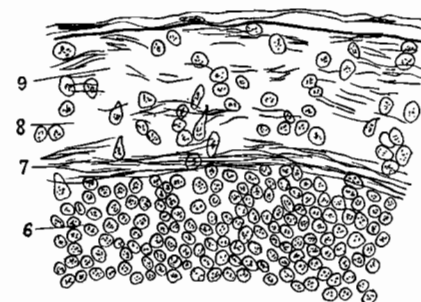


Fig. 7 Portion of optic lobe of normal 18-mm. *Hyla*, at level of III roots, showing elements in the outer layers at this stage. Hem. and Congo red. Cam. luc. $\times 375$.

In the experimental series, corresponding parts of the affected optic lobe, as compared with the normal side, show a considerable reduction in cross-section area, as illustrated in figures 8, 9, and 10. Study of the sections indicates that the most marked deviation from normal is found in the opticus layer (figs. 9, 10, 11, 12, and table 2). In this layer there is a reduction in thickness from 0.048 mm. in both series, VI-2 and II-3, on the normal side, to 0.021 mm. and 0.024 mm., respectively, on the affected side. Other series of sections prepared from experimental larvae at various stages of metamorphosis show a corresponding reduction of this layer on the side affected by excision of the eye. The eighth layer is also reduced in thickness and in number of cells, as shown in figures 9 to 12 and in tables 1 and 2. The sixth

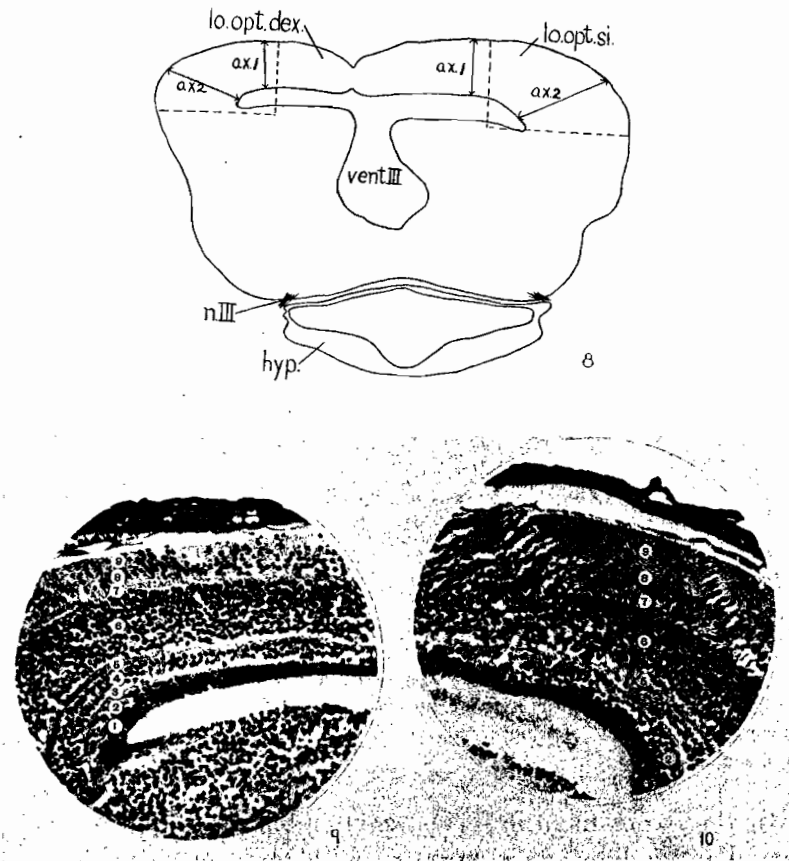


Fig. 8 Outline drawing of cross-section of midbrain at level of III roots of *Hyla* II-1, *Exp.* The left eye had been excised at 20-mm. total-length stage, and the young frog was killed shortly before completion of metamorphosis (2.5-mm. tail stage). $\times 45.5$. The lines *ax.1* and *ax.2* represent the axes of measurement in table 2. The broken lines bound the areas in the right and the left lobe from which the photomicrographs of figures 9 and 10 were taken, but these were taken from series VI-2, *Exp.*

Fig. 9 Photomicrograph of right optic lobe at rostral level of III roots (region bounded by broken lines in fig. 8, left side), showing the effect produced on the development of the various layers following excision of the left eye at an early stage. Series VI-2, *Exp.* Eye excised at 18-mm. total-length stage. Specimen fixed at 3-mm. tail stage. Leitz 4 per. oc., 4 mm. apo. obj. Hem. and Congo red after Carnoy fixation. Reduced to one-half.

Fig. 10 Photomicrograph of the left (normal) optic lobe. Same section and same optic system as figure 9. Reduced to one-half.

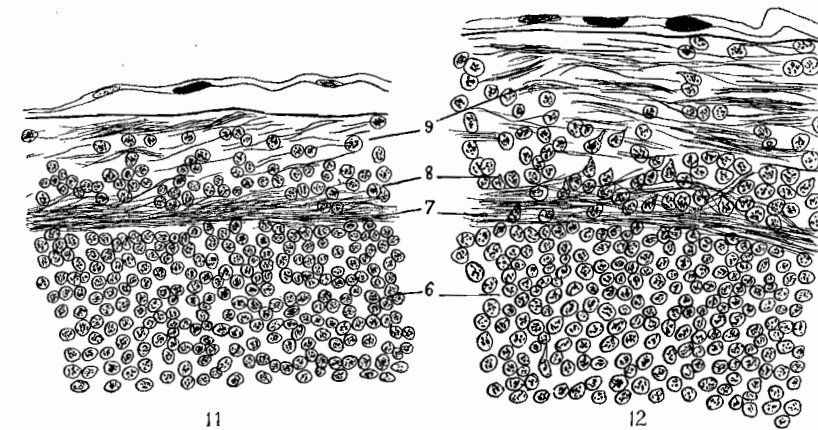


Fig. 11 Portion of section illustrated in figure 9, showing sixth, seventh, eighth, and ninth layers and the presence of nerve fibers not only in the deeper layers, but also in the optic layer. Cam. luc. $\times 375$.

Fig. 12 Corresponding region in left (normal) lobe in same section, showing relative thickness of seventh, eighth, and ninth layers as compared with the affected side, and the relative number of fibers in the optic layer. Cam. luc. $\times 375$.

TABLE 2

Measurements of several of the layers of the cortex of the optic lobes in experimental series VI-2 and II-3, *Hyla regilla*, from which the left eye had been excised at an early stage, and of the corresponding regions in a Weigert series of adult *Hyla regilla*. Measurements were made in axis 1 of figure 8, except the measurements of total thickness of the cortex, which were in axis 1 and axis 2. Measurements are stated in millimeters

SERIES	AXIS	TOTAL THICKNESS OF CORTEX		THICKNESS OF SIXTH LAYER		THICKNESS OF EIGHTH AND NINTH LAYERS		THICKNESS OF NINTH LAYER ALONE	
		Experimental side	Normal side	Experimental side	Normal side	Experimental side	Normal side	Experimental side	Normal side
VI-2 (Carnoy fixation)	(2)	0.185	0.257						
	(1)	0.161	0.218	0.069	0.069	0.035	0.076	0.021	0.048
II-3 (vom Rath fixation)	(2)	0.221	0.284						
	(1)	0.188	0.221	0.066	0.072	0.042	0.078	0.024	0.048
Adult (Weigert series)	(1)		0.377		0.072		0.132		0.102

layer appears virtually unaffected. This layer blends to some extent, medially on the affected side, with the eighth layer, indicating a slight reduction of the seventh layer, which is fibrous. The second layer is less developed on the affected side, but the remaining layers, namely, the first, third, fourth, and fifth, appear alike in both lobes.

A comparison of the development of the eighth and ninth layers at various stages was made by counting, in transverse sections, the number of nuclei contained in them both together and in the optic layer separately, in a portion of the optic lobes represented by a rostro-caudal thickness of 0.1 mm., beginning at the rostral level of the oculomotor roots and continuing caudally. In the series of the 16-mm. and 18-mm. larvae it was necessary to begin three or four sections rostral to the oculomotor roots, in order to avoid including sections of the caudal pole of the optic lobe, in which part the cross-section area is not so great and the differentiation of layers is not so definite at these earlier stages. In the several series cut at $10\ \mu$ the number of nuclei contained in the eighth and ninth layers of ten sections were counted; in sections cut at $15\ \mu$, the nuclei were counted in seven sections, the number for 0.1 mm. being obtained by adding to the total count in six sections, two-thirds the number found in the seventh and caudalmost section. In series cut at $5\ \mu$, the number of nuclei in the layers under consideration was counted in twenty sections. Only those nuclei of the ninth layer which were definitely detached from the eighth layer were counted as belonging to the optic layer.

Because of the much smaller volume of the brains in the early stages, the number of cells found in the portion of the optic lobe in which the cells were enumerated represents a much larger percentage of the total number in the entire optic cortex than is the case in the older and larger brains. A closer comparison would appear possible from individual sections of the early and later stages, cut at corresponding levels and at the same thickness. Such comparison is frustrated by the considerable variation in the number of cells in individual

sections, making averages necessary. But in the younger stages, and to some extent in the older ones, there is a diminution in the number of nuclei in the sections used as one proceeds caudally from the level of the oculomotor roots. To overcome these difficulties, enumeration was made of the nuclei in ten sections cut at $10\ \mu$, or the nuclei in an equivalent thickness of cortex in section series cut at other thicknesses. This gives an impression of quantitative results which is not intended. The results are qualitative only, and show that the number of nuclei in the eighth and ninth layers of the affected side remains much nearer the number found in these layers at the stage of operation than is the case in the normal side.

The results of such counts in larvae of 16-mm., 18-mm., and 22-mm. total-length stages and in 8-mm. and 0.5-mm. tail-length stages of metamorphosing larvae are shown in table 1, together with similar counts in both normal and affected sides of experimental series VI-2 and II-3. In the experimental brains the total number of nuclei found in both the eighth and the ninth layers, in a rostro-caudal thickness of 0.1 mm., was counted on both sides, as stated. It will be noted that the total number of nuclei on the affected side is much smaller than on the normal side.

To determine whether or not this disparity in numbers in the two lobes was due entirely to diminished cross-section area of the affected lobe (fig. 8) or to a difference in number of cells in corresponding parts of the two lobes, the number of nuclei in a measured portion in each of the layers, namely, the eighth and the ninth, was counted on the normal and on the affected side. Corresponding locations were selected in the two lobes, with axis 1 of figure 8 as the midline. The fields in which counts were to be made were delimited by means of an ocular micrometer. An ocular field bounded by the 5-mm. scale in a $10\times$ Leitz ocular, in combination with an 8-mm. apochromatic objective, was found most suitable and most representative for each of the two sides. It should be noted that the fields thus bounded do not represent cor-

responding areas, since the thickness of the optic layer, as noted above, is much greater on the normal side than on that experimentally affected, as shown in figures 9 and 10. The fields, however, include all the cells in the eighth and ninth layers in both sides, for a measured distance mediolaterally in the sections. Counts were made in both optic lobes through a rostro-caudal thickness of 0.1 mm., as described above, in experimental series II-3 and VI-2. The result, shown in table 1, indicates that the number of nuclei in both the eighth and the ninth layers of the affected side is much reduced not only in total number for the entire sections, but also in relative numbers for measured portions of the sections, as compared with the normal side.

That the reduction in cross-section area of the optic layer in the experimental animals is not due entirely to absence of the myelinated fibers of the optic tract is shown by the relatively small area, in proportion to the total thickness of the optic layer, which is occupied by the myelinated fibers, as shown in Weigert series (fig. 142, S. Ramón y Cajal, '11) of the brain of *Rana*, and also in my Marchi series of adult *Rana* in which one eye had been enucleated. My Weigert series of adult *Hyla* are quite similar to Weigert series of *Rana*. So far as I can judge from study of the optic lobes of adult *Rana*, stained by the method of Ramón y Cajal, and from various larval stages of *Hyla*, up to metamorphosis, stained by the same method, unmyelinated terminal branches of the myelinated optic fibers are collectively not of sufficient volume to account for the remaining difference.

As already noted, counts of cells in the optic layer (table 1) show that the number on the experimental side is much smaller than on the control side. These cells appear to migrate into the optic layer from the eighth layer. I have not been able to find any evidence of mitosis in the optic layer, so the cells are obviously not increased in number in this layer by proliferation within it. In the younger larvae studied (16 to 22 mm.), it is difficult to delimit the cells of the optic layer from the eighth layer. The fact that these cells

are much fewer in the experimental side than in the control side accounts, in part, for the reduced cross-section area of the ninth layer. With this reduction in number of cells goes an absence of the dendritic processes of the missing cells, which further accounts for reduced area of the section. Dendritic processes and axones from cells of the second, fourth, and sixth layers reach the optic layer in considerable volume (fig. 2, and also fig. 143, Cajal). To what extent, if any, the development of these processes is affected by the experimental procedure remains to be demonstrated by a more successful application of special methods of technique.

Our results, in general, are in accord with those of von Monakow ('85) and others, who used very young dogs, cats, and rabbits in their experimentation. They found atrophy of the gelatinous substance of the superior colliculus and of part of the lateral geniculate body, after enucleation of the eye. In the present experiment the further point is added, however, that on the affected side of the optic lobe in the tadpole, which corresponds to the superior colliculus of mammals, cells do not migrate in normal numbers from the eighth layer into the optic layer, which receives the optic nerve fibers normally, after these fibers have been destroyed. As may be seen by comparison of figures 7 and 9 and by comparison of the number of nuclei at various larval stages with the number found in the experimental animals (table 1), the number of cells remains about the same for a given field of the ninth layer on the affected side as at the stage of operation.

It does not appear probable that the reduced migration of cells after excision of the eye is due alone to absence of the fibers of the optic nerve themselves, for fibers from other sources are found in the optic layer (fig. 11) in the experimental series. The source of these fibers I have been unable to determine. Some, without doubt, are axones from cells of the deeper layers of the optic lobe, as described in normal *Rana* by Gaupp and P. Ramón, and as shown in figure 2, while others of these fibers appear to come into the optic

layer from other parts of the brain, but they did not show in Wlassak's degeneration experiments. Possibly they are efferent optic fibers in part (compare Herrick, '25 a, p. 440). The fact that fibers are present, whatever their source, coupled with the fact that there is a much smaller number of cells than normal in the optic layer of the affected side in the experimental larvae, makes it appear that removal of the principal incoming stimulus, namely, that from the retina, must be regarded as the chief factor in the reduced migration outward of cells from the eighth layer. The explanation of this fact appears to lie in the law of neurobiotaxis of Kappers.

The results of the experiments here reported appear, at first sight, contrary to those of Burr on *Amblystoma* larvae, but this interpretation does not necessarily follow. It should be recalled that Burr operated on very young larvae during a stage of very active cell proliferation and before functioning by the parts involved had begun, or even any great degree of cell differentiation had taken place. In the present application of von Gudden's method to larval amphibian material, the optic fibers were caused to degenerate after they had penetrated the optic lobe and had begun to function. The results were similar in general effect, although probably not in detail, to those of the classic experiments of von Gudden ('70) and von Monakow ('85) in mammals, namely, arrest of development of the parts entered by the optic nerve. The results on mammals and the present results on frog larvae were both undoubtedly due to direct involvement of the optic-tract fibers (and stimuli from these, in the frog larvae at least) and the structures with which this tract comes into immediate relationship, in both cases.

In a careful study of the brain of the blind and deaf Laura Bridgman, Donaldson ('90, '91) found, among other things, that the occipital lobes were reduced in size, especially in the region of the cuneus. The right lobe was more defective than the left and also had a thinner cortex. Quoting Doctor Donaldson ('90, p. 290):

It must be recalled here that although at the age of two years Laura became completely blind in her left eye, yet she retained some remnants of vision with her right eye up to her eighth year. This has left its mark on the entire central apparatus for vision. The right optic nerve is larger than the left.

The human brain, in which all of the fibers of the optic radiation to the occipital lobes have their origin in the optic nuclei of the thalamus, involves relayed optic stimuli. The fibers of the optic radiations must have been pretty well laid down in the brain in question before the onset of the disease which resulted in blindness. Any stimulus of the occipital lobes due to ingrowth of such fibers must already, in large measure, have manifested itself. The fibers themselves appear not to have been directly involved in the course of the disease which destroyed vision, but optic stimuli passing through them were greatly reduced. Doctor Donaldson reports that the number of large cells in the cortex of the cuneus was also considerably reduced, and to a greater degree in the right lobe than in the left.

Reference must also be made to the study of Ramsey ('01) on the brain of the blind-fish, *Amblyopsis spelaeus*, in which, macroscopically and microscopically, reduction of the optic tracts and optic lobes was described. Ramsey reports atrophy or entire absence of many of the layers of the lobe, as compared with normal fishes, and a total reduction of the dorsal walls of the optic lobe of one-third to one-half in thickness.

In his review of the morphogenetic factors in the differentiation of the nervous system, Herrick ('25 a) summarizes with three stages of development, namely, cell proliferation, cell differentiation, and the stage of influence by functional transmission of nervous impulses, upon further development. In the stages of *Hyla* subjected to excision of the eye, the third phase, namely, that of influence upon the developing optic lobes, of stimuli from the retina, had been reached. The first two stages were not completed, especially that of differentiation of nerve cells. Occasional mitotic figures in or near the ependymal layer indicate that some proliferation of

cells was also taking place. The fact that the neurones were still in process of differentiation is in keeping with Coghill's statement ('24) that nerve cells begin to function before their differentiation is completed. Whether or not degenerating nerve fibers in the developing brain would retard its growth is not known, so far as I am aware, but it appears that the chief factor in the failure of the optic lobes to develop to normal size and of the cortical layers to differentiate normally must have been the absence of functional stimuli and their effect on the differentiating neurones from the time the bulbus oculi was excised. This view appears to be in agreement with the results obtained by Detwiler ('20, '21, '23) of hyperplasia of sensory elements of the nervous system by peripheral overload, and of hypoplasia from peripheral underloading of the sensory elements, pointing to the functional factor as responsible.

SUMMARY

Frog larvae from which one eye was excised after function of the retina had become established and optic nerve fibers had entered the optic layer of the optic lobes show a reduced development of the optic lobe, to which fibers from the excised retina normally pass, on reaching the stage of metamorphosis. The optic layer is reduced in thickness, in number of contained fibers, and in number of cells. The eighth layer, which lies just beneath the optic layer, also shows a hypoplasia, both in number of cells and in thickness. The failure of normal development is apparently due to absence of the principal functional stimulus to the optic layer during much of the period of larval development.

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