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The Structural Organization of the Intracerebral Giant Fiber System of Cephalopods

The Chiasma of the First Order Giant Axons*

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Summary. The course of the two intracerebral first-order giant axons of cephalopods at their chiasma, and the fine structure of the contact area between the crossing axons are examined by light and electron microscopy in species of three taxonomic groups (*Loligo vulgaris, Sepia officinalis, Illex coindeti*). In addition to the well known chiasma of the adult *Loligo* in which the two axons are fused (YOUNG, 1939), three other chiasma types are described. In each of them there are synapse-like contact areas that suggest a passage of impulses from one axon to the other. (1) The larval *Loligo* shows a chiasma with crossed axons and contralaterally descending branches; at the apposed membranes in the chiasma there are clusters of electron-transparent vesicles. (2) In the adult *Sepia* each crossing axon has an ipsi- and a contralaterally descending branch; the apposed membranes of the decussating axons show symmetrical synapse-like areas characterized by a monolayer of electron-transparent vesicles on each side and a regular cleft of 100 Å width. (3) In the adult *Illex* each axon has only an ipsilaterally descending branch and there are at the point of decussation two crossed collaterals; large masses of electron-transparent vesicles are found on each side of the apposed membranes of the collaterals and the membranes show an increased electron density. It is argued that the four chiasma types serve the same function, i.e., the establishment of functional bilaterality of the giant fiber system. By structural analogy with other, both structurally and functionally known synapses it is suggested that in the decussation of *Sepia* impulses pass in both ways from one axon to the other. Data of the embryological development of the giant fiber system are summarized.

Two crossing nerve fibers, one from each body side, theoretically may take the following three courses (Fig. 1): each fiber may cross over to the other side and end on the contralateral side (I), it may end, by bifurcation, on the ipsi- and the contralateral sides (II), or, ending ipsilaterally, it may have two crossed collaterals (III).

As will be shown in this paper each of these cases is realized in the chiasmata of the first order giant axons in the caudal suboesophageal brain of different

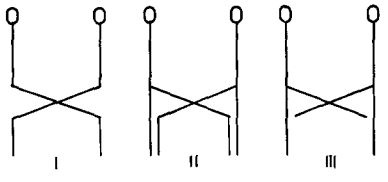

Fig. 1. The three possible decussation types of two axons

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cephalopod species. In addition, a modification of case (I) occurs which has been well known for some time: in the squid the two decussating fibers are fused, forming a syncytium (YOUNG, 1939). Notwithstanding the structural diversity, evidence will be presented for the assumption that the four chiasmata serve the same function by means of various synaptic contact areas connecting the two crossing axons at the decussation.

The structure of these chiasmata is investigated here by histological methods and by electron microscopy in species of the three taxonomic groups that possess a giant fibre system: the Loliginacea, the Sepiacea (excluding the Sepiolidae) and the Architeuthacea.

The Localization of the Chiasma in the Whole Giant Fiber System

In contrast to the extensive literature on structural and functional aspects of peripheral elements of the giant nerve fiber system of cephalopods (second and third order cells and axons) the knowledge of the intracerebral elements is based on a few studies, mainly the work of YOUNG (1939). The course of the first order axons in developmental stages of *Loligo* and of *Sepia* has been studied by MARTIN (1965) and MARTIN and RUNGGER (1966).

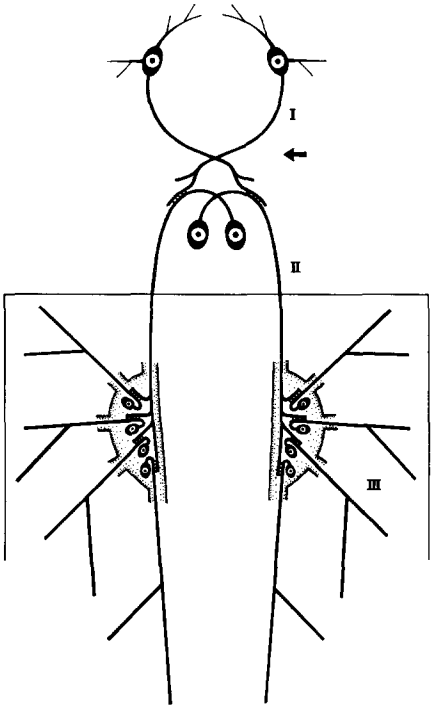

Fig. 2. Schematic representation of the main elements of the giant fiber system in cephalopods, combined after findings in different species. *I, II, III*, first, second and third order giant cells. The first and second order cell bodies lie in the caudal suboesophageal brain, the third order cell bodies in the stellate ganglia. The arrow points to the chiasma of the first order giant axons (= chiasma I)

Fig. 2 is a schematic drawing of the general arrangement of some of the elements in the giant fiber system of cephalopods. The cell bodies of the two first order cells are situated at the lower inner border of the neuropil in the paired magnocellular lobes of the brain. Afferent synaptic boutons cover the cell soma and the dendrites of these cells. The axons of the first order cells run in a caudal direction to the palliovisceral lobe where they decussate (= chiasma I) in the lower anterior part of the neuropil. After the decussation they synapse with several second order axons, the cell bodies of which are lying in the dorsal and ventral cellular layer of this lobe. While only two of the second order axons (g f 2 b: YOUNG, 1939) leave the brain through the mantle connectives to join the paired stellate ganglia, other second order cells (not represented in Fig. 2) innervate the muscles of the head and the funnel directly.

The second order axons that run to the stellate ganglia decussate too in the palliovisceral lobe. However, in contrast to chiasma I, the decussation lies proximal to the synaptic input area from the first order axons.

Possibly a third cross-connection exists between the two bilateral stellate ganglia: the stellate commissure. It is not known whether or not its fibers are connected with the giant fibers of the stellate ganglion. The stellate commissure is present in architeuthacean and in loliginacean species, but has not been found in *Sepia* (HUXLEY and PELSENEER, 1895; HILLIG, 1912). Since *Sepia* possesses a well developed giant fiber system, it seems that this commissure is not indispensable for the functioning of the giant fiber system.

Material and Methods

Earlier histological studies using silver and gold impregnated (Bodian method) sections of the developing giant fiber system of *Loligo vulgaris* and *Sepia officinalis* have been published elsewhere (MARTIN, 1965; MARTIN and RUNGGER, 1966) and the chiasma diagrams drawn here are based on this work. The diagrams of the chiasma I in *Illex coindeti* and the adult *Loligo vulgaris* have been reconstructed from serial 2 μ sections of Durcupan embedded material.

Fixation Methods for Electron Microscopy

a) Larvae of *Loligo vulgaris* LAM. Egg strings, collected in an aquarium, were kept in seawater which was changed daily, on a rocking apparatus until the larvae hatched. For fixation the freshly hatched larvae were transversely cut into slices. The slices containing the caudal part of the brain were immersed for 2 h at 4°C either in a chrome osmium (1) or a potassium permanganate (2) fixative of the following compositions: (1) 100 ml of a physiological solution for cephalopods (NECCO and MARTIN, 1963) with: NaCl 1.39 g, KCl 0.06 g, $CaCl_2$ 0.06 g, $MgCl_2 \times 6\ H_2O$ 0.31 g, $NaHCO_3$ 0.02 g, Na_2SO_4 0.09 g; in this solution the amount of NaCl is reduced to adjust the osmolality to ca. 1000 mosmols; OsO_4 2 g, $K_2Cr_2O_7$ 1.25 g, KOH 0.56 g: pH ca. 7.5. (2) 100 ml of physiological solution (see 1, but NaCl 1.57 g); $KMnO_4$ 1 g, Na — acetate 0.38 g, Na — veronal 0.58 g.

b) *Loligo vulgaris* LAM. (20—30 cm mantle length),
Sepia officinalis L. (8—11 cm mantle length),
Illex coindeti STEENSTRUP (ca. 30 cm mantle length).

Fixative. 3.5% triple distilled glutaraldehyde (Fisher) in diluted (H_2O: seawater = 1:3) filtered natural seawater; pH adjusted to 7.6 by 1 n NaOH. Addition of NaOH produces a precipitate that must be filtered off. When the fixative was stored, phenol red was added as an indicator. Perfusion of this fixative through the aorta gave better results than glutaraldehyde fixatives in which seawater or sucrose were used in different concentrations and either veronal or phosphate buffers were applied at pH between 7 and 8. Perfusion by osmium or

permanganate fixatives did not give satisfactory results, mainly because these fixatives did not penetrate the tissue uniformly.

Perfusion Technique. The animals were placed in 2.5% urethane in seawater for anesthesia (ca. 4 min.). Then they were put under ice and the aorta exposed near the central heart. A polyethylene tube was inserted into the aorta and tied in place. Then the branchial hearts were opened to allow a rapid outflow of the perfusates.

After rinsing the blood system with 20 ml of cooled natural seawater, 80 ml of the cooled (+4°C) fixative were injected at a flow rate of ca. 10 ml per minute. By this procedure the brain soon turns yellow if the fixation is successful. Then the palliovisceral lobe of the brain was dissected out and immersed in the fixative for 2 h, after which the lobe was cut into two sagittal halves, that were further trimmed into smaller blocks, washed for 4 h in seawater and postfixed in OsO_4 (2%) in diluted (1:3) natural seawater.

Embedding Procedure, Ultramicrotomy and Electron Microscopy. The blocks were dehydrated in acetone and included in Durcupan (Fluka). They were oriented in three different planes in order to achieve sections of the decussating axons in the transverse, the horizontal and the longitudinal planes (= sagittal, horizontal and transverse planes in respect to the whole animal). Technically the easiest way to find the chiasma is to take the sagittal plane of the palliovisceral lobe as section face. In these sagittal sections the crossing first order giant axons are transversely cut. They are easily recognised by their size, being three to four times as large in diameter as the accompanying axons next in size. Horizontal and longitudinal sections of the chiasma are produced by slowly approaching the chiasma in 2 μ serial sections that are observed in the light microscope. The same method was used with the blocks of freshly hatched squids.

When the desired area was reached, ultrathin sections were cut with a diamond knife, mounted on carbon coated or uncoated grids and stained with lead citrate (VENABLE and COGGESHALL, 1965). The sections were observed at 80 kV with a Philips EM 200 electron microscope, equipped with a 25 μ objective aperture.

Results

Chiasma I of the Adult Squid, Loligo vulgaris

Much attention has been paid to the fact, found by YOUNG (1939), that in the squid the two crossing axons are fused, forming an axoplasmic bridge between the two cells. Transverse sections through the chiasma (the sagittal plane with respect to the animal) reveal only one axon profile (Fig. 3). By electron microscopy, in the axoplasm of this single profile no membrane remnants are found that would allow to distinguish between two crossing axons. Thus, in the squid, an H-shaped bicellular unit occurs with the cell bodies at the anterior and the efferent synapses at the posterior ends (Fig. 4).

As will be shown below, in the chiasma of the freshly hatched *Loligo vulgaris* there are instead two crossing axons. It is concluded that the fusion of these axons takes place during larval or postlarval development. Unfortunately these developmental stages are usually not obtained from the sea living, nor has it been possible to raise them in our laboratory. The only successful rearing of squids has been reported by YASUO OSHIMA and SANG CHOE (1961) in Japan. The processes leading to fusion are therefore not known.

Chiasma I of the Freshly Hatched Loligo vulgaris

Considering the swimming behaviour of the freshly hatched squid it seems obvious that the giant fiber system is already functioning. Occasionally the larvae show rapid spurts over considerable distances, that are evoked by a few maximal

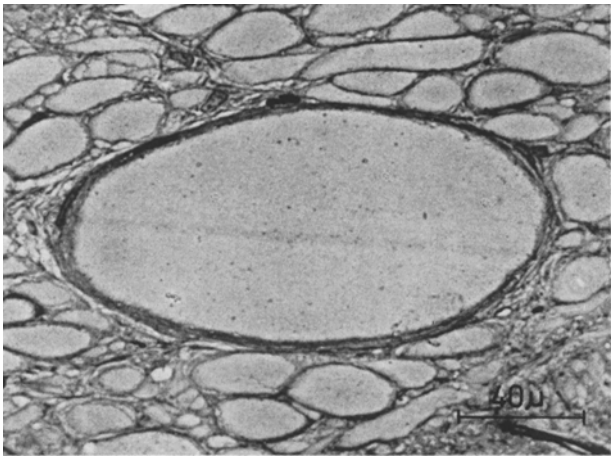

Fig. 3

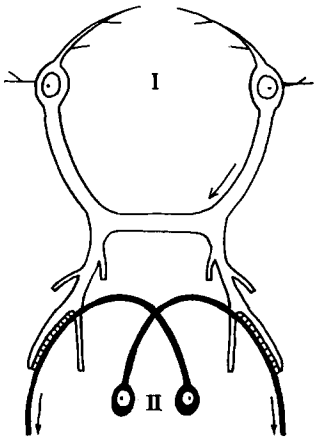

Fig. 4

Fig. 3. Transverse section through the chiasma in the adult squid, *Loligo vulgaris* (= sagittal plane of the animal), showing that the two original decussating axons are completely fused into one axon profile. Toluidine blue stained Durcupan section, phase contrast, ×400

Fig. 4. Schematic representation of the course of the first order giant axons in the adult squid, *Loligo vulgaris*, reconstructed from histological sections. In the chiasma the axons are fused. *I* first order; *II* second order giant cells

mantle contractions. These spurts are, by their velocity and strength, distinctly different from the constant slower contractions that maintain the animal on the same level in the water. Often simultaneously with the fast spurts the larvae eject small clouds of ink.

At hatching, the giant fiber system is extraordinarily well developed. This fact becomes evident when the size of the giant axons is compared with that from other, neighbouring axons. The ratio: diameter of first order axon at the chiasma to diameter of the largest accompanying (non-giant) axons, at hatching, is 8.8:1, in the adult squid 4.6:1. It has been concluded that the giant fiber system differentiates allometrically in the squid during embryonic development (MARTIN, 1965). This allometric development has not been found in *Sepia officinalis* where the diameter ratios increase steadily during development (MARTIN and RUNGGER, 1966). The different growth rates of the two giant fibre systems reflect the different life habits of the two species.

By contrast to the condition in the adult, in the chiasma I of the *Loligo* larva the first order axons decussate unfused, each of them synapsing on the contralateral side with the second order axons (Fig. 5). The apposed membranes of the decussating axons have been found to be intact in all of our sections examined. In rare instances very thin glial processes are found between the crossing axons, but as a rule the two axon membranes are naked. The contact area of the decussating axons appears specialized (Fig. 6): while the width of the gap between the membranes of non-giant axons in osmium fixed material is normally about 150 Å, between the apposed membranes of the decussating giant axons the gap is in many zones doubled (Fig. 6b) and the cleft contains electron dense material that

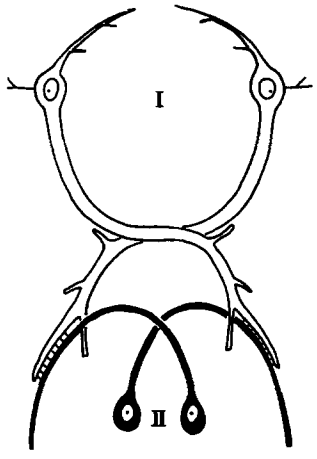

Fig. 5. Schematic representation of the course of the first order giant axons in the freshly hatched squid, *Loligo vulgaris* (after MARTIN, 1965). Each axon has one contralaterally descending branch. There is no fusion

may form an intermediary dense rim, a characteristic feature of desmosomal areas. In permanganate-fixed material this membrane specialization is less evident, the extracellular space generally being narrower.

At both lateral extremes of the chiasma I (Fig. 7a), on the side of the incoming fiber, axonal organelles are found that may play a role in the transmission of impulses from one axon to the other. These are several smaller groups of electron-transparent, membrane-bounded vesicles that measure up to 1000 Å in diameter (Fig. 7b). Although they are somewhat larger, they structurally resemble synaptic vesicles. These vesicles are found next to the membrane, which otherwise does not show the density characteristic of synaptic membranes.

On the side of the crossed axon, mitochondria are found lying near the membrane. They appear to be more frequent in this area than over the length of the chiasma.

No features have been found in animals of this stage that would suggest the subsequent fusion of the axons.

Chiasma I of the Adult Sepia officinalis

The *Sepia* chiasma I consists of unfused axons. In contrast to the *Loligo* larva and the adult *Illex* (see below) each axon has an ipsi- and a contralateral descending branch (Fig. 8). Thus there are on each side two descending branches that run to the second order fibers. Fig. 8 shows also smaller branches that are not always paired. Presumably they contact those 2nd order giant fibers that run directly to the funnel and head muscles.

The two crossing axons are surrounded by a glial sheath ca. 1 μ thick. However, there are smaller areas (= windows) in which the axonal membranes are not covered by glia, but lie directly apposed to each other (Fig. 9a, b). Over the length of the decussation (ca. 600 μ) in one animal five such areas have been counted. The windows occur also frequently between the two descending branches on each

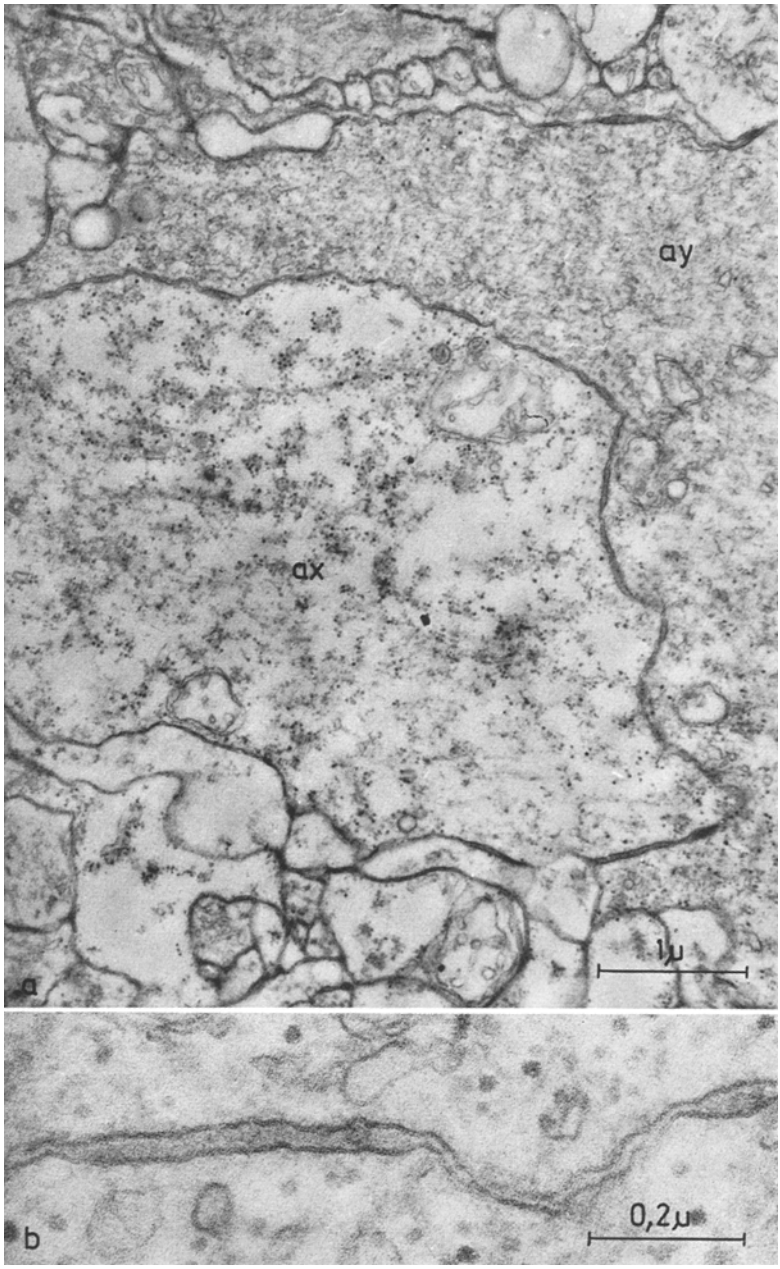

Fig. 6. a Electron micrograph of a transverse section through the chiasma of the squid larva (= sagittal plane of the animal), showing the two unfused axons. *ax* axon from one; *ay* axon from the other body side. The differences in density of the axoplasm are presumably due to varying distances from the cell body, the axoplasm being denser proximally than distally. Chrome-osmium fixation, lead citrate stain, ×20,000. b As Fig. a. In the contact area the apposed membranes are often separated by a ca. 300 Å wide cleft that contains electron dense material. ×100,000

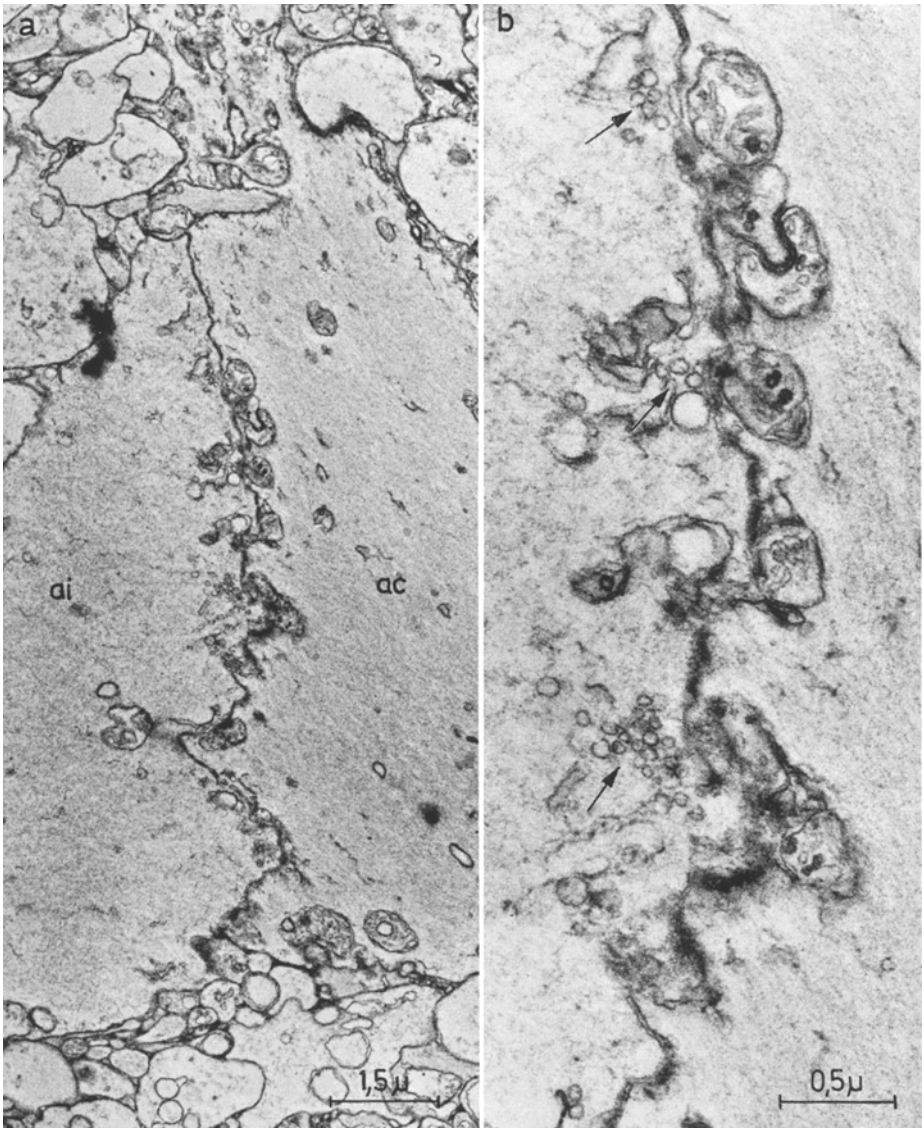

Fig. 7. a Electron micrograph of a horizontal section through the chiasma of the larval squid at the lateral end of the decussation. *ai* incoming uncrossed axon, *ac* crossed axon from the other side descending to the second order fiber. Permanganate fixation. Lead citrate stain. ×10,000. b Enlarged area of Fig. a. On the side of the crossed axon there are several mitochondria; on the side of the uncrossed axon groups of electron-transparent vesicles are seen (arrows). ×30,000

side of the chiasma. In these areas three types of membrane contacts have been found by electron microscopy.

Type one shows several features of a synapse (Figs. 10, 12a, b, c, d). A regular 100 Å wide cleft separates the membranes: The cleft width is very constant in

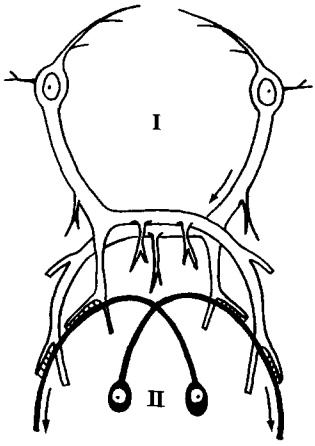

Fig. 8. Schematic representation of the course of the first order axons in the adult *Sepia* (after MARTIN and RUNGGER, 1966). Each axon has an ipsi- and a contralaterally descending branch. The axons are unfused in the chiasma

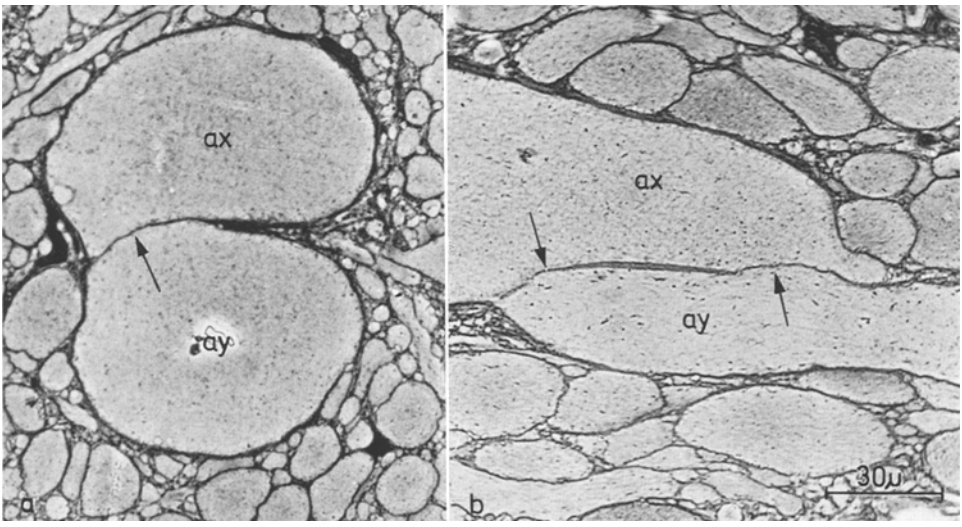

Fig. 9. a Transverse section through the two decussating axons (*ax*, *ay*) of *Sepia*. Over some distance the axons are separated by a glial sheath, but at the arrow the membranes are directly apposed without intermediary sheath (window). Toluidine blue stained, 1 µ section of Durcupan embedded material, phase contrast, ×540. b Longitudinal section of the decussation in *Sepia*. *ax*, *ay* crossing first order axons. At the arrows the axon membranes are directly apposed without intermediary sheath (window). Techniques as in Fig. a

different specimens. Inside each axon membrane is a single layer of up to 15 membrane-bounded, electron-transparent vesicles, which lie close to the axon membrane in an electron-dense ground substance. Transverse, longitudinal and tangential sections of the synapse-like area show that they are spherical with a diameter

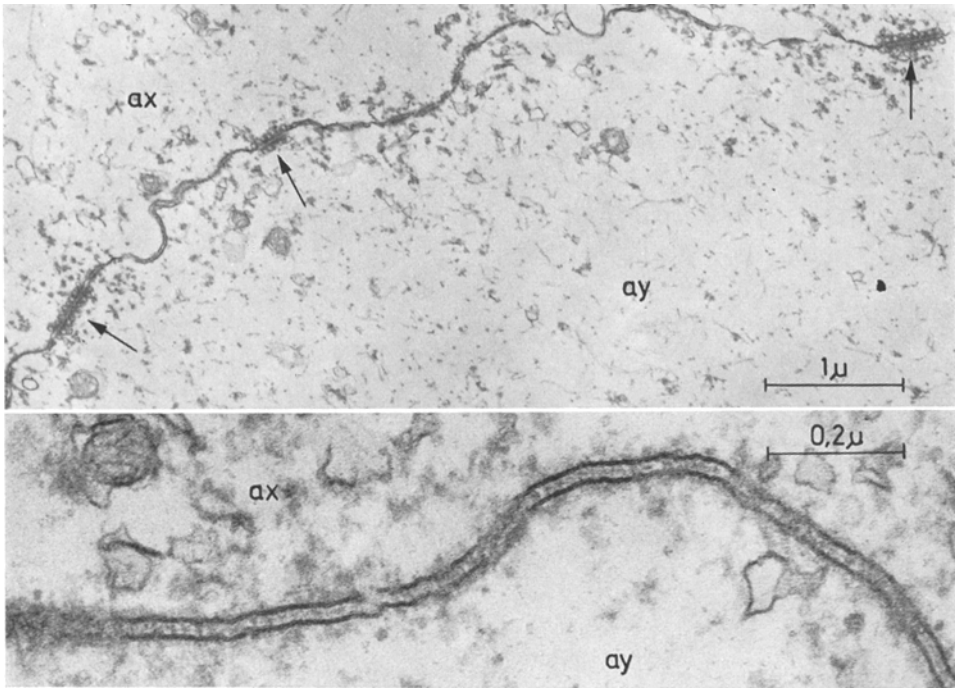

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Fig. 10. Electron micrograph of a window (see arrows in Fig. 9a, b) in *Sepia*. The arrows point to symmetrical synapse-like areas. *ax*, *ay*, decussating axons. ×18,000

Fig. 11. Enlarged desmosome like contact area of a window (*Sepia*). Note the electron dense rim in the cleft. ×90,000

of about 500 Å, including the membrane. In tangential sections the vesicles form densely packed rows (Fig. 12d). No structural polarity of the membrane or in the arrangement of the vesicles is detectable. The synapse-like contact areas appear, from measurements of their size in the three planes, as elongated ovals or ribbons with a plane surface of maximally about 0.30 μ^2, the longitudinal diameter being on the average about twice as long ($<$9,000 Å) as the transverse diameter ($<$4,500 Å). At the most, three synapse-like contact areas have been found per one transverse section of a window (Fig. 10).

The second type of membrane contact at the windows displays some features of desmosomes (Fig. 11). The axon membranes are separated by a regular gap 200 Å wide that contains electron-dense substance often condensed to an intermediary rim in the center of the space. There are no vesicles in the vicinity of these areas. They cover most of the space of the windows. Again the width of the gap does not vary in different specimens.

Between the synapse-like and the desmosome-like areas short zones may be found in which the membranes lie far removed from each other or closely apposed, occasionally showing complete obliteration of the gap. There is no dense ground substance, either in the gap or inside the membranes. The variability of type 3 area is in distinct contrast to the regularity of the type 1 and 2 contact areas.

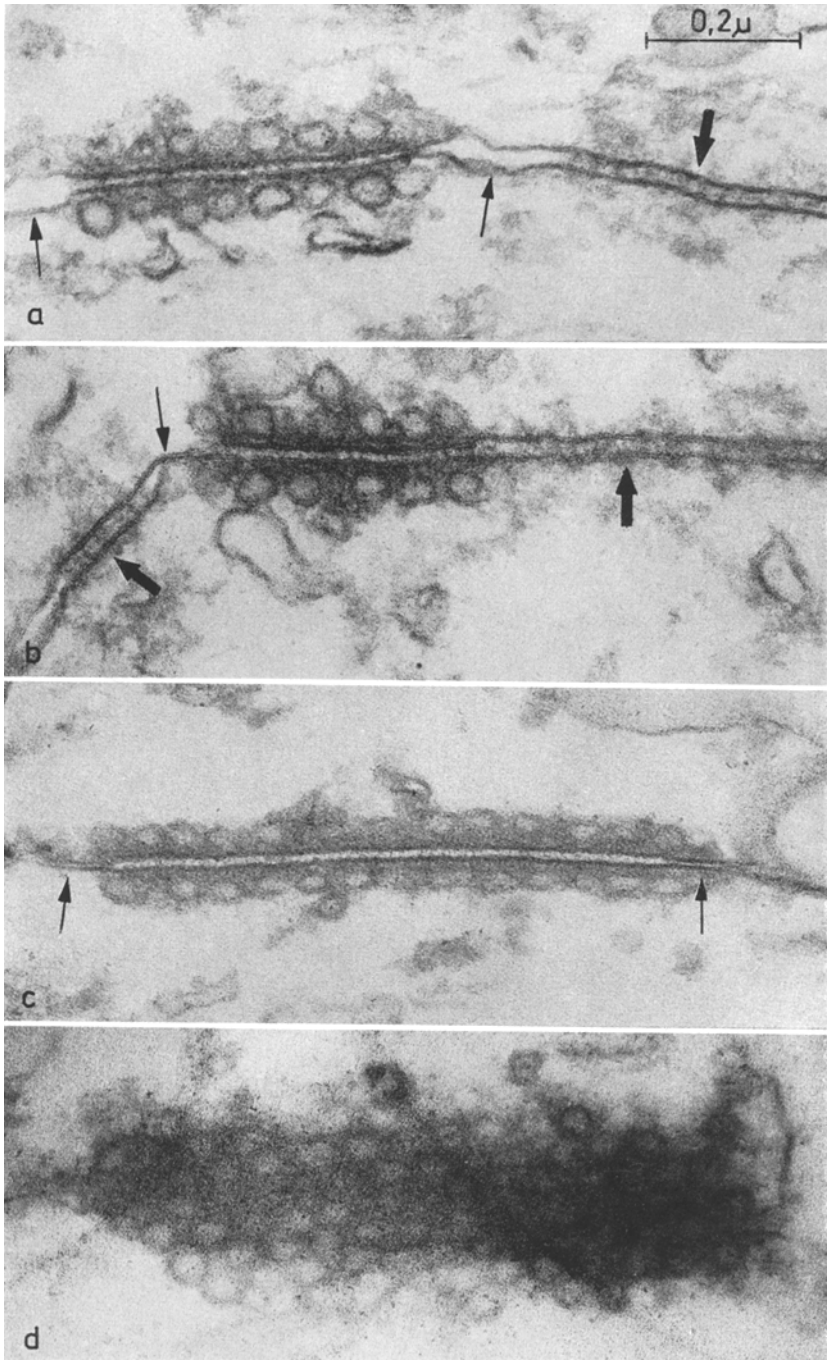

Fig. 12a—d. Enlarged symmetrical synapse-like areas (*Sepia*). a, b, in transverse section; c in longitudinal section; d in tangential section. The small arrows point to marginal zones of the synapse-like areas in which the cleft width is very variable. The thick arrows point to desmosome like contact areas (see Fig. 11)

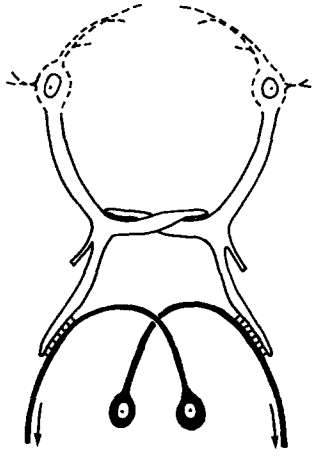

Fig. 13. Schematic representation of the course of the first order axons in the adult *Illex* (reconstructed from series of transverse sections through the chiasma). The axons show ipsilaterally descending branches and crossed collaterals. The morphology of the first order cells has not been studied in this species

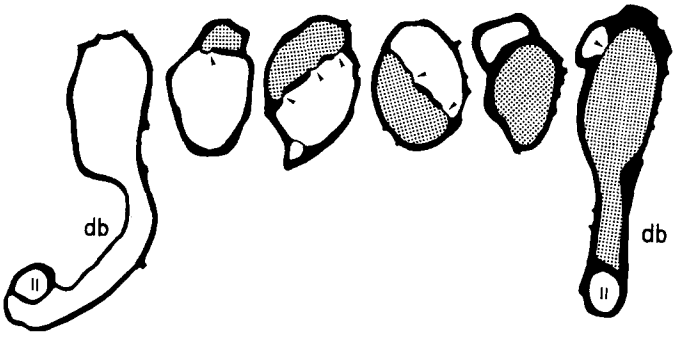

Fig. 14. Series of profiles through the chiasma of *Illex*, showing that the two collaterals cross over each other and end on the contralateral side. Stippled areas = profiles from one axon, white areas = profiles from the other axon. *db* descending branches. *II* second order axons. At the arrows the axons are not covered by a sheath (windows). Drawing from photographs of 1 μ sections

Chiasma I of Illex coindeti

When studying the course of the first order axons in the chiasma of *Illex coindeti*, an architeuthacean squid, it was a surprise to find that this decussation is constructed according to still another scheme. Each giant axon has only an ipsilaterally descending branch, that contacts the 2nd order fibers (Figs. 13, 14). At the place of the chiasma there are two collaterals, one from each axon, that end blindly on the contralateral side. The collaterals are crossed: the proximal part lies ventrally to the other collateral; in the center of the chiasma the collaterals turn around each other from the ventral to the dorsal side; and the blind distal endings lie dorsally.

As in *Sepia* the collaterals in most areas are ensheathed by glial processes, but there are again areas in which the naked membranes of the two axons are directly

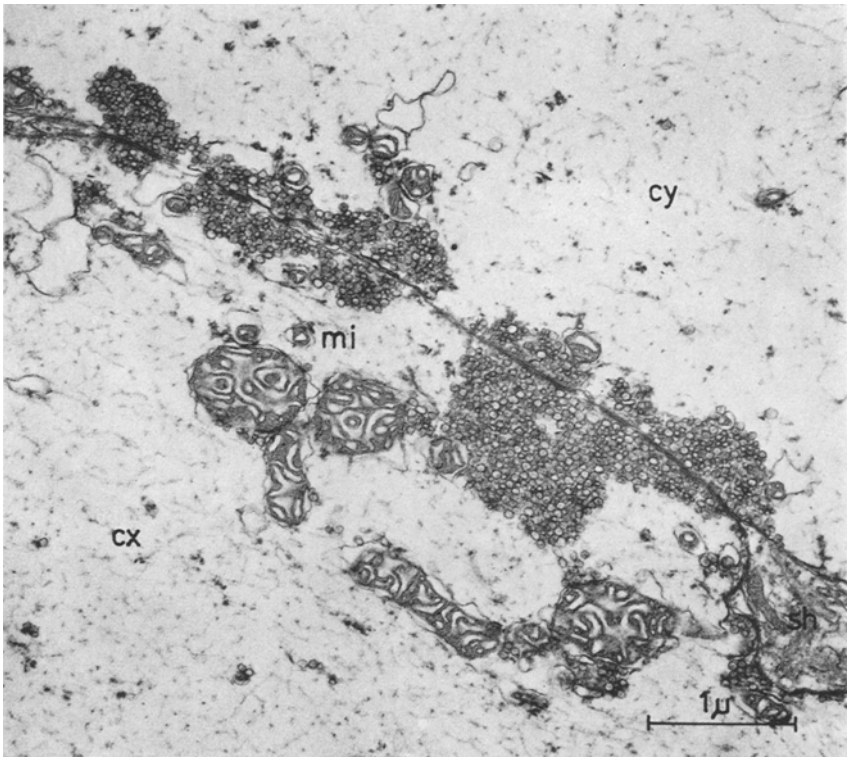

Fig. 15. Electron micrograph of a transverse section through a window in the *Illex* chiasma. *cx* collateral from one; *cy* collateral from the other body side; *sh* glial sheath; *mi* mitochondria. Note the masses of transparent vesicles on both sides of the two membranes and the increase in electron opacity of the membranes in the areas with vesicles. ×20,000

apposed. Again in the windows specialized zones occur with characteristics that are usually attributed to synapses. However, they differ in important features from the synapse-like areas of the *Sepia* chiasma.

Inside both apposed membranes there are large masses of vesicles lying in an electron-dense ground substance. These vesicles are electron-transparent, membrane-bounded, and measure up to 800 Å in diameter. The two membranes are separated by a ca. 150 Å wide cleft that contains electron-dense ground substance. Both membranes appear more electron-dense where surrounded by vesicles. No polarity, either in the structure of the membranes or in the arrangement of the vesicles has been observed. There are large mitochondria near the masses of vesicles (Figs. 15, 16).

Discussion

Specialized Contacts between the two Decussating Axons in Chiasma I of Loligo, Sepia and Illex

Various features in the structural organization of the four chiasmata, as described in this paper, strongly suggest that in chiasma I impulses are passed on from one decussating axon to the other. This seems to be especially evident in the

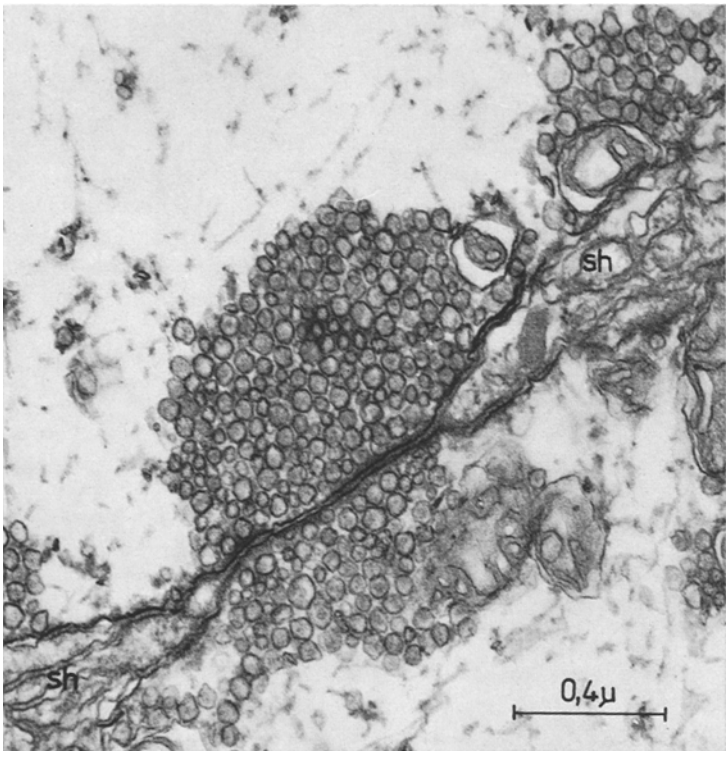

Fig. 16. See Fig. 15, but ×50,000

chiasma of the adult squid in which the two axons are fused. Moreover, the specialized contact areas in the *Sepia* and the *Illex* chiasmata show structural features that are normally found in synaptic areas. In the absence of electrophysiological evidence, one way to ascribe, tentatively, a function to these structures is to look elsewhere for structurally similar areas for which physiological data are available.

Symmetrical Synapse-like Areas in the Sepia Decussation

The ribbon-shaped specialized contact areas with a monolayer of transparent vesicles on either side and a 100 Å cleft, in the *Sepia* decussation structurally resemble the septal synapses of the lateral giant axons of the crayfish and of the septate giant axons of the earthworm (HAMA, 1959, 1961, 1966). Both in the *Sepia* area and in the septal synapses, no structural polarity is discernible, either in the arrangement of the vesicles or in the structure of the two membranes. According to HAMA (1959, 1961, 1966) the cleft width in the two septal synapses is about 100 Å, as it is in the *Sepia* area. However, PAPPAS, ASADA and BENNETT (1967) and PAPPAS (1968) found that in the crayfish septal synapse tight junctions do occur. There are also differences in the size of the vesicles: in the septal synapses they measure 150—200 Å (HAMA, 1961) while in the *Sepia* area they are 500 Å. Moreover, the septal synapses cover a much larger space than the *Sepia* synapse-like area. The

cross-sectional area measures ca. 1,000 μ^2 in the septal synapses (FLOREY, 1966), against 0.3 μ^2 in the synapse-like *Sepia* area. However, in the *Sepia* windows there are several such areas, a few microns (2—5) distant from each other.

Electrophysiological recordings from the septal synapses in the crayfish have shown that electrotonic currents spread freely across them, presumably through the tight junctions, and that they are capable of bidirectional transmission, i.e., they are not rectifying (WIERSMA, 1947; FURSHPAN and POTTER, 1959, see chapter "methods"; WATANABE and GRUNDFEST, 1961). Based on the structural analogies between the synapse-like contact areas in the *Sepia* chiasma and the septal synapses it may be speculated that also in the *Sepia* area impulses are transmitted bidirectionally.

In the *Sepia* windows it is the small areas with narrowed or obliterated extracellular space, which lie next to the synapse-like areas, that may play a part in transmission. However, since the cleft width varies so considerably in different specimens it appears possible that we have to deal with a fixation artefact. It has been shown by KARLSSON and SCHULTZ (1964, 1965) that in mammalian brain after glutaraldehyde perfusion there occurs an occlusion of membranes that is not found in osmium fixed material. Also, dehydration with acetone may be responsible for membrane apposition (JOHNSTON and ROOTS, 1967). At present it appears that the distinction between what is real and what is artefact can not be drawn.

Specialized Contacts in the Chiasma I of the Larval Loligo

The occurrence of clusters of electron transparent vesicles in the incoming axon at the lateral end of the chiasma I, by structural analogy with synaptic sites, suggests that impulses are passed on to the crossed contralateral axon by synaptic transmission. Unfortunately, however, the organization of the membranes in these sites has not been seen in detail. Also the diameter of the vesicles is somewhat larger than is usually found in synapses.

While considering this developmental stage it should be pointed out that in embryonic stages of the squid, *Loligo pealii*, cells of the eye and the skin are electrically coupled with the yolk mass (POTTER, FURSHPAN and LENNOX, 1966). This coupling disappeared four to five days before hatching, i.e., distinctly before the stage examined here. Intercellular coupling has been shown also in chick embryos (SHERIDAN, 1966), and TRELSTAD, REVEL and HAY (1966) showed a possible structural correlate in form of tight junctions between the electrically coupled cells (after osmium fixation).

In our osmium-fixed larvae we never found tight junctions in chiasma I but, from this negative evidence, it cannot be excluded that tight junctions exist. Over large distances the extracellular space between the apposed membranes is extraordinarily wide (300 Å). With respect to the occurrence of electron-dense rims in the cleft, these areas resemble to some extent desmosomal structures. We suggest that these areas serve for the attachment of the two decussating axons during embryonic differentiation of the chiasma.

Specialized Contacts of the Collaterals in the Illex Chiasma I

The high number of electron transparent vesicles, the increase in electron density of the membranes in the vicinity of the vesicles, and the regular 150 Å

cleft between the membranes in the contact areas of the *Illex* collaterals are characteristics that are usually attributed to chemical synapses. It should be pointed out that in these areas again there is no structural polarity. The structural findings suggest that impulses are transmitted from one collateral to the other, possibly in either direction.

A Hypothesis Concerning the Role of the Chiasma I in Respect to the whole Giant Fiber System

Any functional interpretation of the chiasma I is at present purely hypothetical since reports on electrophysiological recordings from intracerebral giant fibers are not available.

The only work presumably dealing with a giant fiber response after intracerebral stimulation is due to BOYCOTT (1961, and personal communication). In preparations of *Sepia*, direct electrical stimulation unilaterally in the ventral magnocellular lobe was followed by strong symmetrical expiratory spasms of the whole mantle. Possibly this means that impulses originating unilaterally in the giant fiber system have spread symmetrically to both sides of the mantle.

The hypothesis already put forward by YOUNG (1939) that the chiasma I mediates functional bilaterality of the whole system seems to be compatible with the construction of all four chiasmata described in this paper. In the case of the adult *Loligo* it appears obvious that impulses spread along the fused membranes to the second order fibers of both sides. Similarly, the fact that the first order axons in the *Sepia* chiasma have ipsi- as well as contralaterally descending branches appears to guarantee bilaterally symmetrical transmission of impulses from the chiasma I downwards. In *Sepia* there may be a second set of structures serving the same function. In the symmetrical synapse-like contact areas, as in the septal synapses of the crayfish, impulses may be exchanged in both directions from one decussating axon to the other. In this chiasma, connections between the crossing axons may be important because not all of the descending branches of the chiasma, especially the smaller ones, are paired.

In two chiasmata (larval *Loligo* and adult *Illex*) neither a fusion nor a bilateral ramification of the first order axons occurs. However, there are structural characteristics of synapses in contact areas of the crossing fibers. Excitatory transmission of impulses in these areas would mediate a bilaterally symmetrical spread of unilaterally originating impulses. In these cases it is likely that there is a delay imposed by synaptic transmission, between the impulses spreading downward on the two body sides. However, since this delay presumably is of the order of magnitude of a few milliseconds it may not be important in the functioning of the system.

The hypothetical role, attributed here to chiasma I, agrees with what is known of the function of the giant fiber system. A maximal water jet through the funnel is achieved if the circular muscles at any point of the mantle contract simultaneously. YOUNG (1938) has shown that simultaneity of contraction in the longitudinal direction is guaranteed by the fact that the shorter axons of the third order giant fibers are smaller in diameter than the longer ones. By means of the greater velocity in conductance of the thicker axons, impulses arrive at the same

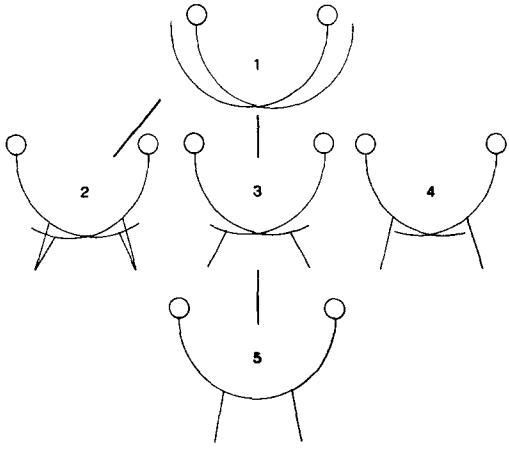

Fig. 17. Diagrams of the chiasma I structure in different developmental stages of three species. *1* embryonic *Loligo* and *Sepia*; *2* adult *Sepia*; *3* larval *Loligo*; *4* adult *Illex*; *5* adult *Loligo*

time in remote areas of the mantle and in areas nearer the stellate ganglion (PUMPHREY and YOUNG, 1938; HODES, 1953). However, third order giant axons of one body side do not cross to the other side. Since the chiasma of the second order axons in the palliovisceral lobe is situated proximally to the synaptic input area, the bilaterality of the system can possibly be mediated only in the chiasma of the first order axons, which is structurally suited to perform this function.

It is interesting that the hypothetical role of chiasma I in cephalopods, as outlined here, is exactly opposite to the functioning of the Mauthner system of teleosts. In the latter, stimulation of the VIIIth nerve on one body side produces excitation of the ipsilateral Mauthner cell and simultaneous inhibition of the contralateral cell (RETZLAFF, 1957; FURSHPAN and FURUKAWA, 1962; FURUKAWA and FURSHPAN, 1963). This is in agreement with the muscular responses to impulses from the Mauthner system, which consist in alternating tail "flips", first to the one then to the other side.

The Embryonic Development of Chiasma I in Sepia and Loligo

Earlier histological examinations of the development of the first order giant fiber system in *Sepia officinalis* and *Loligo vulgaris* have shown that the chiasma structure can be deduced from a common basic scheme. In early embryonic stages of both species (stages XII—XIV of NAEF, 1928) the first order axons cross each other in the palliovisceral lobe, then each axon turns backwards to the opposite magnocellular lobe, thus forming a commissure between the two lobes (Fig. 17) (MARTIN, 1965; MARTIN and RUNGGER, 1966). In later embryonic stages (stage XVI—XVII of NAEF, 1928), in *Sepia* the ipsi- and contralateral, and in *Loligo* the contralateral, descending branches appear and the commissural part disappears, presumably by degeneration. In freshly-hatched larvae a remnant of the commissural part still runs parallel to the other axon over some distance (see Figs. 5 and 17). In *Loligo*, therefore the chiasma I passes through three stages of

differentiation during development: a commissure from magnocellular lobe to magnocellular lobe in early embryos, a chiasma of crossed axons, that presumably are connected to each other by synapses, at hatching, and an axoplasmic bridge formed by fusion of the axons in the adult. It appears that these developmental steps represent stages of functional improvement of the system.

Unfortunately the embryonic differentiation of the giant fiber system in architeuthoid squids is not yet known, due to the impossibility to obtain eggs from the sea or to keep adults in captivity. Speculatively we can derive the chiasma structure of the adult from the same basic scheme if we assume that the degeneration of the commissural part of the axon proceeds further than in *Sepia*, and includes also the contralateral descending branches. In this way the peculiar crossing of the collaterals and the occurrence of only an ipsilaterally descending branch would be explained (Fig. 17).

Considering the fact that in each of the three taxonomic groups chiasma I differs in construction it seems evident that during evolution in each group the giant fiber system has been elaborated separately, possibly from a common commissural connection of the bilateral magnocellular lobes.

References

BOYCOTT, B. B.: The functional organization of the brain of the cuttlefish *Sepia officinalis*. Proc. roy. Soc. B **153**, 503—534 (1961).

FLOREY, E.: An introduction to general and comparative animal physiology. Philadelphia and London: W. B. Saunders Co. 1966.

FURSHPAN, E. J., and T. FURUKAWA: Intracellular and extracellular responses of the several regions of the Mauthner cell of the gold fish. J. Neurophysiol. **25**, 732—771 (1962).

—, and D. D. POTTER: Transmission at the giant motor synapse of the crayfish. J. Physiol. (Lond.) **145**, 289—325 (1959).

FURUKAWA, T., and E. J. FURSHPAN: Two inhibitory mechanisms in the Mauthner neurons of gold fish. J. Neurophysiol. **26**, 140—176 (1963).

HAMA, K.: Some observations on the fine structure of the giant nerve fibres of the earthworm *Eisenia foetida*. J. biophys. biochem. Cytol. **6**, 61—66 (1959).

— Some observations on the fine structure of the giant fibres of the crayfishes (*Cambarus virilus* and *Cambarus clarkii*) with special reference to the submicroscopic organization of the synapses. Anat. Rec. **141**, 275—294 (1961).

— Studies on fine structure and function of synapses. Progr. Brain Res. **21** A, 251—267 (1966).

HILLIG, R.: Das Nervensystem von *Sepia officinalis* L. Z. wiss. Zool. **101**, 736—800 (1912).

HODES, R.: Linear relationships between fiber diameter and velocity of conduction in giant axon of squid. J. Neurophysiol. **16**, 145—154 (1953).

HUXLEY, T. H., and P. PELSENEER: Report on the specimen of the Genus *Spirula* collected by H.M.S. CHALLENGER. In: Report on the Scientific Results of the Voyage of H.M.S. CHALLENGER during the years 1872—1876. A Summary of the scientific results — Appendix (Zool. Part LXXXIII). p. 18 (1895).

JOHNSTON, P. V., and B. J. ROOTS: Fixation of the central nervous system by perfusion with aldehydes and its effect on the extracellular space as seen by electron microscopy. J. Cell Sci. **2**, 377—386 (1967).

KARLSSON, H., and R. L. SCHULTZ: Plasma membrane apposition in the central nervous system after aldehyde perfusion. Nature (Lond.) **201**, 1230—1231 (1964).

— — Fixation of the central nervous system for electron microscopy by aldehyde perfusion. I. Preservation with aldehyde perfusates versus perfusion with osmium tetroxide with special reference to membranes and the extracellular space. J. Ultrastruct. Res. **12**, 160—186 (1965).

MARTIN, R.: On the structure and embryonic development of the giant fibre system of the squid *Loligo vulgaris*. Z. Zellforsch. **67**, 77—85 (1965).

—, u. D. RUNGGER: Zur Struktur und Entwicklung des Riesenfasersystems erster Ordnung von *Sepia officinalis* L. (Cephalopoda). Z. Zellforsch. **74**, 454—463 (1966).

NAEF, A.: Die Cephalopoden, Bd. 2, Teil 1. Embryologie. Fauna und Flora des Golfes von Neapel. 35. Monographie, S. 1—357. Berlin: Friedländer 1928.

NECCO, A., and R. MARTIN: Behaviour and estimation of the mitotic activity of the white body cells in *Octopus vulgaris*, cultured in vitro. Exp. Cell Res. **30**, 588—623 (1963).

PAPPAS, G. D.: Morphological aspects of synaptic function. Electron microscopy 1968: Pre Congress Abstracts of IVth Europ. Region. Conference Rome, vol. 2, p. 531—536 (1968).

— Y. ARADA, and M. V. L. BENNETT: Morphological and physiological changes in junctional sites of crayfish septate axons. Anat. Rec. **157**, 297 (1967).

POTTER, D. D., E. J. FURSHPAN, and E. S. LENNOX: Connections between cells of the developing squid as revealed by electrophysiological methods. Proc. nat. Acad. Sci. (Wash.) **55**, 328—336 (1966).

PUMPHREY, R. J., and J. Z. YOUNG: The rates of conduction of nerve fibres of various diameters in Cephalopods. J. exp. Biol. **15**, 453—466 (1938).

RETZLAFF, E.: A mechanism for excitation and inhibition of the Mauthner's cells in teleosts. A histological and neurophysiological study. J. comp. Neurol. **107**, 209—225 (1957).

SHERIDAN, J. D.: Electrophysiological study of special connections between cells in the early chick embryo. J. Cell Biol. **31**, C1—C5 (1966).

TRELSTAD, R. L., J. P. REVEL, and E. D. HAY: Tight junctions between cells in the early chick embryo as visualized with the electron microscope. J. Cell Biol. **31**, C 6—C10 (1966).

VENABLE, J. H., and R. COGGESHALL: A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. **25**, 407—408 (1965).

WATANABE, A., and H. GRUNDFEST: Impulse propagation at the septal and commissural junctions of crayfish lateral giant axons. J. gen. Physiol. **45**, 267—308 (1961).

WIERSMA, C. A. G.: Giant nerve fibre system of the crayfish. A contribution to comparative physiology of synapse. J. Neurophysiol. **10**, 23—28 (1947).

YASUO, OHSHIMA, and SANG CHOE: On the rearing of young cuttlefish and squid. Bull. Japan. Soc. Sci. Fisheries **27**, 979—986 (1961).

YOUNG, J. Z.: The functioning of the giant nerve fibres of the squid. J. exp. Biol. **15**, 170—185 (1938).

— Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods. Phil. Trans. B **229**, 465—505 (1939).

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