Nervous Structure of the Spinal Cord of the Young Larval Brook-Lamprey

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With two Plates

It is intended to describe here the spinal cord of recently hatched ammocoetes of Lampetra planeri (Bloch). Particular attention will be given to those neurons which may be involved in the somatic sensori-motor arc. It is hoped to base upon this description a subsequent account of the embryo and young ammocoete, which will deal with the successive stages of nervous structure and their relation to the successive patterns of behaviour.

The neurology of the ammocoete, or pride, should be of particular interest, because great importance attaches to all aspects of the morphology of this animal in discussions of the phylogeny of vertebrates. For although the gnathostome vertebrates are not regarded as derived from some palaeozoic ammocoete, the morphology of the ammocoete certainly resembles that of the prototype from which higher vertebrates may be said to have evolved. This point of view is supported by the accounts of the general morphology of the nervous system of the young stages, and is borne out by the detailed neurology of the brain in the older stages and in the adults.

With very minor exceptions, the description of the nervous system of the younger stages has hitherto been made without the use of metallic impregnation or any other method of staining nervous tissue specifically, except in animals of above 4 cm. in length, that is in animals more than 1 year old (cf. Hardisty, 1944). But knowledge of the very youngest stages is urgently required; for the nervous organization of these could be compared with that found in amphibian embryos: and it is by a study of that organization in the embryos of the urodele that Coghill (1914, 1929, &c.) and Youngstrom (1949) have erected a far-reaching system of correlation between the successive structural patterns of the neurons during development and the stages of development of behaviour.

It is important to know how far Coghill’s and Youngstrom’s conclusions are applicable to other classes of vertebrates. Similar investigations to those of Coghill have been performed very effectively upon placentals (Barron, 1941). Upon fishes similar lines of research have been less successful. Descriptions of nervous structure almost all deal with a late period of development, when swimming movements are already an integral part of behaviour; when the most critical period in the relation between structure and function is already
past history. The few accounts at appropriately early stages of development
deal chiefly with sensory or motor neurons which have peripheral processes.
This applies to that of Neal (1914) on *Squalus* and to Harrison's classical
account of the Rohon-Beard cells of *Salmo* (1901).

On the other hand, good descriptions of the early stages of behaviour are
also rare. The few that exist, such as that of Wintrebert (1921) on *Scyliorhinus*,
are difficult to assess in the absence of knowledge of the contemporary
nervous structure.

In fishes we lack an adequate picture of the sensori-motor arc in the
nervous system during the period when locomotor movements or those of
the pectoral fins and visceral arches are being developed. In cyclostomes the
position is even less satisfactory. For this group there is no description of
the relation between the neurons of the spinal cord in the young. Yet such a
description in vertebrates so near the prototype is clearly fundamental.

The present paper attacks this problem in the Petromyzontia. The detail
of the nervous structure will be described at the period in which locomotion
by swimming movements has just been achieved. The animal is then at a
stage roughly comparable with the stages described in *Amblystoma* by
Youngstrom.

**Previous Work on the Petromyzontid Nervous System**

The extensive literature on the neurology of lampreys has been well sum-
marized in Kappers-Huber-Crosby (1935). The descriptions of the brain
are relevant to a study of the spinal cord in that they show that the brain, at
least, appears to be organized in a very primitive fashion. The simple form
of the brain in the young ammocoete can be seen from von Kupffer (1906),
figs. 47 to 57. The primitive and surprisingly unspecialized neural archite-
cture and tracts of the adult brain are clearly described in Johnston (1902).
Later work, such as that of Barnard (1936), Woodburne (1936), and Pearson
(1936), has confirmed his conclusions.

Work on the neurology of the brain has been mainly devoted to adult or
advanced ammocoete preparations. For instance, Tretjakoff (1909b) gave an
extensive account of the brain of ammocoetes of length 10 to 18 centimetres.
In passing, it is important to note that fig. 43, Tretjakoff (1909b), is repro-
duced in Kappers-Huber-Crosby (1935), fig. 316, where it is wrongly
described as part of the brain of an 'Embryo'. But Larsell (1947) has recently
described some parts of the brain in younger ammocoetes: the smallest of his
silver-impregnated specimens was 42 mm. in length.

In contrast to the brain the spinal cord of the older ammocoete and adult
lamprey is not of the form to be expected in a prototype, and the function
and homology of the constituent neurons are difficult to understand. Kolmer
(1905) and Tretjakoff (1909a) have been the chief contributors on the subject.
Their conclusions are discussed by Kappers-Huber-Crosby, but the original
figures should be consulted. The unusual characters of the spinal cord may
be summarized as follows. The cord is so broad and flat as to be almost
ribbon-like; the positions of dorsal, lateral, and ventral funiculi are therefore abnormal. Dendrites of motor neurons extend across their own half of the spinal cord, and may extend to the contralateral side, either above or below the neural canal: the peripheral motor fibre is derived from a collateral of a longitudinal fibre. The chief form of intercalary cell is very large: its dendrites extend into the contralateral dorsal funiculus, while its axon runs longitudinally and then crosses into the contralateral ventral funiculus. Giant fibres descend in the ventral and lateral part of the cord from the giant cells of Müller in the midbrain and hindbrain; these are believed (e.g. by Stefanelli and Camposano, 1946) to be co-ordinating fibres comparable to the Mauthner fibres of teleosts and urodèles, but they occupy a considerable part of a transverse section of the cord. In a dorso-medial position in the grey matter are found some very large cells, termed *Hinterzellen*, whose axons run in the dorsal funiculus. There are normal dorsal ganglia, but the dorsal and ventral roots do not unite in the usual manner.

Two points in the description of the cord of adults and the older larvae require special attention. Firstly, in a Viennese journal, Sigmund Freud (1877) described somatic-sensory fibres which he claimed and figured as originating from the Hinterzellen, i.e. from cells of the dorsal horn. Now in the larvae of fishes and amphibia there are large neurons within or just above the dorsal part of the spinal cord which have the relations of somatic-sensory dorsal ganglion-cells; these neurons are termed Rohon-Beard cells. If Freud's figures were correct, there would be a strong presumption that the Hinterzellen are homologous with Rohon-Beard cells, especially since Rohon-Beard cells are persistent in many adult teleosts. This relation between the Hinterzellen and the dorsal root was subsequently denied by Tretjakoff (1909a). But it was again described by Beccari (1909), who supported his case with some very clear figures.

Secondly, there is a well-developed Commissura Infima Halleri at the anterior limit of the cord. This commissure was described in the lamprey by Johnston (1910), but his paper was not mentioned by Kappers-Huber-Crosby. The importance of the functions of the Commissure of Haller are emphasized by Herrick (1944), and it can be seen, from a comparison of Johnston's account of its structure with the description by Herrick (1908) of its relations and its functions in fishes, that in lampreys this commissure is probably concerned with a correlation between the left and the right spinal somatic-sensory components, and also between these and the cranial nerves.

Turning to the embryo and the young pride, we find three descriptions of the large dorsal cells in the spinal cord; the first two of these have been overlooked in Kappers-Huber-Crosby's account. Kupffer (1894) showed a 'Rohon'sche' cell, i.e. a Rohon-Beard cell, lying just dorsal to the cord in a 3-mm. embryo. Studnicka (1895) figured Hinterzellen in *L. planeri* of lengths between 3 mm. and 30 mm. In his younger stages, the Hinterzellen now lie just below the dorsal surface of the cord; in the older stages, they have sunk still farther into a position ventral to the dorsal sectors of the white matter.
The axon runs longitudinally in the white matter; the peripheral process appeared to him to be independent of the dorsal root, and free from metameric organization. Studnicka concluded that the Hinterzellen were probably motor in function. Beccari (1909), using a silver impregnation, described the same cells in prides of 20 mm. His account confirms that of Studnicka, except that he found that the peripheral fibre left the cord as part of the dorsal root. It seems clear from the three papers that the large dorsal cells are true Rohon-Beard neurons, for they have very large cell-bodies which begin in a very dorsal position in the cord, an axon running longitudinally in the dorsal white matter, and a peripheral fibre which is presumably sensory, since in the older stages it accompanies the other dorsal-root fibres.

The only other type of neuron described in the spinal cord of these stages of Lampetra is the motor neuron. Kupffer (1890) described the first motor axons running from the spinal cord to the myotomes in 3-mm. embryos (his fig. 81).

The peripheral nerves of the trunk in the newly hatched pride have been described by Sagermehl (1882), Shipley (1887), Dohrn (1888), and von Kupffer (1890). Von Kupffer states that the ventral spinal nerve, on leaving the cord, passes ventrally between the notochord and the inner face of the myotome. He describes the dorsal nerve as emerging from the cord and dividing into a lateral and a median branch: the median branch turns ventrally, to pass between notochord and myotome, while the lateral branch passes outward across the upper surface of the myotome. The dorsal ganglion-cells are figured as lying on the course of the median branch of the dorsal nerve, e.g. his figs. 81 and 82.

The relation between the dorsal ganglion and the dorsal nerve was a matter of dispute between these authors. But it would have been difficult to establish the facts with certainty by the aid of the stains they were using: von Kupffer, for instance, used borax-carmine. Certainly, the cells of the dorsal ganglia of the spinal cord are still in a very early stage of development (see Shipley). Methylene blue preparations of peripheral nerves in much older ammocoetes have been described by Tretjakoff (1929). His fig. 25 shows a dorsal spinal nerve dividing into four branches. Two of these branches correspond to the median and the lateral branches of von Kupffer's account: but the other two run, one dorsally and the other dorso-laterally, quite clear of the myotome to reach the skin. His fig. 26 shows a motor neuron.

The position reached by previous work is, then, that in the newly hatched larva the sensory and motor somatic nerves have reached their end-organs; that the sensory fibres are at least partly derived from Rohon-Beard neurons or other intramedullary neurons very like them; and that the dorsal ganglion-cells on the other hand are at an early, probably only neuroblastic, stage of development. In short, the peripheral relations of the nervous system in the trunk have already been considerably elucidated. On the other hand, very little is known about the neurology of the spinal cord. Some of the central
relations of the sensory neurons might be deduced from Studnicka's work and Beccari's work on older animals. But nothing is known about internuncial neurons and the correlating mechanism; nothing is known about the development of descending fibres from the brain which might by this stage be coordinating the motor tracts; nor have either the dendrites or the longitudinal processes of the motor neurons been described.

**Methods**

Two methods of staining neurons specifically were used, namely, vital staining with methylene blue and ‘silver on the slide’.

Methylene blue does not appear to have been used previously on the nervous system of vertebrate embryos. It has many advantages.

1. Much younger material may be used than will adequately take other nerve-specific stains, or impregnations.
2. Total preparations may be made of the more transparent embryos such as *Salmo*. These show much that would be difficult to perceive or reconstruct from sectioned material. This is especially true of nervous structures which are segmentally repeated.
3. The neurons which have taken the stain may be examined either in the living animal or in permanent preparations.
4. Methylene blue and Golgi preparations have many similar characteristics, as Polyak (1941) has pointed out. The sections may be relatively thick, 30\(\mu\) to 60\(\mu\). Only a limited number of neurons are shown up, but these take the stain throughout, in nucleus, cell-body, axon, and dendrites. Methylene blue and Golgi sections are therefore relatively simple to interpret.

In the case of lamprey material, the presence of opaque white food-material in the cells of the younger embryos makes it difficult to observe the central nervous system in the living animal by means of methylene blue.

Silver was used to complete the picture given by the methylene blue. Since silver impregnates all neurons a more quantitative account is possible. Its use also ensures that no entire category of neuron shall be omitted from any reconstruction, for it is exceptional for methylene blue to colour some categories, e.g. the Müller and Mauthner giant fibres.

A silver method where reduction takes place on the slide is more easily controlled than one of the Ramón y Cajal methods, and is therefore particularly suited to very young material, for which adjustments in any technique are usually necessary.

As a control method embryos fixed in Susa were stained by a variety of standard techniques. The most effective was Mallory’s phosphotungstic acid haematoxylin, which gave good differentiation and also picked out the neuroglial cells.

*Methylene blue*. The stain may be applied either as a coloured solution, or as a colourless solution of Rongalit-methylene blue. The former appears not
to penetrate so well. The results with Rongalit-methylene blue are, on the other hand, less predictable, so that it is less easy to determine beforehand what nervous elements shall take the stain. In each case, the quality of the methylene blue is important. In this work, Gr"ubler's methylene blue for 'Vitalfarbung, nach Ehrlich' was used.

The coloured solution was applied to embryos such as trout and lamprey at about 10° C. at dilutions of about 1:1000. The embryos were completely immersed. The first staining, apart from any tissue which might have been cut or damaged, usually occurred after 2-5 hours. Usually nerve-cells, especially those with long peripheral fibres, were the first to appear blue. Coloration is intense, so that there is usually excellent contrast between stained and unstained elements. Where there is a large yolk-sac, as in the trout, it is advisable to cut away the ventral part so that the dye can reach the coelom directly.

Rongalit-methylene blue was applied by the method used in this laboratory by Dr. C. F. A. Pantin, F.R.S., and by Dr. J. E. Smith upon invertebrate material. Their suggestions concerning its use were very helpful. The method is described by Smith (1946).

Whether using methylene blue or its colourless leucobase, the resulting preparations may be made permanent. The procedure has been developed from that of Cole (1936). Only on neurons which are still intensely stained should fixation be attempted, because some time elapses before the fixative affects the dye. Stages 1-6 are carried out at between 0° and 3° C.

1. The embryos are fixed for 2 hours in 8 per cent. ammonium molybdate. If it is necessary to fix overnight, difficult material should be given a preliminary fixation in saturated ammonium picrate of 5 minutes' duration.
2. Wash in water for 1 minute.
3. Transfer to the following n-butyl alcohol mixtures in the Lang (1937) series for 30-40 minutes each: 30, 57, 82, 91, 97 per cent. total alcohol.
4. Leave in absolute n-butyl alcohol for 2-12 hours.
5. Transfer to equal parts of methyl benzoate and n-butyl alcohol, then to pure methyl benzoate, for 1 hour in each.
6. Transfer to a mixture of methyl benzoate and liquid paraffin for 1 hour.
7. Transfer to ice-cold liquid paraffin. The embryos may now be allowed to come to room temperature. They may be left in liquid paraffin indefinitely, before they are examined as total preparations, or embedded in wax and sectioned.

The purpose of this procedure is to avoid the leaching effect of water or ethyl alcohol so far as possible. The following additional points may be helpful. (i) Longer immersion in ammonium picrate, as in the technique of Bethe (1898), causes the nerves to appear green and the background yellow. (ii) Diethylene dioxide has been used for dehydrating methylene blue material, but has not been found so satisfactory as the n-butyl alcohol technique. (iii) If the specimens are not to be sectioned, they may be brought from
the Young Larval Brook-Lamprey

methyl benzoate through a mixture with xylene and Canada balsam into balsam. Balsam preparations of Salmo embryos with stained neurons have kept excellently for the period of the war.

Silver. Embryos, narcotized in weak urethane, are immersed for 2 days in the alcohol-chloral hydrate fixative used by Nonidez (1939). They are then dehydrated with 57, 82, 91, 97 per cent., and absolute alcohols. These alcohols are made up according to the procedure of Lang (1937), which utilizes the properties of $n$-butyl alcohol, and is less damaging to delicate tissue than ethyl alcohol dehydration. The specimens are brought slowly through methyl benzoate, then liquid paraffin, and then imbedded in paraffin wax. The methods of Nonidez and Lang enable considerably better impregnation to be achieved than is attained on similar material by more orthodox procedure.

The subsequent treatment followed the technique of Holmes (1943), which is one of the best of the many variations of the method of Bodian (1936). On the ammocoete material, good results were obtained using a 1 : 30,000 solution of silver nitrate in distilled water, buffered at pH 8.3 at 37° C. for 2–3 days. Bayer's German pre-war Protargol was also used in place of silver nitrate, and gave good results. This form of Protargol is now difficult to obtain. The Protargol should either be used with addition of metallic copper, as in Bodian's method, or should be buffered to about pH 8.0 with the borax-boric acid buffer used in the Holmes method.

Professor J. E. Harris, Department of Zoology, Bristol University, has given me much important advice upon the silver procedure.

Counterstaining, e.g. with Orange G, is sometimes an advantage.

Material

The prides used in this study were between 7 and 10 mm. in length. They correspond to the developmental stage VIII of Hatta (1896), in which the stomodaeum has just opened. The general morphology of the central nervous system of these specimens closely resembles that figured by von Kupffer (1906) for the 6-mm. animal: no appreciable change toward the form described by him for the 20-mm. ammocoete has been observed.

The material was obtained from two sources:

(i) Prides of Lampetra planeri from a tributary of the Lymington river, in the New Forest. I am greatly indebted to Mr. A. R. Hockley, Lecturer in Zoology, University of Southampton, for the living and the fixed material from this source, and also for demonstrating to me his methods of collection. Two batches from the New Forest were used for the silver preparations. Batch 'A', average length 7 mm., was fixed 10 days after fertilization. Batch 'B', average length 8 mm., was fixed 16 days after fertilization.

(ii) Lampetra prides, of 8–10 mm. in length, received alive from Professor J. E. Harris. These were used for methylene blue preparations.

Sagittal and transverse serial sections were prepared of both batches for the silver method: horizontal sections were prepared only of the 'B' batch.
Impregnation and differentiation of nervous tissue both in brain and spinal cord and peripherally was satisfactory in all sections.

THE NERVOUS SYSTEM OF THE TRUNK REGION OF YOUNG AMMOCOETES

Sensory System

The chief sensory system at this stage consists of neurons having the form and relations of Rohon-Beard cells: they will be described under that name.

_Rohon-Beard neurons._ The cell-body lies in the dorsal grey matter, with its median surface close to the neural canal. It is rounded and large, its diameter being about 6\(\mu\), or about twice that of any other type of neuron in the dorsal part of the spinal cord. Each neuron has three large processes: two turn outward into the white matter, where one fibre proceeds towards the head, and the other caudally: the third process runs outward, leaves the cord at the dorso-lateral edge, and runs to the somatic muscle or to the skin (Text-fig. 1).

At anterior levels the peripheral fibre usually lies in about the same transverse plane as the cell-body, and the cell-body is unipolar, as in Text-fig. 1. At posterior trunk levels the peripheral fibre leaves the cord some distance caudal to the cell-body, and is derived, as a collateral, from the descending fibre. The descending and ascending fibres originate from the cell-body separately, so that at posterior trunk levels the cell-bodies are seen in horizontal section to be consistently bipolar.

The longitudinal fibres can be traced for a distance equal to several somites: they give off slender branches, which appear to be quite short. The dorsal...
white matter seems at this stage to be largely made up of the longitudinal Rohon-Beard fibres. The ascending fibres of Rohon-Beard cells lying near the head appear to run into the Descending Trigeminal tract of the medulla. The peripheral fibre normally runs out in the dorsal root, of which it is the largest, and at this stage the most numerous, component. Such a typical condition is seen in Text-fig. 1. But frequently one fibre runs out separately from those in the dorsal root. When such a separation is in the horizontal plane the outlying fibre has a quite normal course after reaching the endochondral layer (Text-fig. 2).

The cell-bodies lie in two rows, one on each side of the midline, for the whole length of the trunk. They are at various dorso-ventral levels: some project above the general outline of the grey matter, while others are as far ventral in it as the area which in gnathostomes contain the visceral sensory component. No difference in the peripheral destination of the more dorsal and the more ventral cells could be observed. Presumably, in older ammocoetes, the cells of the two rows would settle into a single dorso-ventral level.

The dorsal root passes through the endochondral tissue at the intersegmental position, opposite the myocomma. On the outer side of the endochondral layer the root traverses the area of the dorsal ganglion, which lies at a dorso-ventral level midway between dorsal and ventral roots. Some Rohon-Beard fibres of the dorsal root pass beside or even between the cells of the dorsal ganglion without being in any way connected with them. This independence could be seen in transverse and parasagittal silver sections; it is probably true of all Rohon-Beard fibres, although the dorsal ganglia are so compact that it was not possible to be certain of the independence of every one of the many Rohon-Beard fibres examined (Text-fig. 5a). In methylene blue preparations no stained dorsal ganglion-cells have been found: stained Rohon-Beard fibres are seen to pass from the endochondral layer directly into the interface between successive myotomes.

Between the myotomes the Rohon-Beard fibres spread out and branch in lateral or ventral directions. The larger branches reach the skin. Small branches ending as proprioceptors on the ends of the myofibrils remain in the myosepta. No instance was found of branches penetrating in a longitudinal direction to end in parallel with the myofibrils (Text-fig. 3).

The Rohon-Beard fibres to the ventral part of the myotome and to the skin of the ventral surface do not penetrate between myotomes until they have passed well below the notochord: for this reason a parasagittal section just lateral to the notochord shows both the motor nerve and the ventrally directed Rohon-Beard fibres. The motor nerves lie in a mid-segmental and the Rohon-Beard fibres in an intersegmental position in remarkably consistent fashion through the whole length of the trunk.

On reaching the skin the Rohon-Beard fibres divide further; some subdivisions reach the dermis and may penetrate the basement membrane of the
epidermis (Text-fig. 4). Others continue down the outer side of the inter-
somite, where, by meeting other similar branches, they become an appreciable
aggregate of fibres.
Just as some Rohon-Beard fibres run out separately from the dorsal root,
slightly caudal or cephalad to it, so others proceed outward independently
and dorsally to the dorsal root. Most of these fibres reach the dorsal tip of
the myotome, where they cross the muscle in an intersegmental position, or
pass round the top of the myotome to reach the skin. Since such fibres run

singly, and since they are clear of the dorsal ganglion on the whole of their
course, they are easy to examine (Text-fig. 5b). Large branches of these fibres
reach the skin, but the smaller branches turn back to end on a myofibril.
Such Rohon-Beard fibres correspond closely to those described by Coghill
in Amblystoma.
Others of the separate, latero-dorsally directed Rohon-Beard fibres do not
reach the myotome at all, but pass from the dorsal surface of the spinal cord
through the endochondral layer at only a slight angle to the vertical, so that
they reach the skin of the dorsal surface. This third type of Rohon-Beard cell
has been noted previously by Studnicka, Tafel III, fig. 11. He regarded the
type as aberrant: but it occurs in small numbers at all trunk levels, and is
evidently the only sensory neuron which innervates the dorsal areas of the
skin in the young pride (Text-fig. 5c).
The Rohon-Beard fibres which proceed independently of the dorsal root
are confined, like those in the dorsal root, to an intersegmental position.
When they occur at an intersegment in pairs they are arranged symmetrically about the midline. They are probably the 'pathfinders', the earliest sensory neurons to have developed at that intersegment, and neither of the independent types should be regarded as aberrant.

**Dorsal Ganglion-cells.** Some of the cells of the dorsal ganglia are round or pear-shaped neuroblasts: others are bipolar neurons which have slender peripheral and central processes. Neither form of process could be traced to its destination, but there are several fine fibres in each dorsal root, which have presumably come from the dorsal ganglion-cells. The peripheral process is usually directed ventrally, but was never traced far enough to show to which functional component these early dorsal ganglion-cells belong.

**Correlating System**

**Large Internuncial Cells.** In the lateral part of the grey matter there occur some large cells whose axons run to the ventral motor tract, and whose dendrites are in clear relation with the dorsal tracts of the same and also of the opposite side of the cord (Text-figs. 6 and 7).

Dendrites to the lateral, dorso-lateral, and dorsal areas of the white matter arise from the cell-body separately. Those to the lateral area are short: those to the dorso-lateral area are longer. Dendrites to the dorsal areas have a characteristic course: they run directly dorsally, entering the white matter at an oblique angle: they then turn medially in a wide curve which keeps them clear of the Rohon-Beard cell-bodies. Most of the dendrites to the dorsal area continue medially, so that they enter the contralateral dorsal area by way of the dorsal commissure. The contralateral process runs directly across the
cord, so that the cell-body and the crossing dendrite may appear in a single section, as in Text-fig. 6. The contralateral process can also be seen in sagittal sections at 20 μ, for it is quite large, and can be followed from the cell-body to the midline by adjusting the optical section. The axon enters the ventral area of the white matter, where small collaterals branch off into the ventrolateral area. The main process turns forward to run with the longitudinal fibres. Although it is now near the midline, it does not cross to the contralateral motor areas, and no collaterals from the axon have been observed to cross (Text-fig. 8).

These large internuncial cells usually occur singly, one per segment, somewhat caudally to the origin of a ventral root, with which the axon is no doubt in synaptic relation. But the distribution of these cells is not quite so constant as might be inferred from the region figured.

**Small Internuncial Cells.** The Rohon-Beard and the large internuncial cells are only a small proportion of the nerve-cells in the dorsal half of the cord. Many of the remainder are neuroblasts, some of which do not appear to have yet formed any process from the cell-body: those which do have dendritic and axonic processes have not, however, achieved a definitive or constant structure, though they all appear to be developing into small internuncial cells which link the ipsilateral sensory and motor fields and which have no longitudinal process. Cell-bodies of this type of neuron lie at the dorsolateral edge of the grey matter. There is a thick, ‘protoplasmic’ rather than fibrillar axon, which runs down between the grey and the white and ends on...
the cell-body or immediately adjacent dendrites of a motor cell. The dendrites are short, and are usually directed into the dorso-lateral white, but they are diffuse in form and very lightly stained. It is probable that the dendrites are growing towards the incoming central fibres of dorsal ganglion-cells, which we know to be at the same stage of outgrowth. The contrast between the diffuse, almost amoeboid form of these cells, and the constant and well-defined form of the large internuncial neurons is marked.

**Oblique Fibres.** At fairly regular intervals along the mid-trunk region of the cord a single large fibre swings out of the dorsal funiculus and descends obliquely towards the ventral funiculus: it reaches the latter about four somites more caudal than its origin from the dorsal funiculus: the fibre continues caudally in the inner and medial part of the ventral white, internal to the Müller fibres. The oblique fibres can be picked out in transverse sections, where it can be seen that during the oblique part of their course they pass medial to all the longitudinal fibres of the lateral white. In parasagittal sections the oblique fibres stand out sharply, because the other large fibres all run longitudinally in the cord. Since they are on the inner side of the white matter they necessarily pass close to at least one of the large internuncial neurons; but no synaptic relation was observed between them. Individual oblique fibres were followed towards the head in the dorsal white matter; but the cell of origin has not yet been found, nor has any large cell of different form from the Rohon-Beard cell been noticed in the dorsal white at an interval of one per four somites (Pl. Ic).

In horizontal sections of the ventral part of the cord, the oblique fibres are seen to continue in a ventral and caudal direction until they are directly medial to the Müller fibres; they may run parallel with the Müller fibres for a short distance, but they soon swing abruptly out of line, turning ventrally
and medially to reach the ventral white of the opposite side by way of the ventral commissure. Each oblique fibre turns to run parallel with the contralateral Müller fibres, but becomes rapidly attenuated and terminates (Text-fig. 9). The oblique fibres seen in horizontal section appear at fairly regular intervals, about four somites' distance separating successive fibres of the same side. Those of the two sides are not paired. Oblique fibres have not been seen in either sagittal or horizontal sections to occur in the anterior third

![Diagram](image)

**Text-fig. 9.** Horizontal section, 16\(\mu\), 16-day *Lampetra*; silver method. (Plane of section is slightly more ventral towards the lower left side of drawing.) Fibres drawn as if viewed from dorsal aspect; shows the course of two oblique fibres in the ventral commissure.

of the spinal cord: it seems probable that, at this stage of development at least, they do not occur in that region.

**Cells of the Commissura Infima Halleri.** In a dorso-medial position, in the most anterior part of the cord, there are on each side of the midline about twenty neurons with characteristic bipolar, spindle-shaped cell-bodies. All are oriented transversely: from the medial end of the spindle a single dendritic process crosses the midline close to the dorsal surface of the cord to end in the contralateral dorsal part of the cord. The outer end of the spindle is formed by the axon, which runs laterally into the dorso-lateral part of the white matter (Pl. 1a).

These neurons can be seen in horizontal and transverse sections of 16-day prides, when they have the form described: but in 10-day prides the cells are still unipolar neuroblasts. They occur in a length of about 60\(\mu\) of the cord, posterior to the choroid plexus of the medulla oblongata: the most caudal is at the same transverse level as the most anterior Rohon-Beard neurons. The diameter of the cell-body, measured across the spindle, is about one-third that of the Rohon-Beard neurons. The centre or nucleus, constituted by
these cells, lies dorsal to the ordinary cells of the dorsal grey, and median to
the two dorsal funiculi, which at this level are separated from each other by
this centre.

Unfortunately, the longitudinal relations of the centre are not yet known.
A few of the axons can be seen to turn caudally after reaching the dorsal
funiculus, while a small collateral runs anteriorly. But the details already
established show that these neurons constitute by the sixteenth day a Com-
missura Infima Halleri and its Nucleus.

Müller Fibres. The axons of the Müller cells of the brain run toward the
tail for the whole length of the spinal cord. In transverse sections of the

![Text-fig. 10. 12μ, 16-day Lampetra; silver method. The ventral commissure.](image)

trunk they are seen below and lateral to the floor-plate cells and median to
the other longitudinal fibres of the ventral funiculus (Text-fig. 10).

On each side of the cord there are about eight Müller fibres which are
larger in diameter than the other longitudinal fibres of the cord, and about
another eight are of the same diameter as the biggest of the fibres in other
tracts. Each fibre remains on the same side of the cord as its cell of origin,
and closely parallel to its fellows, so that the pattern made by the Müller
fibres remains constant through many transverse sections. The fibres do not
divide, nor have they been seen to give rise to any collaterals.

This description agrees with the accepted account of older animals, except
that the Müllerian fibres in the young pride do not have the extraordinarily
enlarged diameter found in older prides, and the tract still retains the position
to be expected in a motor co-ordinating system. The cells of origin in the
brain show extremely well in the present material: their form and the relations
of their dendrites correspond with the figures given by Tretjakoff. The Müller
cells lie in two groups, one in the midbrain and the other in the hindbrain:
the dendrites of the two groups bring the cells into synaptic relation with
all cranial sensory nerves except the optic and olfactory. A single large cell on
each side can be seen in the present material to reach most parts of the fore-
brain with its dendrites, and to have an axon descending to the region of the
Müller cells of the midbrain. There are optic fibres in the optic chiasma at
this stage; and Studnicka and Walls (1944) have stated that there is a primitive
functional lens at this period. Presumably, therefore, the Müller fibres of the spinal cord may be affected by stimulation of any of the cranial nerves, including those of the forebrain group.

At the most anterior levels of the spinal cord a pair of the Müller fibres, apparently of similar cranial origin to the others, turns laterally and dorsally, reaches a position median to the lateral funiculus, and continues caudally, one on each side, in this position for the whole length of the trunk. This dorsalward movement of one pair of Müller fibres is very striking in the young ammocoete.

Motor System

Somatic Motor Neurons. Some of the larger axons in the ventral root can be followed, in a single transverse section, peripherally to motor end-plates on the myotome and centrally to cell-bodies lying in the ventral area of the grey matter. In such cases the axon grows from the cell-body into the ventral motor tract; from the tract its course to the motor root is horizontal (Text-fig. 11). The majority of motor neurons are not so easily traced, because the cell-body and the ventral root to which it contributes are in widely separate transverse planes; but almost all the large axons can be traced from myotome to ventral root and thence horizontally as far as the ventral motor tract.

The motor neurons of the ventral grey area are of two forms. Those lying in the outer part have a 'protoplasmic' area of the cell-body on the lateral aspect of the nucleus: this area extends dorsally between the grey and the white, giving origin, on its surface against the white, to many branching dendrites which terminate on the lateral and ventrolateral surface of the spinal cord. The axon originates separately, from the outer and ventral part of the cell-body (Text-fig. 12). On entering the ventral motor tract
it turns longitudinally, giving off the peripheral fibre to the motor root as a collateral.

Neurons of the inner part of the ventral grey matter effect the same relations in the cord, but the processes from the cell-body are thin, cylindrical fibres until they reach the boundary between grey and white, when they also form a protoplasmic extension at the base of the dendrites, though it is less extensive (Text-fig. 13).

These ventral motor neurons usually have a separate process on the ventral or ventromedial surface of the cell-body (Text-figs. 10–13). This process can sometimes be traced into the ventral motor tract of the opposite side; sometimes it appears to originate as a collateral of the axon. These commissural fibres are best seen in cases where their whole course is through the white matter, as in Text-fig. 10. They appear to have no relation with the Müller fibres of their own side; their relation with the Müller fibres of the opposite side may be closer, but has not yet been determined. The commissural fibres usually pass ventral to both sets of Müller fibres, but sometimes they pass dorsal to those of their own side; two of this latter type can be seen in Text-fig. 16.

The neurons of the ventrolateral grey matter which are developed by this stage probably also belong to the somatic motor component, of which they would then constitute a second type. As in the case of those just described, the dendrites from neurons lying deep in the grey are thin fibres, while the dendrites of cells on the outer limit of the grey matter take origin from a broad ‘protoplasmic’ area of the cell-body. The dendrites reach an extensive area of the lateral funiculus, but have not yet been found to extend into the ventral funiculus. A great proportion of the branches of the dendrites run a short distance longitudinally in the lateral funiculus, so that they must represent a large part of it. Unlike the first type these neurons have some dendrites which extend dorso-laterally to come into contact with the longitudinal fibres of the Rohon-Beard cells. The axon is directed ventrally before leaving the grey matter, so that they enter the ventral funiculus close to the axons of somatic motor neurons of the first type. Instances in the second type where the cell-body, the intramedullary and the peripheral part of the axon lay in

Text-fig. 12 and 13. 34μ, methylene blue method. Primary motor neurons.
the same transverse section have not so far been found. The motor neurons of the second type appear to be somewhat younger than the first type, and did not show up so well in the silver material, which was prepared from slightly younger embryos (Text-figs. 14 and 15).

**Visceral Motor Neurons.** The innervation of the splanchnic muscle of the gill-region appears to be derived entirely from the vagus nerve, which can be seen on the ventral and outer side of the anterior cardinal veins. The segmental ventral branch of each spinal motor nerve of this region passes close to the vagus: but the fibres of the branch remain adherent to the myotome and none appear to join the vagus nerve or to innervate splanchnic structures directly, or to reach a sympathetic ganglion: nor have sympathetic ganglia yet been identified. It is possible that a small viscero-motor component may, however, exist and have been overlooked, because the organs lying in the area between the pharynx or the intestine and the myotomes are surrounded by stellate melanophores. Consequently, in silver preparations, the black nerve-fibres are extremely difficult to follow among the melanophores. Evidently the viscero-motor component of the spinal nerves, so far as it exists at this stage, can be concerned only with the innervation of blood-vessels, and perhaps of the anal region of the gut (Dohrn, 1888). It appears improbable that the motor neurons of the ventrolateral grey belong to the viscero-motor component.

In older *Lampetra* the hypoglossal nerve is built up by the union of ventral branches from each motor root of the pharyngeal region. These branches individually enter and run a short distance with the vagus nerve before
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segregating into a hypoglossal nerve (Neal, 1897; Johnston, 1905). The association between the hypoglossal and vagal nerves is certainly brought about by the backward extension of the gill-region during development. It is interesting that at the present stage of development the association has hardly commenced.

Ventral Commissure (Text-fig. 10). Some of the fibres in the ventral commissure have been described already as originating directly from motor cells of the ventral grey matter. But the great majority of the fibres crossing in the ventral commissure do not arise from a cell-body at the same transverse level, but from a longitudinal fibre of the ventral funiculus. In these cases, a longitudinal fibre swings towards the midline, passing under the Müller fibres of its own side, and crosses the midline in the extreme ventral position: the fibre then becomes much thinner: it can be followed as it passes ventrally to the Müller fibres of the opposite side. Some of the fibres are too fine to be followed any farther, but others can be traced laterally and dorsally within the ventral funiculus before they also attenuate entirely (Text-fig. 16).

The crossing fibre may approach the commissure from in front or from a caudal direction. As it passes under the ipsilateral Müller fibres, it usually gives off a collateral which turns away from the midline and continues in the ventral funiculus. At the midline the crossing fibre is almost or quite in the transverse plane. It does not usually branch in the opposite funiculus. The number of crossing fibres in the ventral commissure is of the order of thirty in a length of cord equal to one somite. All, or almost all, of the fibres crossing in the ventral commissure probably originate from the somatic motor neurons, either from a process direct from the cell-body or as the termination of a longitudinal fibre in the ventral funiculus.

No outgrowth has been found from any of the floor-plate cells in the present material, at any level of the spinal cord.

In the most anterior segments of the trunk of the 16-day pride, a few of the crossing fibres in the ventral commissure appear to have a synaptic relation with the contralateral Müller fibres, to which they pass very close. These fibres of its own side, and crosses the midline in the extreme ventral position: the fibre then becomes much thinner: it can be followed as it passes ventrally to the Müller fibres of the opposite side. Some of the fibres are too fine to be followed any farther, but others can be traced laterally and dorsally within the ventral funiculus before they also attenuate entirely (Text-fig. 16).

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branches on to Müller fibres are extremely minute; this detail requires confirmation on older material.

The Tail Region

The very short tail region is omitted from the present account because the anatomical relations have proved very difficult. In particular, it has not been possible to find out the relations of the Müller fibres in the tail region.

COMPARISON OF THE SPINAL CORD OF ADULT LAMPETRA WITH THAT OF THE NEWLY HATCHED LARVA

Possible homologies between the neurons described here and those in the spinal cord of older animals will be discussed only briefly, because the forms of neuron to be found in the latter were the subject of furious disagreement between Kolmer, Tretjakoff, and Johnston (1910). Knowledge of the sensori-motor arc in the adult is therefore very uncertain.

It is clear from the foregoing account that the Hinterzellen of the adult must be persistent Rohon-Beard cells. Both the Rohon-Beard cell of the young pride and the Hinterzell are very large dorsally placed intramedullary cells: they occur in two rows for the whole length of the spinal cord: no other large neuron occurs near them. They both have a large fibre ascending and a large fibre descending in the dorsal funiculus. The Rohon-Beard cell has a somatic-sensory peripheral process. A similar process is found on the Hinterzell of older prides (Freud and Beccari), but is reduced or even lost in the adults (Johnston, 1902). Correspondingly, the dorsal ganglion-cells have become the predominant source of somatic sensory peripheral fibres in the adult.

The Rohon-Beard neuron has the form of a large dorsal ganglion-cell, in spite of its intramedullary position; but in the young animal it appears to combine the functions of a ganglion-cell with those of a dorsal horn cell in the somatic sensory column. As a Hinterzell in the adult it has partly lost the first of these functions.

The large internuncial neurons of the young pride are difficult to identify among the cells described in the adult or in the older larvae; but the ‘Type II motor cells’ of Tretjakoff’s figures appear to me to be the probable homologue. The small internuncial cells probably correspond to Tretjakoff’s ‘amacrine’ cells.

It is difficult to say which type of cell in the adult corresponds to the oblique fibres.

The somatic motor neurons have similar relations to those described in the adult by Tretjakoff (1927). But the dendrites of these neurons do not have the bizarre arborizations at this stage, which they have acquired in the adult.

The relatively immense Müller fibres of the adult correspond in the young pride to more normal-sized fibres confined to the area in which the medial longitudinal bundle is found in Gnathostomes.
Although the individual neurons of the Commissura Infima Halleri are less developed than Johnston (1910) found them in the adult, the centre and commissure as a whole has established by this stage its relation with the brain and with the spinal cord.

In short, the spinal cord of the newly hatched ammocoete does not exhibit the unusual characters of morphology and of the shape and relations of the constituent neurons which make it difficult to regard the cord of the adult as a prototype of the spinal cord of gnathostome vertebrates.

**Comparison with Spinal Cord of Gnathostome Embryos**

Reference is particularly made to Coghll’s and to Youngstrom’s accounts of *Amblystoma*. But the result would not be different if the comparison were made with earlier work on fish larvae, such as van Gehuchten’s account of *Salmo* alevis (1895). Similarities between the *Amblystoma* larva and the young pride are:

1. There is a well-defined central grey and an outer white matter. The spinal cord is more or less cylindrical. The dorsal root, dorsal ganglion, and ventral root occur in corresponding positions.

2. The Rohon-Beard neurons are organized in the same relation to other neurons, occupy the same position, and have the same form. (Cf. Coghll, 1929, figs. 9, 10, and 11.)

3. The ‘dorsal intercalated’ neuron is probably homologous with the large internuncial cell of the young pride. The only notable difference is that the latter has not yet developed any process crossing in the ventral commissure. (Cf. Youngstrom, 1940, fig. 1, and Text-fig. 17 of this paper.)

4. Two forms of somatic motor neuron are found in both larvae. The primary motor neuron of *Amblystoma* is similar in all essentials to the motor neuron in the ventral grey of *Lampetra*: both have a ‘protoplasmic’ extension between the grey matter and the white and have dendrites extending through the lateral and ventrolateral white areas; in both the peripheral fibre is given off as a collateral of a fibre in the ventral motor tract; both have a process into the contralateral ventral funiculus; in both the cell-body does not usually lie at the same transverse level as the ventral root. Possibly they both have the same functional relation to a giant-fibre system descending from the brain. For the primary motor neuron of *Amblystoma* and of other anamniote Gnathostomes using the trunk-muscle as the locomotor organ, including teleosts, dipnoans, and anuran tadpoles, is in synaptic relation with Mauthner’s fibre which originates in the contralateral hindbrain. In *Lampetra* the Miiller fibres are derived from cells in the ipsilateral part of the brain, but the fibres appear to be developing a synaptic relation with the ventral motor neurons of the opposite side. If this should be established the Miiller fibres might prove to have the same function in the trunk region of the lamprey as is effected by Mauthner fibres in fishes.

A more dorsal group of somatic motor neurons has been described by Youngstrom, the ‘secondary’ type. They also probably occur in the
other Gnathostomes of the anamniote division. They are concerned with local movements, while the primary type effect the total movements. Movements of paired limbs, independent of trunk movements, are believed to begin when the secondary type first appear at the appropriate level of the cord.

The ventrolateral group of motor neurons in Lamproptera are probably also somatic motor, and have some characters in common with the secondary type in Amblystoma. They are in synaptic relation with the afferent neuron, they have apparently no relation with the longitudinal giant-fibre system, nor with the ventral tracts of the opposite side of the cord, and the axon is very slender. But the ventrolateral group have not been investigated in sufficient detail for these similarities to have much weight. Their relations with other neurons at present suggest that they are concerned with some very local form of movement.

The Commissura Infima Halleri has not apparently been described in very young urodeles: it is not mentioned in Coghill's summary (1929) of his work. In amniotes the commissure is considered to correlate the viscero-sensory columns of the two sides: it was regarded as having that function in the adult Lamproptera by Johnston (1902). But Herrick (1908) showed that in fishes the commissure has a viscero-sensory and a somatic-sensory component: in fish such as the trout the somatic-sensory part of the commissure is the more important. In the trout embryo the commissure is developed at a very early stage, before the embryo can swim, and when the viscero-sensory system of the cord has hardly begun development. It is legitimate to suppose that in the young Lamproptera the Commissura Infima is an important part of the correlating mechanism involved in swimming movements.

### The Somatic Sensori-Motor Arc in Young Pride (Text-fig. 17)

It seems clear that at this stage the Rohon-Beard cells are the chief sensory mechanism. They receive stimulation from sensory endings of very simple nature in the skin and, to a less extent, in the muscle. There are no muscle-spindles nor any proprioceptors in parallel with the myofibrils. Some Rohon-Beard neurons are entirely exteroceptive in function. Centrally, the impulses of sensory origin in the trunk pass up and down the trunk without any strict segmental organization.

The excitatory impulses can pass from the Rohon-Beard tract to the Commissure of Haller or to the Descending Trigeminal centre, thereby affecting either the other side of the trunk or the brain: or the impulse may pass to motor neurons by way of the large internuncial cells, or directly through the dendrites of the ventrolateral motor neurons, or by way of the oblique fibres. Of these paths the most developed at this stage, and that about which most detail has been elicited, is that through the large internuncial cell. But even here it is difficult to see how an apparently simple locomotor system should be related to so complex and precisely organized a system of dendrites, axons, and axon collaterals.
Consideration of the dorsal ganglion-cells, small internuncial cells, and ventrolateral motor cells is not profitable until slightly older material is available, in which these types of neuron would be more differentiated. Equally, the earliest stages in the development of the somatic sensori-motor arc are not covered in the present study.

TEXT-FIG. 17. Stereogram of spinal cord of *Lampetra*, 10–16 days after fertilization, to show the relations between some of the neurons described. The figure is constructed to show a length of cord viewed from an anterior and dorsal position.

Clearly there is already in this stage of the ammocoete a complex and precisely organized structural pattern, both of the form of the neurons and of their relation to each other. Any satisfactory description of the function of this nervous system must account for the intricacies of this pattern.

I wish to thank Professor James Gray, F.R.S., for his kind encouragement and advice on problems of neuro-embryology, and Professor J. E. Harris, Department of Zoology, Bristol University, for his helpfulness in considering the problems of function which are implicit in the description of nervous
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**SUMMARY**

1. A method for the study of the nervous system of vertebrate embryos by methylene blue vital staining is described. A reliable technique for rendering the preparations permanent is described.

2. An adaptation of the silver ‘on-the-slide’ method is given.

3. Three types of sensory intramedullary neuron are described in the spinal cord of recently hatched ammocoetes, or prides, of *Lampetra planeri*. All three are regarded as types of Rohon-Beard cell.

4. Four contemporary correlating types of cell are described in the cord: large internuncial neurons with a dendritic system which reaches the contralateral dorsal funiculus; cells of the Commissura Infima; oblique fibres, descending caudally from the sensory to the motor tracts; and small internuncial neurons with short dendrites.

5. The relations of the Müller fibres in the trunk are described in part.

6. Two types of motor neuron have been found; the more fully developed corresponds to the primary motor neuron of aquatic larvae of other anamniote vertebrates.

7. The peripheral fibres of the somatic system of the trunk are described.

8. The neurological pattern revealed is compared with that in adult *Lampetra*: the divergences from the vertebrate pattern found in the cord of the adult are not found in the young ammocoete, which in this, as in so many respects, is a good prototype of gnathostome vertebrates.

9. The probable functional pattern is compared with that found in a similar stage of *Amblystoma*.

Neurons of the correlating and motor system appear not to have been described before in ammocoetes less than 1 year old.

**LIST OF REFERENCES**

Van Gehuchten, A., 1895. La Cellule, 11, 111.
DESCRIPTION OF PLATES

PLATE I

A. Transverse section showing the Commissura Infima Halleri. \( \times 1150 \).

(a) One of the cells of the Commissure.

B. Horizontal section showing several Rohon-Beard neurons with longitudinal and peripheral processes. \( \times 400 \). Compare Text-figs. 1 and 2.

C. Parasagittal section showing oblique fibre in the lateral part of its course. \( \times 370 \).

PLATE II

D. Parasagittal section showing relation of large internuncial cells to the segmental motor nerves and to the intersegmental sensory nerves. \( \times 370 \). Compare Text-fig. 8.

E. Transverse section, \( 34/x \), showing motor neuron stained with methylene blue. \( \times 1700 \). Compare Text-fig. 12.

F. Parasagittal section showing a large internuncial cell. \( \times 740 \).
A

white

gray

B

Rohon-Beard neuron

dorsal root

C

spinal cord

anterior

notochord

WHITING—PLATE I