

**Anatomy.** — *The pars magnocellularis of the nucleus preopticus in Amphibia, particularly in Urodela.* By H. H. CHARLTON. University of Missouri School of Medicine and Central Dutch Institute for Brain Research, Amsterdam, Holland. (Communicated by Prof. C. U. ARIËNS KAPPERS.)

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Although HERRICK ('10) described in *Amblystoma tigrinum* a vertical sulcus dividing the preoptic nucleus into rostral and caudal portions which he termed the pars anterior and the pars magnocellularis of the preoptic nucleus, the fact seems to have been lost sight of, particularly in view of the extensive work done on this nucleus by RÖTHIG ('11), who failed to observe a pars magnocellularis in his *Urodele* material. The only figure showing the large-celled part of the preoptic nucleus is one by HERRICK ('17) showing its position in *Necturus maculatus* as seen in a cross section through its caudal portion.

In a recently prepared series of *Cryptobranchus alleghaniensis* and of *Amphiuma* means, cut transversely 20 micra in thickness and arranged in an alternate series, one stained in DELAFELD's haematoxylin and the other by the method of WEIGERT, the pars magnocellularis is exceedingly well defined. In addition to these two series prepared by the writer, there was at the Central Dutch Institute for Brain Research a beautiful Cajal silver series of the Giant Salamander of Japan and China, *Cryptobranchus japonicus*, as well as other good slides of *Urodela* and *Anura*.

Fig. 1 is a low power drawing through the brain of *Cryptobranchus alleghaniensis* in the region where the pars magnocellularis is most prominent. Here the nucleus extends from the level of the sulcus diencephalicus ventralis nearly to the floor of the preoptic recess. In order to bring out the details of the cells, the area shown in the small rectangle in fig. 1 is reproduced in fig. 2 under higher magnification. The large cells are spheroidal or oval in shape and measure in their longest diameter about 24 micra, contrasting with the small cells, which measure 12 micra.

The cells stand out not only because of the difference in nuclear size, but to a certain extent because of their faintly stained appearance in contrast to the more pycnotic appearance of the cells of the pars parvocellularis. The nucleus magnocellularis as a whole begins high up on the wall of the preoptic recess and descends to the bottom almost immediately. Its upper limits follow the sulcus diencephalicus ventralis and its depth becomes less with each succeeding section until it terminates near the level of the caudal part of the habenular commissure. At first the pars magnocellularis

is located between the ependymal cells of the preoptic recess and the pars parvo-cellularis. Later, as the nucleus leaves the floor of the recess most of

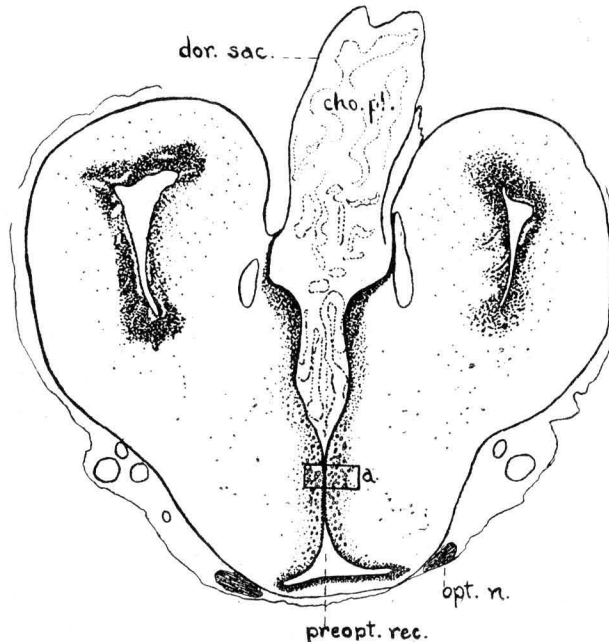


Fig. 1. *Cryptobranchus alleghaniensis*. Cross section through the diencephalon near the entrance of the optic nerves.  $\times 18$ . *a.*, Rectangular area through the preoptic nucleus shown under higher magnification in figure 2; *opt. n.*, optic nerve; *preopt. rec.*, preoptic recess; *cho. pl.*, choroid plexus; *dor. sac.*, dorsal sac.

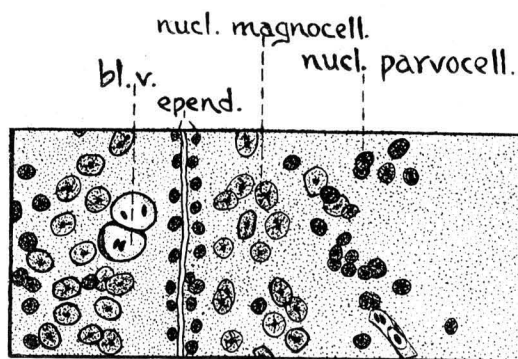


Fig. 2. *Cryptobranchus alleghaniensis*. Area shown in rectangle (*a*) of figure 1.  $\times 180$ . *nucl. magnocell.*, nucleus preopticus pars magnocellularis; *nucl. parvocell.*, nucleus preopticus pars parvocellularis; *bl. v.*, blood vessels; *epend.*, ependyma lining the preoptic recess.

the cells seem to be of the large cell type, with but a slight intermingling of the smaller cells. In my slides most of the cell nuclei seem to be bare

of cytoplasm, but in a few cells a small quantity seems to partially surround the nucleus.

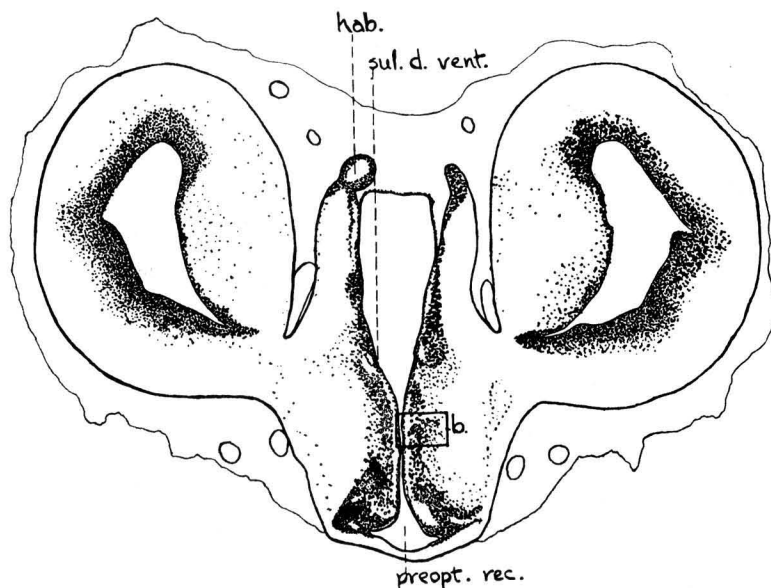


Fig. 3. *Amphiuma means*. Cross section through the preoptic recess at the level shown by line A—B in figure 7.  $\times 17$ . *b.*, Rectangular area through the preoptic nucleus shown under higher magnification in figure 4; *preopt. rec.*, preoptic recess; *hab.*, habenula; *sul. d. vent.*, sulcus diencephalicus ventralis.

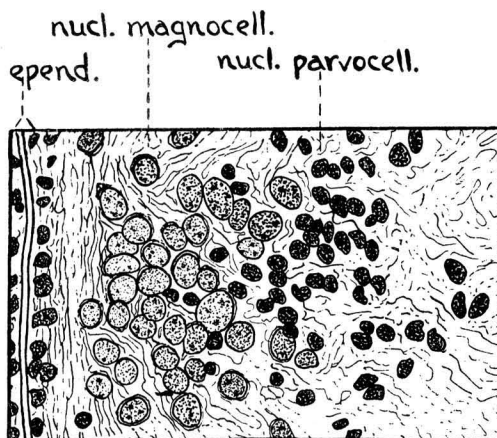


Fig. 4. *Amphiuma means*. Area shown in rectangle (*b*) of figure 3.  $\times 180$ . *nucl. magnocell.*, nucleus preopticus pars magnocellularis; *nucl. parvocell.*, nucleus preopticus pars parvocellularis; *epend.*, ependyma lining preoptic recess.

A similar series of drawings has been made of the brain of *Amphiuma means*, (figs. 3 and 4). Here the contrast in cell size is considerably

greater than in *Cryptobranchus alleghaniensis*. The diameter of the pars magnocellularis cells is from 20 to 30 micra, the size increasing as one follows the nucleus caudally. The pars parvo-cellularis cells measure 15 micra in their greatest diameter. The large cells are for the most part spherical, showing a narrow ring of cytoplasm around the nucleus. Although the nucleus at its most prominent region extends practically to the floor of the preoptic recess, it does not extend along the lateral branch of the preoptic recess at all.

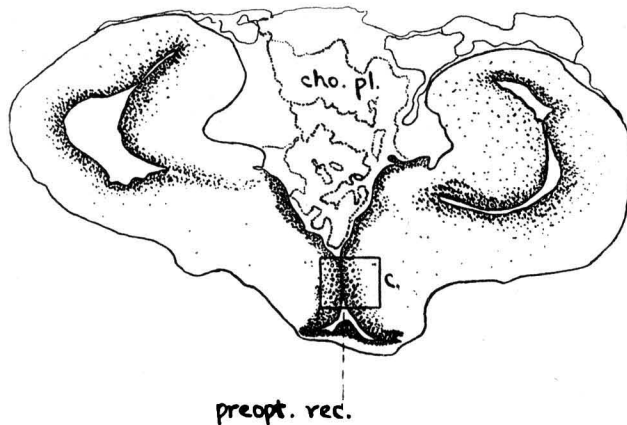


Fig. 5. *Cryptobranchus japonicus*.  $\times 18$ . Cross section through the diencephalon at about the same level as shown in figure 1. c. Rectangular area through the preoptic nucleus shown under higher magnification in figure 6. Cho. pl., choroid plexus; preopt. rec., preoptic recess.

In *Cryptobranchus japonicus* the nucleus magnocellularis is shown under high magnification in fig. 6, (for level and position of drawing see fig. 5). The large cells are first seen anteriorly near the middle level, dorso-ventrally of the recess wall. Traced caudally they soon reach the floor and run along the lateral pockets of the preoptic recess. This ventro-lateral extension of the nucleus, greater here than in either *Cryptobranchus alleghaniensis* or *Amphiuma means*, is the usual arrangement in fishes, where the nucleus usually begins in front in this ventro-lateral position.

Tracing the nucleus caudally, it has the same characteristics as seen previously in *Cryptobranchus alleghaniensis* and *Amphiuma means* except that it ends almost as soon as it reaches the caudo-dorsal position instead of extending caudally for some distance as a thin strand of cells.

The nucleus, however, differs quite markedly from that of the other *Urodela* described, in at least two particulars. First, the cells are few and scattered among the numerous pars parvo-cellularis cells of the nucleus preopticus. In the region figured, the cell number is about one half that of *Cryptobranchus alleghaniensis* and only one fourth that of *Amphiuma means*. The variations in cell size are not great, since we find

here a diameter roughly of 26 micra, compared to 24 in *Cryptobranchus alleghaniensis* and 30 in *Amphiuma*, measuring the longest observed cells

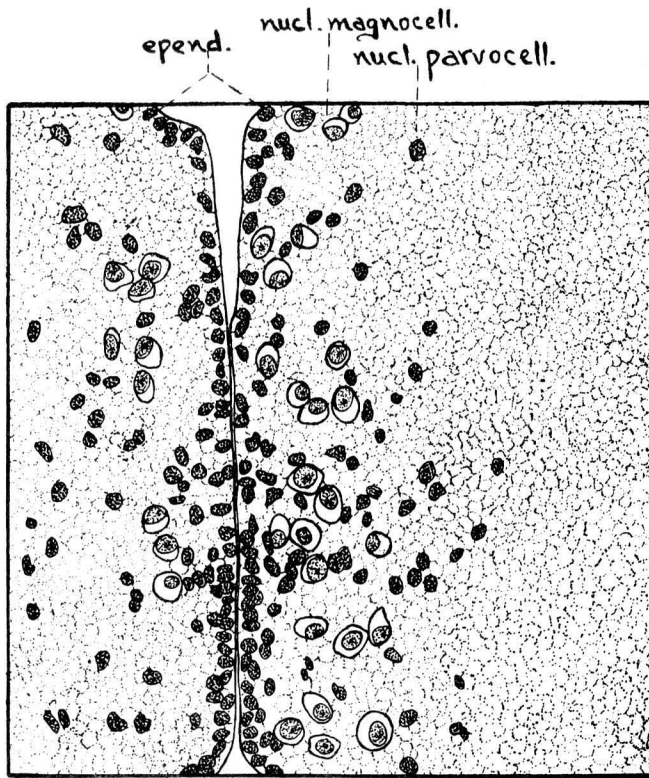


Fig. 6. *Cryptobranchus japonicus*. Area shown in rectangle c of figure 5.  
 ×150. *Epend.* ependyma; *nucl. magnocell.*, nucleus preopticus pars magnocellularis; *nucl. parvocell.*, nucleus preopticus pars parvocellularis.

in each case. Much more striking, however, is the second difference, i. e., nuclear size. Here the nucleus has a diameter of 15 micra, or only about one half the size of the cell, while in *Cryptobranchus alleghaniensis* it is 24 and in *Amphiuma* means 28. In the last two forms the cytoplasm is reduced to a mere vestige. We have, therefore, here in *Cryptobranchus japonicus* a small nucleus surrounded by a relatively large amount of cytoplasm standing in sharp contrast to the large nucleus in *Amphiuma* means which on the contrary has but little cytoplasm. The nuclei of the large cells of *Cryptobranchus japonicus* do not differ greatly in size from those of the pars parvocellularis. This may explain why RÖTHIG ('11) did not report the presence of the nucleus in this form, for unless one were particularly fortunate in getting a good cytoplasmic fixation, the nucleus magnocellularis would be lost among the cells of the pars parvocellularis.

HERRICK ('10, fig. 22) has indicated the position in *Amblystoma tigrinum* of a number of sulci seen on the lateral wall of the thalamic and

hypothalamic regions. According to this writer the sulcus limitans ends somewhere in the floor of the preoptic recess. In order to compare the

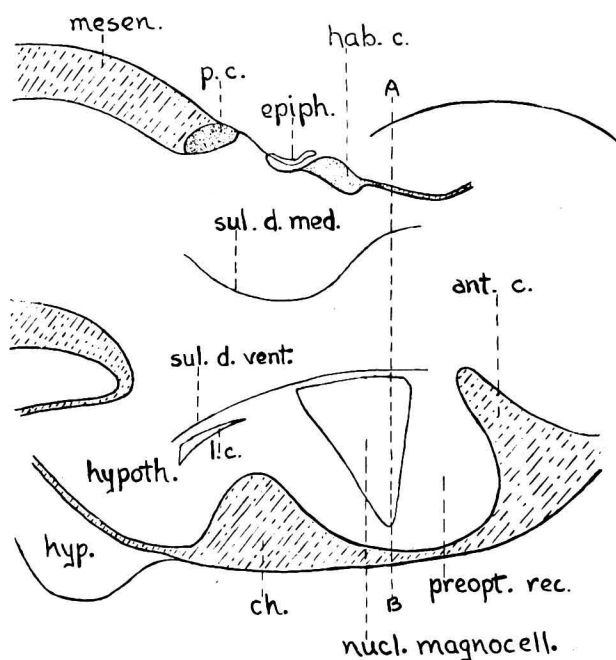


Fig. 7. *Amphiuma means*. A schematic sagittal section.  $\times 17$ . p. c., posterior commissure; hab. c., habenular commissure; epiph., epiphysis; mesen., mesencephalon; sul. d. vent., sulcus diencephalicus ventralis; sul. d. med., sulcus diencephalicus medialis; ant. c., anterior commissure; preopt. rec., preoptic recess; nucl. magnocell., nucleus preopticus pars magnocellularis; l.c., large cells; ch., optic chiasma; hyp., hypophysis; line A—B represents plane of section of figure 3. hypoth., hypothalamus.

preoptic region in *Amphiuma means* with HERRICK's *Amblystoma* figure, a schematised sagittal section is shown in fig. 7. This may also be compared to OSBORN's ('83) figure *H* of a sagittal section through the brain of *Amphiuma*. The sulcus limitans does not appear with any degree of certainty in any of the forms described above, but it is of some interest to note that the postero-ventral limits of the nucleus in *Amphiuma means* seem to coincide almost exactly with the ventral limb of the sulcus limitans as figured by HERRICK. This being true, we can describe the nucleus as holding an antero-dorsal position with reference to the sulcus limitans, and if we can still speak at this level of alar and basal plates, then the pars magnocellularis is located in the dorsal, or alar, division.

The cells of the pars magnocellularis are in *Amphiuma means* of so striking a character that the appearance of similar cells located just below the sulcus diencephalicus ventralis as one traces it into the hypothalamus is clearly recognizable. These cells are relatively few in number and reach

their maximum near the level of the beginning point of the recessus lateralis hypothalami. Their position is indicated in fig. 7. HOLMGREN ('20) has called attention to large cells holding the same position in Teleostei. These he finds to be similar in type to the cells of the nucleus magnocellularis and suggests that they may have a similar function.

The nucleus magnocellularis is of such prominence in fish that rarely indeed does one have trouble in recognizing it. In the Urodele group of Amphibia this does not seem to be so easy, for RÖTHIG ('11) did not observe it in *Spelerpes fuscus*, *Cryptobranchus japonicus*, *Necturus maculatus*, *Siren lacertina*, *Diemyctylus viridescens*, and *Hynobius*. In his paper on the phylogenesis of the hypothalamus RÖTHIG ('11) writes as follows: "Der Nucleus praeopticus ist die Zellansammlung, die den Recessus praeopticus umgibt. Sie besteht bei den Urodelen aus gleichartigen, kleinen, runden Zellen, während die Anuren eine kleinzellige und eine grosszellige Abteilung besitzen; die erstere liegt mehr frontal, die letztere mehr caudal". His figures for the size of the large cells in *Rana* are given as 15 micra, and for the small cells 6 to 8 micra. In *Amphiuma* means, as previously noted, the magnocellular cells may reach fully 30 micra in diameter and the parvocellular cells about 12 micra.

Yet the nucleus magnocellularis is present in *Necturus maculatus* and in *Amblystoma tigrinum*, HERRICK ('10 and '17). By the aid of the camera lucida, cell size differences have been noted by the writer in *Necturus maculatus* (corroborating the findings of HERRICK), in the cave salamander, *Typhlotriton spelaeus*, and in *Molge cristata*, but the differences in the first two were not sharp enough to make a complete nuclear study and in *Molge cristata* only a few large cells were observed. These differed however quite markedly from the parvocellular cells both in size and appearance. The writer ('24) considered the Urodela exceptional in that they did not possess a pars magnocellularis division of the preoptic nucleus. This view is no longer tenable. That the nucleus may be lacking in some Urodele forms is doubtful in view of the present findings, but that it may be difficult of detection is quite apparent from the previously mentioned work of RÖTHIG and from my own experience. In the present study it has been impossible to make out cell size variations or cells giving a distinctly different stain reaction, in *Bombinator pachypus*, *Proteus anguinus*, *Salamandra maculosa*, and *Axolotl*.

If it should be found that the nucleus magnocellularis appears only late in brain development, it might explain some failures recorded. Either that, or the necessity for a more rapid fixative than the usual formalin mixtures, may be indicated.

In order to compare the position and length of the nucleus as found in the Urodela with that of fish, where it is a constant finding, and with the Anura, the nuclei of several representatives of each group have been charted in relation to the anterior commissure and the place of exit of the third or oculomotor nerve. The method of KAPPERS ('20 and '21) for

comparing the position and shifting of the cranial nerve nuclei has been slightly modified for use here. The results are given in fig. 8.

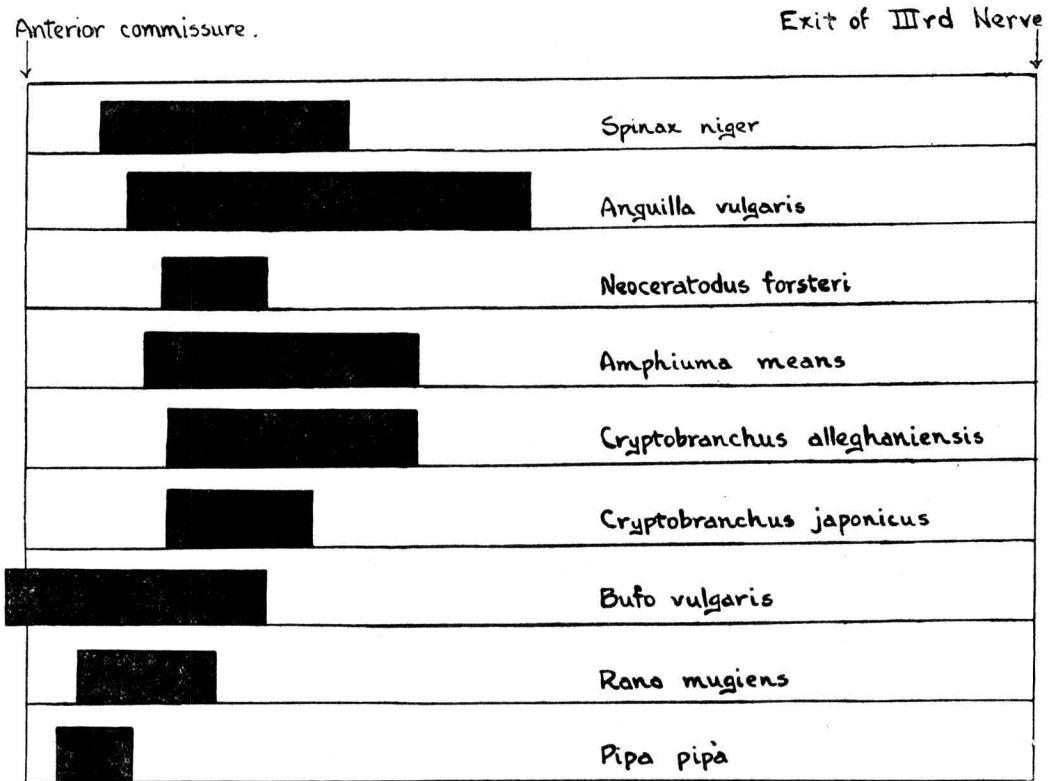


Fig. 8. This chart indicates the position and length of the nucleus preopticus pars magnocellularis in Selachians, Teleostei, Dipnoi, Urodela and Anura in relation to the anterior commissure and the place of exit of the fibres of the third nerve.

In the three Urodela forms charted, *Amphiuma means*, *Cryptobranchus alleghaniensis*, and *Cryptobranchus japonicus*, the nucleus has about the same relation to the anterior commissure but decreases in length in the above order, being in the latter only slightly more than one half as long as in the other two.

The nucleus has been charted in three fishes, *Spinax niger*, a Selachian, *Anguilla vulgaris*, a Teleost, and *Neoceratodus forsteri*, a Dipnoan. The first differs but little from our findings in the Urodela. *Anguilla vulgaris* has a longer nucleus magnocellularis than the average Teleost, and its cells are among the largest found in that group, measuring from 40—60 micra. The nucleus of these giant cells has a diameter of from 10—15 micra. But when compared to the Urodela the chief difference is a longer drawn out strand of cells extending caudo-dorsally from the nucleus proper. An examination of fig. 9 shows the prominent nucleus of closely packed large cells lining the preoptic recess throughout its entire extent. The



nucleus is quite short in *Neoceratodus forsteri*, in fact quite the shortest except for that in *Pipa pipa*.

When we compare the position and length of the nucleus magnocellularis

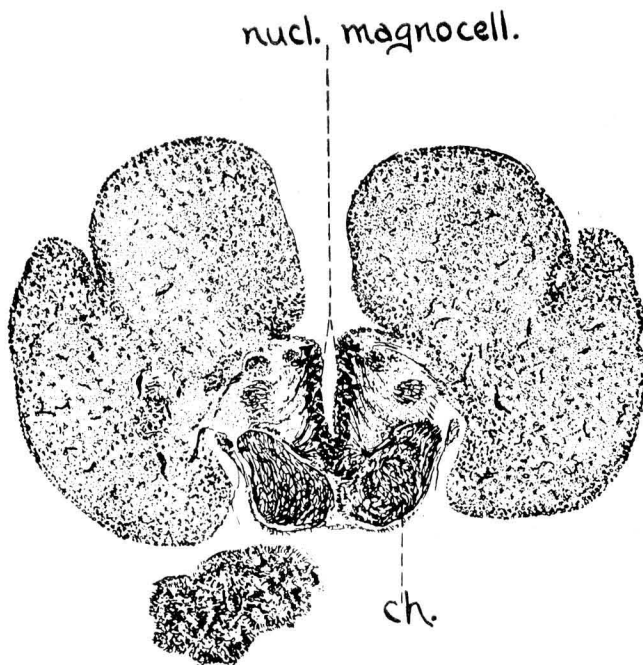


Fig. 9. *Anguilla vulgaris*. Cross section through the preoptic recess.  $\times 60$ . nucl. magnocell., nucleus preopticus pars magnocellularis; ch., optic chiasma.

(fig. 8) in Urodela with that in Anura it will be seen at once to have a more caudal beginning in the Urodela. As for length, that in *Bufo vulgaris* is somewhat longer, but in both *Rana catesbyana* (mugiens) and *Pipa pipa* it is shorter. As the brain structure is much compressed in *Pipa pipa* and in the Anura in general, this compression, reduced in my charts should give rise to a larger extent of its field, thus making the nucleus appear relatively longer. Since this is not the case, its shortness in these two forms of Anura is but accentuated. In *Bufo vulgaris* the nucleus, while long, is narrow dorso-ventrally, never lining the entire extent of the preoptic recess at any one level. Furthermore, the nucleus in *Bufo vulgaris* and *Rana catesbyana* near its caudal termination bends or turns laterally to end quite some distance from the preoptic recess. OSBORN ('83) calls attention to the gross similarity of the infundibulum in *Amphiuma* to that found in Teleostei. It certainly seems true also that the nucleus magnocellularis in the Urodela is more similar to that of Teleostei than to the Anura as judged by length, height or dorso-ventral extent, and the position of the beginning point.

Of what significance is the anterior position of the nucleus in Anura? Is it correlated in any way with a terrestrial life? The fact that it is located

still more anteriorly in birds would seem to confirm this last view point. Or is it perhaps a consequence of the telescoping of the forebrain and the anterior commissure over the betweenbrain as also occurs in birds? (cf. KAPPERS '20, Vol. II). But why should such closely related forms as *Cryptobranchus alleghaniensis* and *Cryptobranchus japonicus* show such nuclear length variations? The nucleus magnocellularis is not only short in *Cryptobranchus japonicus*, but the cells are much fewer in number and are rather scattered.

GADOW ('01) states that *Cryptobranchus japonicus* differs from *Cryptobranchus alleghaniensis* in one essential point only, namely, by the absence of gill openings and of the modifications of the branchial apparatus connected therewith. *Cryptobranchus japonicus* lives in mountain streams and has been found 4500 feet above sea level. The respiration rate under aquarium conditions is, according to TEMMINCK and SCHLEGEL<sup>1)</sup> once every 6—10 minutes. It would be interesting to know the respiratory rate in *Cryptobranchus alleghaniensis*.

## CONCLUSIONS.

I. A distinct and prominent pars magnocellularis of the nucleus preopticus occurs in the Urodele forms *Cryptobranchus alleghaniensis*, *Cryptobranchus japonicus*, and *Amphiuma* means. It has been observed in a lesser degree in *Necturus maculatus*, *Typhlotriton spelaeus*, and in *Molge cristata*, and is probably present in other Urodela.

II. The cells and nuclei of the cells are the largest in *Amphiuma* means. *Cryptobranchus japonicus*, on the other hand, has cells with small nuclei but a much greater amount of cytoplasm.

III. The nucleus is limited dorsally by the sulcus diencephalicus ventralis and its caudo-ventral limits follow a curve quite like that shown by many authors for the rostral termination of the sulcus limitans.

IV. The nucleus in Urodela resembles its counterpart in the fishes in its position, length, and cell size, more than it does that in its nearer relatives, the Anura, which is correlated perhaps with a life spent almost wholly in the water.

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<sup>1)</sup> As quoted by GADOW.

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