THE EYE-MUSCLE NERVES IN NECTURUS

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EIGHT FIGURES

Information concerning the eye-muscle nerves in Amphibia seems abundant. For Salientia these nerves have been partially described by Arnold ('94) and Strong ('95) and the relations in the adult frog given by Gaupp ('99). In Urodela descriptions are fragmentary for many species, but in Amblystoma, Herrick ('94); Spelerpes, Bowers ('00); Amblystoma, Coghill ('02); Triton, Coghill ('06) and Amphiuma, Norris ('08); the origin, course, relations and distribution of these nerves have been quite fully set forth. In Proteida, the classical description of Fischer ('64) in *Menobranchus* (*Necturus*) is incomplete, as is that of Osborn ('88), for *Proteus*, the extremely small size of the trochlear and abducent nerves making them difficult even to identify. In his study of the brain of *Necturus*, Kingsbury ('95) gives descriptions of the origins of all the eye-muscle nerves. The present contribution may serve to supplement the observations of Kingsbury by adding descriptions of all the eye-muscle nerves in *Necturus* from their origins to their distributions in the orbit.

MATERIAL AND METHOD

The specimens of *Necturus maculosus* Rafinesque used were adult animals, varying in length from 36 cm. to 42 cm., supplied by Alex Nielsen, Venice, Ohio.

The extremely small size of the eye-muscle nerves makes them difficult to study in fresh or preserved material; but by staining with methylene blue, intra-vitam, and careful dissection under a stereobinocular microscope, the origin, course and distribution of each of these nerves may be quite easily demonstrated.

The method used is with some modification that given by Wilson ('10). The animals are tied by the legs to a board, the root of the tail being held immovable by a wooden clamp. The tail is cut off and after bleeding the stain is injected through the caudal artery. A 0.066 to 0.075 per cent solution of methylene blue (Grübler's "med. pur." or "rect., nach Ehrlich") in salt solution was found most useful.

Methylene blue (0.5 per cent in water)	13-15 cc.
Salt solution (0.75 per cent in water)	87-85 cc.

About 200 cc. of the stain is usually injected into an animal of 40 cm., outlet being furnished by the caudal vein and by cutting the tips of the gills to which clamps are applied when the vessels are well filled. Pressure is obtained by gravity and for a good injection of vessels of the head a pressure is necessary which is sometimes sufficient to rupture abdominal capillaries. After the vessels are well filled and the outlets cut off, the animal remains untouched for three to five minutes. The head is then cut off, the lower jaw removed and the region to be studied moistened with salt solution and exposed to the air. After a successful injection color appears in the eye-muscle nerves almost immediately on exposure to the air. The dye in the other nerves is oxidised somewhat later.

For fixation, if permanent preparations are desired, a cold solution of ammonium molybdate (8 per cent in water) is used. This is applied in an ice box for eighteen to forty-eight hours. (Some material, thus fixed, has been kept successfully, for purposes of dissection, in 4 per cent neutral formaldehyde—9 parts water, 1 part, 40 per cent formaldehyde—for four or five days without total loss of the stain in the nerves). The molybdate is now removed by washing in cold running tap water or by numerous changes of cold water in the ice box and the tissue then transferred to several changes of 96 per cent alcohol in the ice box and then to absolute alcohol. Clearing is accomplished in xylol and the tissue mounted in balsam or embedded in paraffin. If kept in the dark, preparations will keep for several years. For further details concerning this method, see Wilson ('10).

About twenty-five animals have been used in this study and the eye-muscle nerves on both sides of twelve of them thoroughly dissected. The rapidity with which the dye in the nerves is oxidised makes impossible the completion of a dissection of the fresh material which involves several hours, so that the dissections were continued after fixation, in a cold solution of ammonium molybdate and the relations of some of the larger structures established after the material was transferred to neutral formaldehyde.

The drawings shown in figures 1 and 2 were made from brains of *Necturus* fixed in formaline-Zenker's fluid, in which neutral formaldehyde (40 per cent) is substituted for the acetic acid ordinarily used with Zenker's fluid. Measurements indicate that this fluid produces in this brain less change in the size, shape and relations of parts than other solutions most often used for the fixation of whole brains. In the brain of *Necturus* there occurs usually a very slight swelling in the fore-brain (2.5 to 3 per cent); in the mid-brain and medulla oblongata no marked changes in size due to fixation have been noted after use of this fluid. In the fixation of the brain of *Necturus* it is essential that the brain be left in the skull and removed after hardening. This is particularly well illustrated by the appearance of the hypothalamus, hypophysis and saccus vasculosus after various treatments (fig. 2). Failure by several authors to recognise the saccus vasculosus in tailed *Amphibia* has been due evidently to improper conditions during fixation. If the meninges and blood vessels at the caudal end of the hypophysis are cut, the attachments of the hypophysis and the saccus vasculosus shrink and shrivel during fixation so that the hypophysis comes to lie ventral to the hypothalamus and the lateral dilations of the saccus vasculosus show no longer the relations observed in the fresh condition. In order to insure for the fixing fluid ready access to the brain, the part of the parasphenoid bone which forms the floor of the cranium should be removed from the cephalic tip of the hypophysis to the middle of the olfactory tracts. The bone should be left intact over and caudal to the hypophysis. Removal of the roof of the cranium may disturb the attachment

of the paraphysis, causing more marked change in the form of the fore-brain than occurs when a part of the parasphenoid is removed. Fixation of the brain of any amphibian may preserve more closely the normal relations as seen in the fresh condition if the precautions above mentioned are heeded.

Variability

The optic apparatus in *Necturus*, when compared with that in some other tailed Amphibia, seems poorly developed. The habits and reactions of *Necturus*, so far as known, also indicate the comparatively slight functional importance of the eye. Considerable variability is noted in the eye-muscle nerves in their passage from the brain to the orbit and in their ultimate branches. The relations of the trunks of the oculomotor and abducent nerves in *Necturus* seem fairly constant, but the trochlear nerve shows some remarkable differences in different individuals and often varies greatly on the two sides of the same head. Such variability in the course and relations of the eye-muscle nerves in Amphibia has previously been noted by Coghill ('02 and '06) and by Norris ('08). As pointed out by these authors, conclusions concerning cranial nerves in Amphibia can not be drawn accurately from a study of single or even several specimens.

In no two of the twenty-four eyes of the twelve individuals studied carefully do the eye-muscle nerves appear exactly alike. The descriptions and figures which follow illustrate the average condition, special note being made of some of the most interesting variations.

N. oculomotorius

The oculomotor nerve is formed by two or three bundles of fibers which spring from the ventral surface of the mid-brain about 0.6 mm. cephalad to the lateral pouches of the medulla oblongata and about 0.4 mm. from the midline. The nerve runs directly laterad above the saccus vasculosus (fig. 2) and inclining slightly cephalad reaches its foramen which lies about 3.7 mm. caudad to the optic foramen in the parietal bone (fig. 4). The foramen for the oculomotor nerve lies usually about 1.1 mm.

dorsal to the optic foramen. Running now for a distance of about 3 mm. in its bony canal, which inclines sharply cephalad, the oculomotor nerve emerges from the parietal bone about 4.8 mm. caudad to the emergence of the optic nerve; a point about at the level of the preoptic nucleus in the brain. Here the nerve lies in the trabecular cartilage immediately ventral to the r. ophthalmicus profundus V, and passing cephalad and very slightly laterad in the same relation, divides into its ventral and dorsal rami about 0.7 mm. cephalad to the foramen of exit of the optic nerve from the parietal bone; a point about at the level of the entrance of the olfactory tract in the olfactory bulb.

The dorsal ramus now passes dorsad to the optic nerve and running medial to or piercing the r. ophthalmicus profundus V, reaches the common origin of the recti muscles (figs. 5 and 7). Here it applies itself closely to the superior rectus muscle and breaks up on the dorso-medial surface of this muscle about its middle (fig. 5).

There are usually present one or two small twigs, containing two or three fibers, which leave the dorsal ramus of the oculomotor nerve to enter the bulbar fascia (fig. 5). Occasionally some of these fibers may be traced to the eyeball but most of them are lost among the blood vessels of the bulbar fascia.

The ventral ramus of the oculomotor nerve, running immediately ventral to the r. ophthalmicus profundus V and ventrolateral to the optic nerve, reaches the common origin of the recti muscles and passes directly out on the ventral face of the inferior rectus muscle (fig. 6). Quite frequently the ventral ramus enters the inferior rectus muscle and in several cases was seen to lie entirely dorsal to this muscle (fig. 7).

During its passage cephalo-laterad on the inferior rectus muscle, several twigs rise from the ventral ramus of the oculomotor nerve which enter this muscle and, having reached its ventromedial edge, the ventral ramus splits into two branches one of which runs directly to the medial rectus muscle, and the other, passing along the medial border of the inferior rectus finally enters the inferior oblique muscle near to its insertion on the eyeball (fig. 6).

When the ventral ramus of the oculomotor nerve reaches the common origin of the recti muscles there usually arise from it one or two small twigs of two to four fibers, which, passing into the bulbar fascia, finally reach the eyeball. Very often one of these branches applies itself to the sheath of the optic nerve and thus gains the eyeball (fig. 7). In several cases these twigs have been seen to enter either the inferior oblique or the medial rectus muscle; but usually one at least is present which reaches the eyeball and this very often in company with the sheath of the optic nerve.

In three of the eyes examined there was present a small twig from the ventral ramus of the oculomotor nerve which entered the lateral rectus muscle along with the abducent nerve.

N. trochlearis

The trochlear nerve rises from the caudal border of the dorsal surface of the mid-brain and passes ventro-laterad and slightly cephalad, lying in the angle between the mid-brain and the rostral border of the medulla oblongata and cerebellar commissure (figs. 1, 3 and 8). The root of the nerve rises after decussation immediately cephalad to the cerebellar commissure and contains usually sixteen to twenty-four fibers. A few fibers have been observed which seem to enter the nerve uncrossed. These rise from the caudal border of the optic tectum about 0.1 mm. from the midline and at once seem to enter the main trunk of the nerve. Four to eight such fibers usually appear on either side in preparations stained with methylene blue. On careful examination under high magnification, these fibers seem to be larger fibers than those which make up the trochlear nerve and to belong to the mesencephalic root of the trigeminal nerve which lies here beneath the trochlear nerve. Two or three smaller fibers from the tectum have been observed which appear to enter the trochlear nerve uncrossed.

The work of Tozer and Sherrington ('10) shows that in mammals the eye-muscle nerves contain sensory fibers (proprioceptive). If a similar arrangement exists in *Necturus* and the mesencephalic root of the trigeminal nerve is a sensory root, we may

have here, just after its decussation, the reception by the trochlear nerve of these sensory fibers; but further investigation is required to establish these relations in *Necturus* with certainty.

Figure 8 (right side) shows a case in which the trunk of the trochlear nerve runs at first directly caudad and then laterad, splitting into two bundles, one of which runs through the choroid plexus of the fourth ventricle. One or two fibers are sometimes given off the main trunk of the nerve in its passage laterad, to the lateral processes of the choroid plexus.

Passing directly caudad over the cerebellar commissure and into the choroid plexus of the fourth ventricle there are quite constantly present three to six large medullated fibers which seem to leave the main trunk of the trochlear nerve. These fibers, previously noted by Kingsbury ('95), are lost among the blood vessels of the choroid plexus. The connections of these fibers have not been demonstrated.

There have been observed in the dura mater in many specimens fibers similar in size to those just described. Such fibers have been traced from the choroid plexus of the fourth ventricle cephalad to the pineal region. Their origin has not been determined.

Having reached the dura mater, at this point quite closely adherent to the skull, the trochlear nerve turns, and passing usually about 1 mm. dorsal to the oculomotor nerve runs cephalad in the dura of the lateral wall of the cranial cavity (fig. 3). Passing now dorsal to the optic nerve, the trochlear nerve runs in the dura to a point usually about 0.5 mm. cephalad of the olfactory bulb and, forming a loop, returns to its foramen which in the majority of cases lies in the parietal bone about 2.5 mm. dorsal to the optic foramen. Quite frequently the foramen for the trochlear nerve may lie 0.2 mm. cephalad to that for the optic nerve or it may lie as much as 0.3 mm. behind it. In several cases the two foramina were in the same transverse plane but they are always separated by almost the entire depth of the cranial cavity.

One animal was examined in which, on one side, the foramen for the trochlear nerve in the parietal bone, was 0.6 mm. caudal to the lateral angle of the olfactory foramen.

In two cases, the trochlear nerve running cephalad in the duramater, left the cranial cavity through the olfactory foramen, turning then sharply laterad to reach the superior oblique muscle.

During its course inside the cranial cavity the trunk of the nerve frequently splits into several fascicles. This splitting usually occurs in the region of the loop of the nerve or soon after its origin from the brain and before the lateral cranial wall is reached.

After reaching its foramen the trochlear nerve runs laterad and cephalad in its canal in the parietal bone for a distance of about 2 mm. (fig. 3). Reaching thus the dorsal face of the lateral process of the parietal bone, the nerve runs cephalad close to the bone from which the temporal muscle is arising. Having arrived at the cephalo-medial angle of the orbit, the nerve swings laterad and breaks up on the dorsal face of the superior oblique muscle.

In one animal the nerve on the right side joined the oculomotor nerve, leaving the cranium through the oculomotor foramen. The fibers of the two nerves intermingled so that it was impossible to separate them. In this case the superior oblique muscle was innervated by a branch of the dorsal ramus of the oculomotor nerve, presumably by trochlear fibers.

An anastomosis of the trochlear nerve has been observed in several cases with the medial branch of the r. ophthalmicus profundus V inside the orbit (fig. 5); and in several cases a twig from the same branch of the trigeminal nerve has been seen to enter the sheath of the superior oblique muscle near to its insertion on the eyeball.

N. abducens

The abducent nerve is formed by two or three twigs which rise from the ventral face of the medulla oblongata about 0.3 mm. from the midline and 0.5 to 0.8 mm. cephalad to the root of the glossopharyngeal nerve (fig. 2). The abducent nerve is the smallest of the cranial nerves and contains usually ten to fifteen fibers. Running at first directly laterad, the nerve swings cephalad to the ventral surface of the root of the facial and acoustic

nerves and passing forward in the sheath of these nerves comes to lie on the ventral face of the Gasserian ganglion of the trigeminal nerve. Here the abducent nerve lies in the connective tissue sheath of the Gasserian ganglion, no interchange of fibers ever having been observed between the abducent nerve and any part of the trigeminal. Passing now slightly ventrad, the abducent nerve soon enters the temporal muscle which at this point takes its origin from the lateral surface of the trabecular cartilage. Running in this muscle just ventro-laterad to the r. ophthalmicus profundus V, the abducent nerve reaches the common origin of the recti muscles and then swings laterad to enter the ventro-caudal face of the lateral rectus muscle (figs. 4, 5, 6 and 7).

In four of the eyes dissected, a small twig has been observed running to the sheath of the optic nerve from the abducent nerve as it breaks up in the lateral rectus muscle (fig. 5). In one case this was a large twig of five or six fibers. in the others only two or three fibers were concerned.

Nn. ciliares

There is constantly present a branch of the r. ophthalmicus profundus V, which enters the sheath of the optic nerve (figs. 5 and 7). This fascicle, containing usually about ten fibers, leaves the lateral border of the r. ophthalmicus profundus V just before the orbit is reached, and running dorsal to the lateral rectus muscle, applies itself to the dorso-caudal face of the sheath of the optic nerve. Many of the fibers of this twig reach the eyeball.

Mention has already been made, in the descriptions of the oculomotor and abducent nerves, of twigs from these nerves entering the bulbar fascia and the sheath of the optic nerve. Some of these twigs reach the eyeball.

In none of the specimens examined has there been found any group of cells corresponding to the ciliary ganglion. The condition in *Necturus* may be similar to that in *Squalus acanthias* as reported by Dr. H. D. Senior at the meeting of the American Association of Anatomists, December, 1912. Here no ciliary ganglion is said to exist, the motor ciliary nerves containing,

probably, only vaso-motor fibers. In *Squalus acanthias*, according to Senior, physiological examination reveals great simplicity of accommodation; a fact correlated with the absence of the ciliary ganglion. No similar experiments with *Necturus* are known to the writer.

SUMMARY

In *Necturus* all the eye-muscle nerves have been demonstrated from their origin to their distribution in the orbit.

The trochlear nerve shows frequent variations in its intracranial course and in the position of its foramen in the parietal bone.

No group of cells corresponding to the ciliary ganglion has been found, but there are quite constantly present twigs from the r. ophthalmicus profundus V, the oculomotor and the abducent nerves which enter the bulbar fascia and the eyeball. These twigs most often apply themselves to the sheath of the optic nerve.

LITERATURE EXAMINED

- ARNOLD, G. A. 1894 The anterior cranial nerves of *Pipa americana*. Tufts College Studies, March.
- BOWERS, MARY A. 1900 The peripheral distribution of the cranial nerves of *Spelerpes bilineatus*. Proc. Amer. Acad. Arts and Sci., vol. 33.
- COGHILL, G. E. 1902 The cranial nerves of *Amblystoma tigrinum*. Jour. Comp. Neur., vol. 12.
- 1906 The cranial nerves of *Triton taeniatus*. Jour. Comp. Neur., vol. 16.
- DODDS, G. S. 1906 The cranial nerves of one of the salamanders (*Plethodon glutinosus*). Univ. of Colorado Studies, vol. 3.
- 1907 On the brain of one of the salamanders (*Plethodon glutinosus*). Univ. of Colorado Studies, vol. 4.
- DRÜNER, L. 1900 Über Mikrostereoskopie und eine neue vergrößernde Stereoskopcamera. Zeitschr. f. wissenschaft. Mikroskopie u. f. mikrosk. Technik, Bd. 17.
- FISCHER, J. C. 1864 Anatomische Abhandlungen über die Perennibranchiaten und Derotremen. Hamburg.
- FISH, PIERRE A. 1895 The central nervous system of *Desmognathus fusca*. Jour. Morph., vol. 10.
- GAGE, SUSANNA PHELPS. 1893 The brain of *Diemyctylus viridescens*, from larval to adult life and comparisons with the brain of *Amia* and *Petromyzon*. Wilder Quarter Century Book.

- GAUPP, E. 1899 Anatomie des Frosches. Second edition, part 2.
1911 Über den N. trochlearis der Urodelen und über die Austrittsstellen der Gehirnnerven aus dem Schädelraum im allgemeinen. Anat. Anz., Bd. 38.
- HERRICK, C. JUDSON 1894 The cranial nerves of *Amblystoma punctatum*. Jour. Comp. Neur., vol. 4.
- KINGSBURY, B. F. 1895 On the brain of *Necturus maculatus*. Jour. Comp. Neur., vol. 5.
- KINGSLEY, J. S. 1892 The head of an embryo *Amphiuma*. Amer. Nat., vol. 26.
1896 On three points in the nervous anatomy of amphibians. Jour. Comp. Neur., vol. 6.
1902 The cranial nerves of *Amphiuma*. Tufts College Studies, May.
- MCGREGOR, J. H. 1896 Preliminary notes on the cranial nerves of *Cryptobranchus alleghaniensis*. Jour. Comp. Neur., vol. 6.
- NORRIS, H. W. 1908 The cranial nerves of *Amphiuma means*. Jour. Comp. Neur. vol. 18.
- OSBORN, H. F. 1883 Observations on the brain of *Amphiuma*. Proc. Acad. Nat. Sci., Philadelphia.
1888 A contribution to the internal structure of the amphibian brain. Jour. Morph., vol. 2.
- SCHWALBE, G. 1879 Das Ganglion oculomotorii. Ein Beitrag zur vergleichenden Anatomie der Kopfnerven. Jen. Zeitschr. f. Naturwis., Bd. 13.
- STIEDA, L. 1874 Über den Bau des centralen Nervensystems des Axolotl. Zeitschr. f. wissen. Zool., Bd. 25.
- STRONG, OLIVER S. 1895 The cranial nerves of *Amphibia*. Jour. Morph., vol. 10.
- TOZER, F. M., AND SHERRINGTON, C. S. 1910 Receptors and afferents of the third, fourth and sixth cranial nerves. Roy. Soc. Proc., June.
- VON PLESSSEN, J., AND RABINOVICZ, J. 1891 Die Kopfnerven von *Salamandra maculosa* im vorgerückten Embryostadium. J. F. Lehmann, Munich.
- WALDSCHMIDT, JULIUS 1887 Zur Anatomie des Nervensystems der Gymnophionen. Jen. Zeitschr. f. Naturwis., Bd. 20.
- WILDER, H. H. 1892 Die Nasengegend von *Menopoma alleghaniensis* und *Amphiuma tridactylum*. Zool. Jahrb., Bd. 5.
- WILSON J. GORDON 1910 Intra-vitam staining with methylene blue. Anat. Rec., vol. 4.

Fig. 1 Drawing of the dorsal aspect of the brain of *Necturus* fixed in formaline-Zenker's fluid and removed from the cranium after fixation. The choroid plexus of the fourth ventricle and the paraphysis have been removed. $\times 6$.

Fig. 2 Drawing of the ventral aspect of the brain of Necturus. $\times 6$

Fig. 3 Dorsal aspect of the brain of *Necturus* showing relations of the nasal sac, eye and ear and the course of the trochlear nerve. $\times 4.5$

Fig. 4 Ventral aspect of the brain of *Necturus* showing relations of the nasal sac, eye and ear and the course of the optic, oculomotor and abducent nerves. $\times 4.5$.

Fig. 5 Dorsal view of the right orbit showing termination of the trochlear and abducent nerves and of the dorsal ramus of the oculomotor nerve. $\times 10$.

Fig. 6 Ventral view of the right orbit showing termination of the ventral ramus of the oculomotor nerve. $\times 10$.

Fig. 7 Dorsal view of the right orbit. The superior rectus and the superior oblique muscles have been cut. The optic nerve has been cut close to the eyeball and turned medially so as to show the ciliary nerves on its caudal surface. This drawing shows also a case in which the ventral ramus of the oculomotor nerve is dorsal to the inferior rectus muscle (compare fig. 6). $\times 15$.

Fig. 8 Dorsal view of the mid-brain and medulla oblongata showing relations of the trochlear nerve. On the left side the most usual relations are shown. On the right side appear the relations in a case in which the nerve was split into two fascicles, the main trunk of the nerve passing caudad over the cerebellar commissure to the choroid plexus of the fourth ventricle. The dotted lines indicate the edges of the choroid plexus. The connections of the fibers which appear coming from the trochlear nerve to the choroid plexus have not been determined. $\times 9$.