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ANALYSIS OF HABENULAR NUCLEI IN ELASMOBRANCHS AND TELEOSTS

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INTRODUCTION

1. The diencephalon of the Chondrichthyes: cell masses

1.1 The Diencephalon

The structure and functions of the Chondrichthyan diencephalon is poorly understood. There have been few detailed studies on this region of the brain in these fishes except for the work of Bergquist (1932), who carefully analyzed the diencephalon of *Squalus* at various developmental stages.

Unlike other regions of the brain, the diencephalon in cartilaginous fishes has retained essentially the embryonic tube-like shape. Its thickened walls surround a narrow ventricle that expands ventrocaudally within the hypothalamus to form the lobi inferiores hypothalami.

The location of the precise boundary between the telencephalon and diencephalon is disputed. Kuhlenbeck (1929a, 1977) and Gerlach (1947) recognize the rostral border as passing from the point of attachment of the velum transversum to the commissura anterior. We follow Johnston (1911), Bäckström (1924), and Bergquist (1932) and draw the boundary as passing from the velum transversum to the caudal border of the optic chiasm.

Although the Chondrichthyan diencephalon is dominated by the entrance and crossing of the optic nerve and by the broad corse of this nerve to the tectum mesencephali, other prominent fibre streams such as the fasciculus basalis telencephali, tractus pallii, stria medullaris and thalamotegmental fibres are present in the diencephalon. Fibres ascending from the brain stem and spinal cord are though to terminate within the brain stem and the diencephalic area, for no experimental studies has revealed ascending fibres passing beyond the diencephalic level.

Conversely, the majority of fibres descending from the telencephalon seem to terminate at the level of the diencephalon, though a number of fibres reach to more caudal levels (Ebbesson and Schroeder 1971: Smeets 1983). Clearly, the diencephalon has to be considered as an important relay-center between the telencephalon and other regions.

1.2 Morphological pattern

The diencephalon of the amniotes has served as one of the major battle grounds on which the problem of homology has been fought by numerous authors including Herrick (1910), Kuhlenbeck (1929a), Haller (1929), Bergquist (1932), and more recently, Nieuwenhuys and Bodenheimer (1966).

The problem of establishing homologies is central to comparative neuroanatomy. The giving of a name to a neuronal cell mass implies that it has certain similarities with nuclei of the same name in the brains of other species. Thus a pre-existing anatomical name should only be used for a nucleus when we are certain of its homology. On the other hand the creation of a novel name for a region denies homologies while continual proliferation of novel names inevitably leads to confusion in the literature.

The following procedures have been used by various authors to establish homologies in the nervous system, particularly with emphasis on the diencephalon (for details see Nieuwenhuys and Bodenheimer 1966).

2. Herrick (1910) divided the diencephalon into four longitudinal zones: epithalamus, pars dorsalis thalami, pars ventralis thalami and hypothalamus. These zones are separated from each other by the sulcus dorsalis thalami, the sulcus medius thalami and the sulcus ventralis thalami respectively. Herrick held that the sulci difined regions which are functionally as well as morfologically distinct.

3. Kuhlenbeck (1929a) also used

ventricular sulci to divide the diencephalon of all vertebrates into "Grundbestandteile", which form together the structural plan (Bauplan). In Kuhlenbeck's opinion regions in different brains that occupy a corresponding position in the Bauplan were to be considered omologous, regardless of their structure or fibre connections. Haller (1929) also considered the ventricular sulci as a basis for the homologization of parts of the diencephalon. However, this author believed that the sulci are related to the embryonic transverse neuromeres, and that they imply a vertical rather than horizontal subdivision of the diencephalon. The nearly horizontal appearance of these sulci was accounted for by the rotation of the diencephalic portion of the neural tube about the axis of the tuberculum posterius.

4. A totally different way of determining homologies was established by tha Swedish school of neuroanatomists (Bergquist 1932; Källén 1951), who attempted to homologize areas of the brain on the basis of a study of the early embryological development of cytoarchitectonic units. Thus Bergquist subdivided the diencephalon into areas in the ventricular wall, "Grundgebiete", where the mitotic activity is higher than in intervening areas during early stages in development. He found that a sulcus usually forms in the middle of each "Grundgebiete" just at the point of highest mitotic density and therefore he called these sulci "Proliferationsfurchen".

Thus we have three different procedures for the establishing of homologies in the diencephalon. Herrick felt that sulci, nuclear structure, and fibre connections are all closely related so that a subdivision based on any one of these criteria will conform with subdivisions derived from the others. Kuhlenbeck believed that, at least for amniotes, the sulci are the important determinants of brain organization, rather than the cytoarchitecture or disposition of fibres tracts. Finally, Bergquist and Källén held that nuclear structure as seen in the embryonic brain is primarily important, with sulci and fibre connections being of no assistance in the establishment of homologies.

More recently, Nieuwenhuys and Bodenheimer (1966) enunciated the following working definition of neural homology:"entities in different species which, within an obviously similar structural plan common to all species compared, occupy a corresponding topological position, should be considered homologous" (Nieuwenhuys and Bodenheimer 1966). They tested the practical validity of the three criteria for establishing homologies-the ventricula sulci, nuclear boundaries and fibre connections-in the diencephalon of bony fish and concluded that there is no pan-diencephalic parallelism among the results if all three, or between any two, of the criteria used to establish homologies. They concluded that similarity in the topological position of cytoarchitectonic entities is the most useful criterion.

We shall test the practical validity of these criteria for establishing homologies in the diencephalon of the four Chondrichthyan fish studied, after first providing a description of sulcal pattern, cell masses and fibre connections.

Apart from a subdivision of the diencephalon into epithalamus, dorsal thalamus, ventral thalamus and hypothalamus, the cell masses of this brain ragion can also be subdivided accordino to their degree of migration into: (1) a zone situated immediately lateral to the ventricular ependyma; (2) a zone lateral to the inner ventricular zone but still a part of the ventricular grey, called the outer ventricular zone, and (3) migrated nuclei which are either completely separate from, or which retain

only a slight connection with, the ventricular zones.

CELL MASSES

In Chondrichthyans the cell masses of the diencephalon have a more diffuse arrangement and contain neurons of more uniform size than those in the brain stem. About 20 cell masses could be delineated in this area in some species, like *Squalus acanthias, Scyliorhinus canicula, Raja clavata* and *Hydrolagus collei*)

1.3 Epithalamus

The epithalamus comprises the epiphysis and the ganglia habenulae. The epiphysis or pineal organ of *Scyliorhinus*, is a long thin, tubular structure, the closet distal end of which rests against the roof of the brain case. The proximal end opens into the third ventricle, between the habenular and posterior commissures. Using light- and electron- microscope techniques, Rüdeberg concluded that the pineal parenchima consists of receptor cells, supporting cells and ganglion cells. The latter give rise to the diffuse tractus pinealis, wich passe to the posterior commissure and, to a lesser extent, to the habenular commissure. However, its exact termination site has not been identified. A pineal tract could be recognized in all four species studied, the course of which is in full agreement with the description of Rüdeberg (1969). This author also found that the receptor cells have well developed outer segments similar to that of retinal photoreceptors. Hamasaki and Streck (1971) demonstrated electrophysiologically that the epiphysis of *Scyliorhinus* is a very sensitive photoreceptor organ and a preliminare study by Wilson and Dodd (1973a) has provided some evidence that in *Scyliorhinus* the pineal gland evokes a paling response in darkness either directly through the secretion of e.g. melatonin, or indirectly through nervous connections (perhaps via the MSH-system).

The ganglia habenulae which are well developed in chondrichthyans, constitute the rostral part of the epithalamus. In all species studied the left habenular ganglion was found to be larger than the right, in agreement with Arïens Kappers and Carpenter (1911), Bergquist (1932), Arïens Kappers et al. (1936), Gerlach (1947), Kuhlenbeck (1977), Farner (1978), Kemali et al. (1980), and Miralto and Kemali (1980). The difference in size, mainly produced by the somewhat more frontal extension of the left ganglion, is less in *Squalus* and *Hydrolagus,* than in *Scyliorhinus* and *Raja*. Differences in histological structure between the two ganglia, however, are more considerable. In Klüver-Barrera sections it is particularly striking that the dorsolateral region of the left ganglion is provided with a dense network of myelinated fibres. In the left ganglion, cells lying within the myelinated network are somewhat larger and more loosely arranged than are the other cells of the ganglion. The latter cells, just like all the cells of the right ganglion, are of the characteristic small habenular type and lie tightly packed together. These findings are in full agreement with those of Kemali et al. (1980) in *Scyliorhinus stellaris*.

In many groups of vertebrates the ganglia habenulae can be suddivided into medial and lateral nuclei. In Chondrichthyans, however, such a subdivision of the ganglia habenulae is only vaguely suggested at some habenular levels.

Caudally the two ganglia habenulae fuse across the midline in the area below the habenular commissure. This commissure comprises a cell mass which consists of small cells of the same size and type as the ordinary habenular cells, although they are more loosely arranged. This nucleus has been termed the nucleus of Bellonci by Addens (1945) and was also recognized by Kemali et al. (1980) in *Scyliorhinus stellaris*; however, the latter considered it to be a midline habenular nucleus. In *Scyliorhinus* and *Hydrolagus* we have found a cell mass in the habenular commissure which may be

equivalent to the nucleus of Bellonci of Addens (1945). Such a nucleus could not be distinguished, however, in *Squalus* and *Raja*.

1.4 Thalamus Dorsalis

Previous studies of the diencephalon of cartilaginous fishes merely describe major subdivisions of this brain area without giving details of nuclei (Ariëns Kappers 1906; Ariëns Kappers et al. 1936; Gerlach 1947). However, those cell masses that receive a retinal input have been recently described in more detail by Northcutt (1979b) and Smeets (1981a).

The dorsal thalamus can be suddivided into two parts, a pars medialis that consists of small densely packed periventricular cells and a pars lateralis which contains slightly larger and more diffusely arranged cells. The thalamus dorsalis pars medialis extends from the level of the optic chiasm to the di-mes-encephalic boundary. This cell mass can be further subdivided in *Squalus* and *Hydrolagus* into a pars dorsalis and a pars ventralis because of the greater density and slightly more intensive staining of the cells of the pars ventralis.

The position of the thalamus dorsalis pars lateralis varies somewhat in the four species studied. In *Scyliorhinus, Raja* and *Hydrolagus* the lateral dorsal thalamus lies at the level of a zone connecting the habenular commissure with the optic chiasm, whereas in *Squalus* this cell mass has a more caudal position.

1.5 Thalamus Ventralis

According to Ariëns Kappers et al. (1936), Gerlach (1947), and Kuhlenbeck (1929a, 1973, 1977) the thalamus ventralis of chondrichthyans is separated from the thalamus dorsalis by the sulcus diencephalicus medium. However, it can be concluded from the study of Northcutt (1979a: *Squalus*) and our own observations that this sulcus cannot be used to identify the exact boundary between the dorsal and ventral thalamus because its position varies rostrocaudally with referente to the cell groups that run the lenght of the thalamus.

The ventral thalamus, like the dorsal thalamus, is composed of two parts, a periventricular pars medialis and a migrated pars lateralis. The former consists of some loosely arranged laminae of small cells, the latter of diffusely arranged small cells. The position of these two cell masses differs little in the species studied. The thalamus ventralis pars lateralis caps the fasciculus basalis telencephali.

Rostral to the thalamus ventralis pars medialis a distinct nucleus could be delineated which, following Bergquist (1932) and Kuhlenbeck (1977), has here been termed eminentia talami. This cell mass could be distinguished on account of the lager size and the more intensive staining of its constituent neuronal elements. The existence of such a nucleus could be confirmed in all species studied except *Hydrolagus* and is particularly strongly developed in Raja. In Hydrolagus, in the middle region of the thalamus dorsalis pars medialis, a number of cells are seen that are of the same character as the neurone of the eminentia talami in the other three species.

Before turning to the description of the migrated thalamic cell masses it should be noted that the external part of the ventricular layer contains, in addiction to the cell masses described, numerous diffusely scatterei cells.

The corpus geniculatum laterale is the only nucleus in the chondrichthyan thalamus which can be included in the category of migrated cell masses. This nucleus is embedded in the strema of optic nerve fibres and can be easily recognized because it is the only cell mass within the diencephalon that comprises medium-sized cell.

Comparisons of the lateral geniculate body between species showed that it contains more neurone and has a more compact arrangement in Scyliorhinus ,Raja and Hydrolagus than in Squalus. Another striking difference is

that the lateral geniculate body of the first three species has migrated further lateral-ward in harmony with the suggestion of Northcutt (1979a) that "evolution of primari visual pathways in cartilaginous fishes occurs by migration and an increase in neuronal number, rather than by occurrence of new visual pathways" (Northcutt 1979a, p. 219).

Although this nucleus has been termed the corpus geniculatum laterale in the present analysis, following the terminology of Ariëns Kappers et al. (1936), this does not imply that this cell mass is equivalent to the lateral geniculate body of amniotes. Recent experimental studies of Northcutt (1979a: Squalus), Luiten (1981a, b: Ginglymostoma) and Smeets (1981a,b: Scyliorhinus, Raja) have revealed that there is considerable disagreement concerning the question of which nucleus should be designated as the lateral geniculate body. Northcutt (1979a) avoided this term and labelled the lateral geniculate body of the present authors as the nucleus pretectalis superficialis. Luiten (1981a, b) divided a cell mass, which has about the same position in the diencephalon of the nurse shark Ginglymostoma, into two, calling only the dorsal part the lateral geniculate body.

This portion does not receive direct retinal projections but does have tectal input, whereas the ventral part, i.e. the ventrolateral optic nucleus receives a strong retinal input. Both (sub)nuclei, project to the caudal part of the telencephalic pallium. The degeneration studies of Smeets (1981a,b) in Scyliorhinus and Raja revealed both retinal and tectal inputs to the nucleus, labelled here as corpus geniculatum laterale. These species differences may be explained by considering the brain of Ginglymostoma to be more highly developed (Northcutt 1977, 1978) than that of Scyliorhinus and Raja. According to the parcellation theory of Ebbesson (1980a, p. 183) "neural systems evolve not by the mixing of systems, but by differentiation and parcellation, which involves competition of inputs, the redistribution of inputs, and the loss of connections". Experimental studies, particularly HRP-injections in the caudal part of the pallium of Scyliorhinus and Raja, will be needed to test the validity of this theory with regard to the lateral geniculate body in elasmobranchs.

1.6 Fibre tracts

The fibre systems in the diencephalon of cartilaginous fishes have been studied in normal material by several authors among whom Edinger (1982), Ariëns Kappers (1906), Ariëns Kappers and Carpenter (1911), Ariëns Kappers et al. (1936) and Addens (1945) should be mentioned. Recent experimental studies have confirmed the presence of most of the previously described fibre systems. These experimental studies have revealed, in addition, the presence of a number of previously unknown connections.

In this section we shall first describe the fibre tracts of the diencephalon that we could distinguish in normal material and then discuss the results of experimental studies. The fibre tracts of diencephalon will be subdivided into the following categories: (1) CONNECTIONS BETWEEN DIENCEPHALON AND BRAIN STEM (2), CONNECTIONS BETWEEN DIENCEPHALON AND TELENCEPHALON, (3) INTRINSIC DIENCEPHALIC CONNECTIONS, (4) DIENCEPHALIC COMMISSURE and (5) THE TRACTUS OPTICUS.

1. CONNECTIONS BETWEEN DIENCEPHALON AND BRAIN STEM

Under this heading the following fibre tracts will be discussed: the fasciculus retroflexus; tractus tectohabenularis; tractus tegmentohabenularis; tractus thalamotegmentalis; tractus thalamotectalis; ascending tectal efferents; cerebellar efferents, and the remaining connections of the diencephalon with the brain stem.

The chief efferent system of the ganglion habenulae, the fasciculus retroflexus of Meynert or tractus habenulo-interpeduncolaris, comprises myelinated and unmyelinated fibres. The right and left bundles are asymmetrically developed, just like the ganglia, with the left bundle containing far more myelinated fibres. The right bundle contains some myelinated fibres in Squalus and Scyliorhinus, somewhat fewer in Hydrolagus, and almost none in Raja. Comparable findings have been reported, among others, by Ariëns Kappers et al. (1936) and Kemali et al. (1980). The bundles themselves, however are of about equal size, except in Hydrolagus, where the right fasciculus retroflexus is larger than the left. In Chimera monstruosa, however, Ariëns Kappers and Carpenter (1911) and Kuhlenbeck and Niimi (1969) found that the left and right bundle differ not in overall size but in number of myelinated fibres. The fasciculus retroflexus passes ventrocaudal-ward through the diencephalon, occupying during this corse a position closet to the ventricular wall. During its corse to the interpeduncular nucleus the fibre bundle protrudes into the ventricle as a slight eminence, the ventral border of which is formed by the dorsal component of the sulcus diencephalicus dorsalis in Squalus, Raja and Hydrolagus. This fibre bundle terminates after decussation in the interpeduncular nucleus.

The quantitatively most important afferent system of the ganglia habenulae is the stria medullaris, which is largely of telencephalic origin. But the ganglia also receive fibres from the mesencephalon, the majority of which arise from the tectum mesencephali and constitute the tractus tectohabenularis. This bundle was first recognized by Edinger (1892), who regarded it as running to the tectum and called it accordingly "tractus Ganglii habenulae ad Mesencephalum". Addens (1945) however, recognized this bundle as running from the mesencephalon to the habenular ganglion and termed it tractus tectohabenularis. In sagittal sections of the four species studied we can recognize fibres connecting the mesencephalon with the habenular ganglia, but the direction of the fibres is not certain.

Another group of fibres originates in the tegmentum mesencephali and joins the tractus tectohabenularis before reaching the ganglion habenulae. This tract, described by Addens (1945) as the tractus tegmentohabenularis, could be observed in our sagittal sections as a loosely arranged bundle of fibres. It should be emphasized that the description of the tectohabenular and tegmentohabenular tracts is based entirely on observations in normal material. Experimental studies, involving large tectal lesions, have not confirmed the existence of a tectohabenular connection (Smeets 1981b), so that a habenular origin of these fibres cannot be excluded.

The tractus thalamotegmentalis and the tractus thalamotectalis mentioned briefly by Ariëns Kappers et al. (1936) form a diffuse system of fibres running between brain stem and thalamus. In sagittal section of the four species studied we could also recognize fibres connecting the thalamus with the tectum as well as the tegmentum mesencephali.

Ascending Tectal Efferents: distinct fibre tracts that connect the tectum mesencephali with various diencephalic cell masses are hard to recognize for, apart from the tractus tectohabenularis and some commissural system, no distinct fibre tracts can be distinguished in normal material. However, a degeneration study (Smeets 1981b) on the tectal efferent pathways in Scyliorhinus and Raja, has revealed that the diencephalon receives a substantial tectal input. After lesions that included all six tectal layers, ascending fibres could be traced to the pretectal area and to the dorso-medial region of the thalamus. A few fibres appeared to run further lateralward to the corpus geniculatum laterale and to the torus lateralis (in Raja). These ascending projections are both ipsi- and contra-lateral. The majority of the ascending efferent tectal fibres probably reach the

contralateral pretectal and thalamic region by way of the tectal commissure. However, some fibres run parallel and medial to the optic tract and probably reach to the opposte side, via the commissura postoptica.

Cerebellar Efferents: an important connection between cerebellum and mesencephalic and diencephalic regions, is provided by the brachium conjunctivum or tractus cerebello-mesencephalicus of Ariëns Kappers et al. (1936). This tract arises from the cerebellar nucleus and swings ventromedialward and forward to cross in the most caudal part of the tegmentum mesencephali, ventral to the fasciculus longitudinalis medialis. The majority of its fibres terminate in the nucleus ruber; however, some run to the diencephalic region and terminate reportedly in the thalamus and hypothalamus (Wallenberg 1907; Ariëns Kappers et al. 1936; Gerlach 1947). An experimental study of the cerebellar efferent pathways in the nurse shark, Ginglymostoma; (Ebbesson and Campbell 1973) has confirmed the existence of this pathways with the exception of a cerebellohypothalamic connection.

Remaining Connections of the Diencephalon with the Caudal Regions: some fibre tracts have been described in the older literature; the polarity of these fibre systems is unknown and some authors are convinced that only experimental studies can unravel this network of diffuse fibre systems. According to Ariëns Kappers et al. (1936) and Gerlach (1947), fibre tracts which arise chiefly in the lobus inferior hypothalami form a tract, tractus lobobulbaris, which can be traced to the most caudal regions of the rhombencephalon, where it probably becomes associated with the visceral efferent nuclei of the region, particularly those of trigeminal and facial nerve nuclei. Another tract, the tractus mamillo-interpeduncolaris (Gerlach 1947), courses together with the tractus lobobulbaris from the region of the recessus posterior

or recessus mamillaris to the nucleus interpeduncolaris in the midbrain tegmentum.

The material, especially in sagittal sections, does not exclude the existence of these two tracts. Numerous fibres, both myelinated and unmyelinated, run from the caudal part of the lobus inferior hypothalami and also from recessus mamillaris to the midbrain tegmentum. Probably these fibres include the tractus lobocerebellaris, described by Ariëns Kappers et al. (1936) .

Ebbesson and Hodde (1981) described a direct spinothalamic connection in Ginglymostoma. After a hemisection in the third spinal segment a small number of degenerated fibres could be traced to the dorsal thalamus. Although in normal material, such a pathway could not be recognized, a direct projection from the thalamus ventralis and nucleus periventricularis hypothalami to the spinal cord has also been experimentally confirmed by Smeets and Timerick (1981) in Scyliorhinus and Raja.

2. CONNECTIONS BETWEEN DIENCEPHALON AND TELENCEPHALON

The following tracts will be discussed in this section: the stria medullaris, the tractus pallii, the fasciculus basalis telencephali and the tractus thalamotelencephalicus.

The Stria Medullaris: the most important connection of the ganglion habenulae, passes through the most dorsal part of telencephalon impar and swings upward into the habenular ganglion to form the habenular commissure. The stria medullaris consists of fibres which cross in the habenular commissure to synapse with habenular cells and fibres which constitute a true telencephalic commissure, the commissura superior of Ariëns Kappers et al. (1936) .

According to these authors this commissure contains unmyelinated axons which are surrounded by the myelinated fibres of the decussating olfactohabenular

tract. The stria medullaris can be easily recognized and collects fibres from both the pallial and subpallial regions of the telencephalon. It consists of a thin sheet of fibres that surrounds a bundle of unmyelinated fibres. The various tracts that make up the stria medullaris are known principally from the work of Johnston (1911) who recognized as many as seven components.

The Tractus Pallii: an important fibre tract that originates in the roof of the telencephalon and was first described by Edinger (1982) as "Mantelbündel". This tract, which has been noted by all later students of chondrichthyan brain (e.g. Johnston 1911; Bäckström 1924; Ariëns Kappers et al. 1936; Gerlach 1947), connects the telencephalic pallial region with the hypothalamic areas. According to Johnston (1911) the tractus pallii constitutes a system relating visceral and gustatory centres of the hypothalamus to the olfactory centres of telencephalon. As he stated: "this is equivalent to saying that the massive roof in selachians is an olfactogustatory correlation centre".

The tractus pallii is a well defined tract of predominantly myelinated fibres. It passes immediately ventrolateral to the stria medullaris, through the telencephalon impar, keeping a position lateral to the basal forebrain bundle and then runs superficial to, or intermingles with, lateral fibres of the optic tract. Finally the fibres of the tractus pallii after decussating in part, in the decussato tractus pallii, caudoventral to the chiasma optimum, mostly terminate in the rostral half of the nucleus lobi lateralis inferior.

There are several possibile sites of origin and termination of the fibres of the tractus pallii. Catois (1901) using Golgi material concluded that the tract contains descending fibres, whereas Ariëns Kappers and Theunissen (1907) with normal material, and Wallenberg (1907) with the Marchi technique, held that this pathway consists of ascending fibres. More recent preliminare studies using axon degeneration techniques have revealed that in fact the tractus pallii contains both descending and ascending fibres (Ebbesson 1972; Smeets unpublished), and that most of the fibres cross in the decussate tractus pallii.

The Fasciculus Basalis Telencephali: or basal forebrain bundle, is the largest fibre system that connects the telencephalon with more caudal, and particularly diencephalic centres. This tract was first recognized by Edinger (1892) who called it "das basale Vorderhirnbündel". It has subsequentely been called the tractus striothalamicus (Ariëns Kappers 1906), tractus striohypothalamicus (Ariëns Kappers and Carpenter 1911), medial forebrain bundle (Johnston 1911) and tractus striothalamicus et hypothalamicus (Ariëns Kappers et al. 1936).

In most groups of lower vertebrates two basal bundles can be distinguished, namely the fasciculus medialis telencephali and the fasciculus lateralis telencephali. It has to be emphasized that the medial and lateral forebrain bundles are named according to their topographic position in the telencephalic peduncle and they do not necessarily share similar origins and terminations in different groups (Nieuwenhuys 1969). In Teleosts, two parts of the basal forebrain bundle can be recognized, whereas in primitive Actinopterygians and in cartilaginous fishes this separation is not distinct.

After partly crossing in the commissura anterior the basal forebrain bundle enters the diencephalon to distribute its fibres mainly to the more superior and caudal parts of the lobi inferiores hypothalami. However, some fibres can be traced to the ventral thalamus and to the midbrain tegmentum. Experimental studies by Ebbesson (1972) and Smeets (1983) confirm this route for the basal forebrain bundle.

The Tractus Thalamotelencephalicus: Ebbesson and Schroeder (1971) and Schroeder and Ebbesson (1974) reported that in the nurse shark, *Ginglymostoma*, following thalamic lesions a strong, largely crossed bundle of degenerating fibres can be traced to the pallium. The fibres of this thalamotelencephalic tract

collect in the rostral thalamic region and pass ventrally to decussate mostly in the postoptic commissure, directly dorsal to the decussation of the tractus pallii. Further rostrally the tract moves laterally and finally swings dorsalward to terminate in an area known as the pallium dorsale pars centralis.

A thalamotelencephalic tract could not be distinguished as a separate entity, although it is probabile that the lateral part of the basal forebrain bundle contains the fibres of this tract. Johnston (1911) recognized a thalamocortical tract, which connects the tellencephalon with the lateral geniculate body, nucleus pretectalis and possibly the tectum mesencephali.

3. INTRINSIC DIENCEPHALIC CONNECTIONS

Many diffusely arranged fibres pass dorsoventralward and rostrocaudalward within the diencephalon. Amongst these fibres earlier students of the chondrichthyan brain (Dammerman 1910; Johnston 1911; Ariëns Kappers et al. 1936) recognized a tractus habenulothalamicus, a tractus thalamolobaris, a tractus saccothalamicus, a tractus tuberoposterior, a tractus sacci vasculosi and a tractus preopticohypophyseus.

Tractus Habenulothalamicus: mentioned by Johnston (1911), was stated by Ariëns Kappers et al. (1936) to arise from the subhabenular, rather than from the habenular region and to surround partly the fasciculus retroflexus. These authors were unable to determine its area of termination.

Tractus Thalamolobaris: according to Ariëns Kappers et al. (1936), is formed by diffusely arranged fibres which in more caudal diencephalic regions connect the thalamus dorsalis with the lobi inferiores.

Tractus Saccothalamicus: has been reported by Dammerman (1910) as consisting of those fibres of the tractus sacci vasculosi, that pass beyond the nucleus sacci vasculosi, together with processes from cells of the latter nucleus.

This tract extends frontalward and dorsalward toward the central grey of the dorsal thalamus.

Tractus Tuberoposterior: according to Dammerman (1910) another fibre system, this tract arises from the nucleus sacci vasculosi and passes caudalward from the nucleus through the tuberculum posterius. The termination of this tract is at present unknown.

Tractus Sacci Vasculosi: this is a well developed unmyelinated tract formed by the processes of the cells of the saccus vasculosus. The wall of the saccus vasculosus consists of cubic or cylindric cells (coronet cells) which have sensory hairs with knob-shaped terminations turned toward the lumen of the sac. Between these cells lie smaller, supporting elements. The axons of the coronet cells run in the wall of the sac, forming an evident fibre layer. Frontalward they form a fascicle of fibres. In the region where the wall of the sac passes over into that of the recessus mamillaris this fibre tract enters the grey substance of the tuberculum posterius as a distinct little bundle which ascends to the level of the commessura post-infundibularis pars inferior. After decussating, the majority of the fibres terminate in the nucleus sacci vasculosi, which has already been mentioned.

There is little or no agreement about the function of the saccus vasculosus. According to Dammerman (1910) the fact that the sensory hairs of the crown or coronet cells protrude into the ventricular cavity of the saccus suggests that it is concerned with perceptions related to the ventricular fluid. A secretory function of the epithelium of the saccus vasculosus, based on the presence of inclusions in the coronet cells, has been proposed by Bargmann (1954), van de Kamer and Verhagen (1954) and Altner (1964b, 1965), whereas Mellinger (1964) and Altner (1964a) have stated that these inclusions could not be interpreted as a sign of a secretion process, but are due to differences in endoplasmic structure. Watanabe (1966) and von Harrach (1970)

favoured a receptor function and suggested that the saccus vasculosus could be an organ for chemoreception or for the perception of liquor pressure. A third possibility is that in chondrichthyan fish the coronet cells are functionally involved in a transcellular ion transport process between the cerebrospinal fluid and the blood, in the same manners as proposed by Jansen (1969) for actinopterygians.

An electronmicroscopic study by van de Kamer et al. (1973) has revealed that large quantities of glycogen are present in the coronet cells and that these cells are filled with an extensive system of vertically oriented, smooth endoplasmatic reticulum tubules which might facilitate transcellular ion transport.

Tractus Preopticohypophyseus: the second intrinsic diencephalic tract. Earlier students of the diencephalon have considered this tract to be the afferent connection of the saccus vasculosus and therefore termed it the tractus thalamosaccularis (Dammerman 1910; Ariëns Kappers et al. 1936; Gerlach 1947). However, some sudies (e.g., Meurling and Björnklund 1970; Wilson et al. 1974) have revealed that this tract passes to the pituitary and, hence, deserves the name tractus preopticohypophyseus.

4. DIENCEPHALIC COMMISSURE

Commissura Habenulae: consists of myelinated and unmyelinated fibres of the stria medullaris. The unmyelinated fibres are probably true commissural fibres that form a commissura superior telencephali (Ariëns Kappers et al. 1936), whereas the myelinated fibres arise from the stria medullaris, which decussates in the commissura habenulae and terminates in the contralateral ganglion habenulae. The commissura habenulae also receives fibres from the pineal tract.

Commissura Posterior: consists of a large mass of fibres forming a dorsally convex arch just ventral to the pretectal region. It is compact in the midplane but sprays out laterally, with most of its fibres going in a ventrocaudal direction. The most ventrally directed fibres run near the periventricular grey toward the tuberculum posterius. The most caudally running fibres are lost in the tegmental fibre stream. No definite connections of the posterior commissure could be distinguished but the fibres possibly relate to all of the nearby cellular regions. Connections with the tectum, as stated by Ariëns Kappers et al. (1936), have not been confirmed by the experimental study of Smeets (1981b). The posterior commissure also receives fibres from the pineal tract.

Commissura Postoptica: in addition to the crossing optic fibres, decussations of other pathways are seen ventrocaudal and dorsocaudal to the optic chasm. The ventrocaudal part of the commissura postoptica is formed by crossing fibres of the tractus thalamotelencephalicus, as described above. It can be easily distinguished from the more ventral decussation of the tractus pallii. The dorsal part of the postoptic commissure probably represents a connection between the two sides of the rostral parts of the hypothalamus and may also contain crossing fibres of the commissura transversa.

Commissura Transversa: crosses immediately behind the optic chiasm in the dorsal part of the commissura postoptica. Little agreement exists about the origin and termination of these fibres. Edinger (1892), who considered it more a decussation than a commissure, saw its fibres end under the tectum mesencephali, while Catois (1901) described it as a commissure between the posterior and lateral regions of the mesencephalon. Haller (1898), who called it "commissura postoptica superior", claimed that it contains only decussating fibres of the tectum mesencephali. Ariëns Kappers et al. (1936) stated that the fibres of this commissure pass along the lateral surface of the diencephalon to tectal and tegmental centres with which they are

functionally related.

Although the commissura transversa could easily be traced over some distance, its origin and termination could not be determined with certainly. It seems probable that these fibres are subtectal rather than tectal decussating fibres, though lesions of the tectum show some degenerating fibres which corse to the postoptic commissure.

Commissura Preinfundibularis and Commissura Postinfundibularis: the commissura preinfundibularis lies caudal to the decussation of the tractus pallii in the ventral part of the hypothalamus and consists partly of myelinated dorsally-running fibres, the origin and termination of which are still unknown. The commissura postinfundibularis can be subdivided into a pars superior and a pars inferior: the pars superior lies somewhat more rostral in the tuberculum posterius than the inferior part and probably forms a connection between the caudal cell masses of the hypothalamus. In *Squalus* ans *Scyliorhinus* this commissure extends rather far in the rostrocaudal direction. The pars inferior of the commissura postinfundibularis takes a more ventral position in the tuberculum posterius and has a much smaller rostrocaudal extent. This part is formed by the crossing fibres of the tractus sacci vasculosi. In *Scyliorhinus* the preinfundibular commissure is poorly developed, whereas in *Raja* the postinfundibular commissure is less distinct.

5. THE TRACTUS OPTICUS

The tractus opticus of cartilaginous fish has been the subject of several experimental studies (Ebbesson and Ramsey 1968; Graeber and Ebbesson 1972; Northcutt 1979a; Ebbesson and Meyer 1980; Northcutt and Wathey 1980; Luiten 1981a; Smeets 1981a). These studies shown that the retinal efference are not restricted to the corpus geniculatum laterale and the tectum mesencephali, as was previously believed (Houser 1901; Ariens Kappers et al. 1936), but project to several additional diencephalic and mesencephalic regione.

In Scyliorhinus and Raja (Smeets 1981a) the optic nerve fibres decussate completely in the chiasma optimum with the exception of a very small ipsilateral projection to the caudal part of the preoptic nucleus or to an area immediately ventral to this nucleus, which corresponds to our nucleus suprachiasmaticus. The decussating optic nerve fibre salso terminate for a very small part in the contralateral preoptic area, in the caudal region of which are scatterei cells among the decussating optic nerve fibres. The crossed optic nerve fibres form a lateral or marginal optic tract which courses both dorsally and caudally along the lateral wall of the diencephalon. Some of the optic nerve fibres leave the main stream and curve medialward to terminate in the thalamus dorsalis pars lateralis. At somewhat more caudal levels, the optic nerve fibres or their collaterals terminates within the area of the lateral geniculate body and this is the most prominent diencephalic projection. At the same level the thalamus ventralis pars lateralis also receives a retinal projection from fibres which run in the ventral part of the main stream of optic fibres.

A medial optic tract splits off from the marginal tract and courses dorsomedially, where it divides into fascicles which terminate for a small part in a pretectal nucleus and for the greater part distribute their fibres over the rostral and medial parts of the tectal lobe. In Raja also, a central pretectal terminal field was found. The remainder of the marginal optic tract, the tractus opticus lateralis, courses dorsolaterally and supplies the lateral and caudal parts of the tectal lobe.

Caudal to the division into medial and lateral optic tracts, fibres were observed in Raja leaving the lateral optic tract and these fibres could be traced to the midbrain tegmentum, where they terminate in the basal optic nucleus,thus forming a tractus opticus basalis.

Comparisons between the retinal projections found by some authors are

difficult because there is a little agreement about the diencephalon nomenclature. However, it can be concluded that the preoptic area, the ventral and dorsal thalamus, the pretectal areas, as well as the tectum mesencephali, receive a contralateral retinal input. Ipsilateral projections to the preoptic area or nucleus suprachiasmaticus have been reported by Greaber and Ebbesson (1972: Negaprion), Northcutt (1979a: Squalus), Ebbesson and Meyer (1980: Rhinobatos), Northcutt and Wathey (1980: Platyrhinoidis) and Smeets (1981a: Scyliorhinus, Raja). As far as we know, only Northcutt and Wathey (1980) have observed ipsilateral projections to the other diencephalic and mesencephalic retinal targets.

A tractus opticus basalis has not been easily recognized in chondrichthyan fish. In Galeocerdo (Ebbesson and Ramsey 1968), Negaprion (Greaber and Ebbesson 1972) and Scyliorhinus (Smeets 1981a), following enucleation of an eye, degeneratine fibres are observed leaving the lateral optic tract ventrally and coursing caudalward to the midbrain tegmentum. Their site of termination could not be ascertained, except in Galeocerdo. In Squalus (Northcutt 1979°), Platyrhinoidis (Northcutt and Wathey 1980) and Ginglymostoma (Luiten 1981a) a distinct basal optic tract can be recognized, which partly terminates in a small cell group which is comparable to the basal optic nucleus, and partly continues caudally and medially to terminate in an ill-defined tegmental area, which was termed the ventral accessory optic nucleus by Northcutt and Wathey (1980). According to Luiten (1981a), the close proximity of this terminal area to the oculomotor nuclei strongly suggests that this nucleus is the homologue of the accessory or basal optic nucleus of amniotes. It would be very interesting to know whether the presumed basal optic nucleus in cartlaginous fish project sto the oculomotor nucleus and the cerebellum, as has been demonstrated for some teleosts (Finger and Karten 1978), birds (Brauth and Karten 1977); Brecha et al. 1980) and

reptiles (Reiner and Karten 1978).

2. The diencephalon of the Osteichthyes

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2.1 The preoptic area

The preoptic area surrounds the preoptic recess of the third ventricle. It is bounded rostrally by the anterior commissure, dorsally by the telencephalon, the ventral thalamus, and the posterior tuberculum, and ventrally by the optic chiasm and the dorsal hypothalamus. The anteroventral part of the parvocellular preoptic nucleus and the parvocellular part of the parvocellular preoptic nucleus represent the most rostral preoptic cell masses. They emerge just caudal to the anterior commissure, surrounding the rostral aspect of the preoptic recess. The anteroventral part of the parvocellular preoptic nucleus lies in the ventrolateral pole of the preoptic recess.

The magnocellular part of the magnocellular preoptic nucleus arises dorsal to the parvocellular part of this nucleus. At the same transverse level, the cell bodies of the gigantocellular part of the magnocellular preoptic nucleus lie dorsally to the magnocellular subdivision. Whereas the gigantocellular part of the magnocellular preoptic nucleus reaches the caudal pole of the preoptic area, the parvocellular and magnocellular parts of the nucleus are caudally replaced by the anterior periventricular nucleus. It is limited dorsally by the gigantocellular part of the magnocellular preoptic nucleus and ventrally by the suprachiasmatic nucleus. The suprachiasmatic nucleus occupies the ventral border of the ventricle. At the caudal pole of the preoptic area and coinciding with the rise of the hypothalamus, the anterior periventricular nucleus is substituted by the posterior periventricular nucleus.

2.2 Epithalamus

The epithalamus is composed of the habenula, the habenular commissure, and the epiphysis. The habenula is a paired finger-shaped structure that lies dorsal to the ventral thalamus, in the dorsal area of the rostral diencephalon. The habenular nucleus can be subdivided into dorsal and ventral parts. The dorsal habenular nucleus consists of small and intensely stained cells, disposed near the ventricular surface but also extending out laterally.

2.3 Hypothalamus

The hypothalamus emerges ventral to the caudal pole of the preoptic area; consists of a medial tuberal lobe and two inferior lobes. The tuberal lobe bounds the third ventricle, which is laterally expanded at midrostral levels giving rise to diverticula, known as the lateral recesses. The caudal part of the hypothalamic ventricle forms the posterior recess, which also displays ventrolaterally directed diverticula.

The medial tuberal zone consists of the lateral tuberal nucleus and its subdivisions, the anterior tuberal nucleus, the nucleus of the posterior recess, and the nucleus of the saccus vasculosus. Two cell masses that border the medial evaginations of the lateral recess, the ventral, and the dorsal parts of the nucleus of the lateral recess are also included in this zone. The most rostral cell group of the hypothalamus is the lateral part of the lateral tuberal nucleus. Caudally, the lateral part of the lateral tuberal nucleus migrates slightly laterally along the ventral surface of the hypothalamus. The ventral and medial parts of the lateral tuberal nucleus begin at the level where the hypothalamic ventricle rises. The ventral part of the lateral tuberal nucleus bounds the ventral aspect of the ventricle.

The medial part of the lateral tuberal nucleus is placed laterally to the ventral subdivision of this nucleus; the dorsal aspect of the hypothalamic ventricle is bordered by the cells of the dorsal part of the lateral tuberal nucleus. This nucleus lies ventral to the caudal pole of the preoptic area and dorsal to the ventral part of the lateral tuberal nucleus.

At the caudal level of the anterior tuberal nucleus, the hypothalamic ventricle shows dorsal and ventral sulci, associated with the dorsal and ventral parts of the lateral tuberal nucleus, respectively. These sulci

are the beginning of the mediolaterally directed extensions of the lateral recess. Two cell masses extend laterally from these sulci.

The nucleus of the posterior recess (NRP) arises as a thickening of the ependymal cell layer characterized by the presence of small cells firmly packed in a ventromedial position; caudal to the medial part of the lateral tuberal nucleus, a scattered cell group lies laterodorsal to the nucleus of the posterior recess. Slightly caudal to the level where both sides fuse, the dorsal portion of the hypothalamic ventricle appears covered by the cells of the nucleus of the saccus vasculosus.

The large bilobular inferior lobes of the hypothalamus comprises the diffuse nucleus of the inferior lobe and its subdivisions, the lateral subdivision of the nucleus of the lateral recess, the central nucleus of the inferior lobe, and the medial nucleus of the inferior lobe. Rostrally, the inferior lobes are occupied by the cells of the lateral part of the diffuse nucleus. The rise of the lateral recess determines the onset of two cells masses in the inferior lobes: a medial part of the diffuse nucleus, and a cell group that borders the lateral extensions of the lateral recess. Rostrally, the lateral part of the nucleus of the lateral recess occupies the dorsolateral and ventrolateral portions of the lateral recess. Further caudal, the dorsal and the ventral subdivisions disappear and the lateral subdivision surrounds all the lateral recess. The central nucleus of the inferior lobe arises more caudally, in a position ventromedial to the torus lateralis.

Finally, at the level where the inferior lobes appear separate from the rest of the brain, an almost cell-free region is found in the dorsal area of the inferior lobes. This region represents the caudal part of the diffuse nucleus.

2.4 Thalamus dorsalis

The dorsal thalamus is formed by three nuclei: the anterior thalamic nucleus, the dorsal posterior thalamic nucleus, and the central posterior thalamic nucleus. The most rostral of them is the anterior thalamic nucleus that arises ventrocaudal to the habenula, displacing the intermediate thalamic nucleus laterally from its periventricular position. Slightly caudal, the nucleus becomes V-shaped, showing two ventrolaterally extended arms; further caudal, the anterior thalamic nucleus is displaced laterally by the dorsal posterior thalamic nucleus and the central posterior thalamic nucleus. It loses its bilaminar structure and only a single cell lamina is evident. The dorsal posterior thalamic nucleus starts ventromedial to the fasciculus retroflexus. Caudally, the dorsal posterior thalamic nucleus increases in size and its cell density diminishes. The central posterior thalamic nucleus lies ventral to the dorsal posterior thalamic nucleus; at more caudal levels, the central posterior thalamic nucleus exhibits a characteristic dorsomedial-to-ventrolateral orientation.

2.5 Thalamus ventralis

Four nuclei can be identified in the ventral thalamus: the nucleus of the thalamic eminentia, the ventromedial thalamic nucleus, the ventrolateral thalamic nucleus, and the intermediate thalamic nucleus. The rostral pole of the ventral starts just caudal to the intermediate nucleus of the ventral telencephalon and lies dorsolateral to the gigantocellular part of the magnocellular preoptic nucleus. The ventromedial thalamic nucleus arises rostrally as a thickening of the ependymal layer.

A small cell group forming the ventrolateral thalamic nucleus appears lateral to the ventromedial nucleus. Further caudal, the ventromedial nucleus loses its laminar conformation and exhibits a few scattered cells. The intermediate thalamic nucleus forms a cell group that arises ventral to the epithalamic habenula and dorsal to the ventromedial thalamic nucleus. The nucleus is displaced

laterally and loses its connections with the ventricular surface, lying dorsolaterally to the ventromedial thalamic nucleus.

2.6 Posterior tuberculum

The posterior tuberculum can be divided into a periventricular area and a migrated area. The periventricular area comprises the periventricular nucleus of the tuberculum posterior, the paraventricular organ, the nucleus of the paraventricular organ, and the posterior tuberal nucleus. The periventricular nucleus of the posterior tuberculum lies caudal to the ventromedial thalamic nucleus and ventral to the dorsal thalamus. It is bounded ventrally by the paraventricular organ, and caudally by the commissural preglomerular nucleus.

Further caudal, both sides of the brain fuse medially and the paraventricular organ appears to be separated into a dorsal and a ventral component. Laterally apposed to the paraventricular organ lies the nucleus of the paraventricular organ. The posterior tuberal nucleus begins more caudally in the midline, dorsal to the nucleus of the saccus vasculosus and ventral to the periventricular nucleus of the posterior tuberculum and the commissural preglomerular nucleus.

The migrated area of the posterior tuberculum comprises the preglomerular nuclear complex, the nucleus glomerulosus, the caudomedial nuclei, and the outlying nuclei. The most rostral nucleus of the preglomerular complex is the anterior preglomerular nucleus, which arises in the ventral surface of the brain. Further caudal, at the onset of the hypothalamic ventricle, the lateral preglomerular nucleus appears ventral to the anterior preglomerular nucleus. The tertiary gustatory nucleus emanates between the anterior preglomerular nucleus and the lateral preglomerular nucleus. This nucleus is morphologically characterized by its rounded shape, showing small and medium-sized cells interspersed in the neuropil and lining the border of the nucleus. In a more medial position, the medial preglomerular nucleus begins, surrounding the fibers of the lateral forebrain bundle. The medial preglomerular nucleus enlarges caudally, adopting a more medial position, around the dorsomedial aspect of the lateral forebrain bundle. At the caudal pole, its cells are located between the commissural preglomerular nucleus and the posterior part of the nucleus glomerulosus. The nucleus glomerulosus consists of a rostral subdivision, the anterior part, and a caudal subdivision, the posterior part. The anterior part of the nucleus glomerulosus arises caudal to the intermediate superficial pretectal nucleus, in the pretectal area. It is located ventral to the central pretectal nucleus, dorsal to the accessory optic nuclei and medial to the magnocellular superficial pretectal nucleus.

Slightly further caudally, the anterior part of the nucleus glomerulosus migrates medially, occupying a position ventrolateral to the dorsal posterior thalamic nucleus. Further caudally, it moves ventrally and fuses with the posterior part of the nucleus glomerulosus. The posterior part of the nucleus glomerulosus begins just caudal to the tertiary gustatory nucleus, displacing the medial preglomerular nucleus medially.

The corpus mamillare starts ventrally apposed to the commissural preglomerular nucleus and dorsal to the caudal pole of the inferior part of the lateral tuberal nucleus.

The outlying nuclei comprise the nucleus of the torus lateralis, the posterior thalamic nucleus, and the lateral thalamic nucleus. The most rostral cell mass of the outlying nuclei is the nucleus of the torus lateralis. It emerges dorsal to the rostral pole of the inferior lobes and lateral to the tertiary gustatory nucleus, lying along the lateral surface of the brain. The posterior thalamic nucleus replaces the tertiary gustatory nucleus, adopting a laterodorsal position in relation to the posterior part of the nucleus glomerulosus and a medial position with respect to the nucleus of the torus lateralis. The caudal pole of the

diencephalon is occupied by the lateral thalamic nucleus. Further caudally, LT moves ventrally and occupies the place left by the posterior part of the nucleus glomerulosus; the nucleus of the tractus pretecto-isthmicus is an inconspicuous cell mass that contains very small cells that appear ventrolateral to the fibers of the tractus pretecto-isthmicus, dorsolateral to the posterior part of the nucleus glomerulosus, and lateral to the fibers of the horizontal commissure exiting at the dorsal pole of the posterior part of the nucleus glomerulosus.

The nucleus of the fasciculus retroflexus is ventrolaterally apposed to the fasciculus retroflexus and lies ventral to the nucleus of the medial longitudinal fasciculus. Finally, the dorsal periglomerular nucleus appears at the same transverse level than the nucleus of the fasciculus retroflexus, ventrolateral to it, and dorsomedial to the posterior part of the nucleus glomerulosus.

2.7 Synencephalon

The synencephalon, a region placed between the diencephalon proper and the mesencephalon, according to Braford and Northcutt (1983) is formed by five nuclei. The subcommissural organ emerges caudal to the habenula and lies ventral to the fibers of the posterior commissure. The dorsal periventricular pretectal nucleus lies immediately caudal to habenula, dorsal to the anterior thalamic nucleus, and lateroventral to the subcommissural organ. Slightly caudal, and around the medioventral and ventral zones of the fasciculus retroflexus, the ventral periventricular pretectal nucleus begins. Further caudally, at the end of the posterior commissure, the dorsal and ventral periventricular pretectal nuclei move laterally away from the ventricle. The paracommissural nucleus arises dorsolateral to the posterior commissure and ventral to the rostral optic tectum. Slightly caudal, it lies ventrolateral to the rostral pole of the torus longitudinalis, bordering the lateral extension of the posterior commissure.

2.8 Pretectum

The pretectal area begins in the rostral diencephalon of sea bass with the appearance of the parvocellular superficial pretectal nucleus. This nucleus starts at the rostral pole of the optic tectum. The central pretectal nucleus arises in the rostral pretectal area; the central pretectal nucleus extends more caudally than the other pretectal nuclei.

The nucleus corticalis begins lateral to the central pretectal nucleus, forming a characteristic dorsolaterally elongated cell plate. The nucleus corticalis is recognizable by its large ovoid and rounded cells embedded in the rostral pole of the optic tectum. The intermediate superficial pretectal nucleus starts ventromedial to the caudal end of the parvocellular superficial pretectal nucleus. Finally, the intermediate superficial pretectal nucleus adopts a ventrolateral position in relation to the central pretectal nucleus before disappearing slightly rostral to the rise of the anterior part of the nucleus glomerulosus.

The magnocellular superficial pretectal nucleus represents a nucleus that appears ventral to the nucleus corticalis, lateral to the central pretectal nucleus, and dorsal to the parvocellular and intermediate superficial pretectal nuclei. Further caudally, it lies lateral to the anterior part of the nucleus glomerulosus. The accessory pretectal nucleus arises slightly dorsocaudal to the posterior end of the intermediate superficial pretectal nucleus. This cell mass adopts a central position in the pretectal area and is surrounded by the neurons of the nucleus corticalis, the central pretectal nucleus, the magnocellular superficial pretectal nucleus, and the anterior part of the nucleus glomerulosus.

3. The origins of cerebral asymmetry

A crucial issue in modern neuroscience is that of asymmetry of brain function and its evolutionary origins. A brain is considered to be asymmetrical (or lateralized) if one side (hemisphere or other brain region) is structurally different from the other and/or performs a different set of functions. For instance, in our species language and speech production are a function controlled by the left hemisphere in the large majority of individuals. The right hemisphere, on the contrary, seems to be mainly involved in a variety of emotional and spatial functions. Each hemisphere controls the opposite side of the body, including the muscles of the face. Thus, emotions are expressed more strongly on the left side of the face (controlled by the right hemisphere) whereas during speech (controlled by the left hemisphere) the right side of the mouth moves more than the left (Wolf et al., 1987). Gross anatomical asymmetries of the human brain are visible even to the naked eye: the left hemisphere tends to be wider in the posterior region, whereas the right hemisphere is wider in
the anterior region (Bradshaw et al., 1980). These asymmetries impress themselves on the interior side of the skull, and so have been observed even in the endocasts of the skulls of fossil hominids (Holloway et al., 1982).

For a long time brain lateralization has been considered a characteristic unique to the human species, associated with language and handedness. In the last twenty years, however, a great body of evidence has been accumulating that animals too have lateralization of brain functions. Evidence for brain lateralization is now widespread among both mammals and birds (reviews in: Andrew et al., 1991; Bradshaw et al., 1996; ,Rogers et al. 1993; Ward et al., 1993). It is still unclear, however, whether this should be interpreted as reflecting basic homology, or parallel but independent evolutionary histories.

Various authors have suggested that the functional significance of lateralization may be to prevent conflict of response emission arising from visual input of two laterally placed (largely monocular) eyes (Bradshaw et al., 1993). Laterally placed eyes and relatively small commissural systems may have set a requirement for lateralization of the brain. This is particularly evident in birds, which lack the large corpus callosum of the mammalian brain interconnecting the left and right hemispheres. The main commissural systems of the avian brain (the tectal and posterior commissures) occur at a lower level of neural organization, in the midbrain, and here they appear to have a role in suppressing lateralization (Parsons et al., 1993).

Most species of birds have laterally placed eyes with only a small degree of binocular vision in the frontal field. Furthermore, with the exception of special cases (such as owls), birds frequently present independent use of the two eyes to scan the environment (Wallman et al., 1985). Thus, each eye is able to examine different portions of the visual environment even in the binocular fields. It is much as if each eye acts as an independent unit, with information from each of the two visual fields being processed primarily separately. In this condition, the presence of hemispheric specialization may have the important function of preventing conflicting responses elicited by stimuli perceived simultaneously in two monocular visual fields, each demanding a different response. If one hemisphere is dominant in the control of a certain behaviour, then conflict of response emission would be avoided by having a lateralized brain. Birds, therefore, may have had quite specific reasons to develop brain asymmetry.

In mammals, on the other hand, given the well-developed corpus callosum, perceptual information can be conveyed easily to both hemispheres. Some mammals are also different from birds in that they have both ipsilateral (non-crossing) and contralateral (crossing) projections from each eye to the brain (in

birds there is complete decussation at the optic chiasma). Rodents have laterally placed eyes, and the number of ipsilateral projections from the retina to the brain is relatively small (in rats, only 5% of the optic fibers do not cross over at the optic chiasma). Thus, in rodents visual projections to the lateral geniculate nucleus (the first relay station in the visual system) are largely from the contralateral eye. Transfer of information is of course possible at the higher levels via the corpus callosum, but it should be noted that information that reaches an hemisphere via the corpus callosum is qualitatively different from that which reaches a hemisphere directly.

Even in mammals with frontally placed eyes there are differences in the inputs from one eye to each of the hemispheres. In fact, although each eye relays inputs to both the right and left hemispheres, the fibers from the nasal half of the retina, which cross to the contralateral hemisphere, are larger than those which arise from the temporal half of the retina and go to the ipsilateral hemisphere. Fibers which cross and go to the contralateral hemisphere, therefore, conduct neural signals faster and they dominate the uncrossed fibers during binocular stimulation (Proudfoot et al., 1983). This may explain eye preferences for viewing in humans and nonhuman primates. In the small-eared bushbaby (*Otolemur garnettii*), for instance, Rogers et al. reported a left-eye preference for viewing familiar stimuli and a trend for right-eye preference when viewing novel, arousing stimuli.

Thus, even in mammals there could have been selective pressures to develop brain asymmetries similar to those postulated for birds. In principle, therefore, brain lateralization may have arisen de novo in both the mammalian and the avian classes, but this seems unlikely. The hypothesis of an identical selective pressure for independent evolution of lateral asymmetries in the two phyla contrasts with the observed pattern of convergence in the directions of the asymmetries.

Mice, rats, Japanese macaques (Petersen et al., 1978, Petersen et al., 1984), passerine birds (Nottebohm et al., 1971; Nottebohm et al., 1977) and humans all show a dominance of the left hemisphere in the production and/or perception (e.g., zebra finches (Nottebohm et al., 1990)) of their species-specific vocalizations. Though it is not clear whether the crucial factor is the communicative significance (Petersen et al., 1984) or the temporal processing of auditory sequences, the similarity remains impressive nonetheless. (Obviously, only some aspects of the human language system are left-dominant, a few show a right dominance and most seem to work in parallel. Furthermore, humans, rhesus monkeys, chimpanzees (Morris et al., 1993), and chicks (Vallortigara et al., 1992; Vallortigara et al., 1991) all show a right hemisphere advantage in individual recognition of familiar conspecifics. The right hemisphere is also selectively involved in the production of facial expressions in humans (Sackeim et al., 1978) and monkeys, and it is in general the dominant hemisphere for controlling negative emotional expressions and novelty responding in humans, rhesus monkeys (Ifune et al., 1984), rats (Denenberg, 1981) and chickens (Andrew et al., 1995; Regolin et al., 1996; Rogers et al., 1991). Finally, the right hemisphere is better at spatial processing in humans (De Renzi, 1982), rats (King et al., 1992), chicks (Rashid et al., 1989; Vallortigara et al., 1996), and both food-storing (Parus palustris; P. caeruleus) and non-storing (Garrulus glandarius; Corvus monedula) birds. Hemispheric differences in overall modes of analysis of perceptual information, holding across different sensory modalities, have been suggested for humans, rats (Bianki et al., 1992), pigeons (Güntürkün et al., 1985) and chickens (Vallortigara et al., 1994; Vallortigara et al., 1994).

Thus, a strong argument can be made that brain asymmetry was already present in a common reptilian ancestor shared by birds and mammals. This reptilian ancestor

would have had laterally placed eyes and extremely reduced, if any, commissural systems. However, since behaviour (and the brain as well) does not provide us with fossils, we must turn to present-living 'lower' vertebrates
(fishes, amphibians and reptiles) to find evidence for this evolutionary account.

Left–right asymmetries in brain anatomy in lower vertebrates have long been known. However, evidence of behavioural asymmetries at the population or individual level has begun to appear only recently. In this paper we review the evidence currently available for lateralization in lower vertebrates, and summarize the state of our present knowledge on the evolution of brain lateralization.

4. Asymmetry in the epithalamus

of Vertebrates

The epithalamus has been historically conceived as a distinct neuroanatomical moiety within the diencephalon of all Vertebrates. Named because of its topographical situation "above" ("*epi*") the thalamus, the epithalamus was originally considered as one of the fundamental longitudinal subdivisions of the diencephalon, together with the dorsal thalamus, ventral thalamus and hypothalamus. Recent ontogenetic studies, however, have revealed that at least the dorsal thalamic and ventral thalamic subdivisions are not longitudinal, but instead are oriented perpendicular to the longitudinal axis of the brain. In the context of this emerging neuromeric model of brain organisation (Rubenstein et al. 1998), the epithalamus stems from the same neuromere as the dorsal thalamus, denominated parencephalon posterius or P2.

The epithalamus is constituted by 2 sets of neuronal conglomerates with strikingly dissimilar cytoarchitectonic organisation: the habenula and the pineal complex. Whereas the habenula is formed by a bilateral set of nuclei surrounding the lateral walls of the third ventricle, the pineal complex comprises a pair of median evaginations situated along the diencephalic roof plate. The habenular commissure divides the diencephalic roof plate into a larger rostral and a smaller caudal part. The rostral part gives rise to the saccus dorsalis, a membranous evagination of unknown function that reaches the posterior end of the velum transversum. The caudal part of the diencephalic roof plate gives rise to a pair of saccular or tubular evaginations known as pineal organ or epiphysis cerebri, and parapineal organ or parietal eye. Together, the pineal and parapineal organs constitute the so-called pineal complex.

From the 19th century, a large number of cytoarchitectonic studies have shown that in many species the left and right sides of the habenula display a remarkable asymmetry in size and sometimes also in

neuronal organisation. Other, more sporadic studies have revealed that asymmetry is also seen in the parapineal organ, and that asymmetry in both the habenula and parapineal organ is not restricted to cytoarchitecture but is also reflected in neuronal connectivity, neurochemistry and gene expression during embryogenesis. Despite its widespread occurrence, the functional consequences of epithalamic asymmetry upon animal behaviour remain largely unknown.

4.1 Asymmetry of habenula

The habenula and its associated fibre tracts form a part of a conserved conduction system linking the forebrain and the ventral midbrain (Butler & Hodos, 1996). The habenula of lampreys (Yaňez & Anadòn, 1996), for example, contributes to a system of projections between nuclei of the caudal telencephalon and the interpeduncular nuclei of the ventral midbrain. Similar projections form a subset of the much more complex set of connectivities found in amniotes (Herkenham & Nauta, 1977, 1979; Diaz & Puelles, 1992*a,b*). Indeed, it has been proposed that the habenula of lampreys and teleosts is homologous to the medial component of the habenula of lizards and mammals (Yaňez & Anadòn, 1994, 1996). The lateral habenula may be a late acquisition in the evolution of Vertebrates, perhaps reflecting the increasing importance of cortical circuits in amniotes (Yaňez et al. 1996). The habenula in mammals facilitates functional interactions amongst neural structures in the limbic forebrain and the midbrain (Wang & Aghajanian. 1977; Sutherland, 1982), and roles have been proposed in olfactory responses, mating and feeding behaviours, in the generation of sleep patterns, secretion of hormones (noradrenaline, adrenaline, corticosterone), in the response to stress, and in avoidance learning (reviewed in Sandyk, 1991). In support of a role in reproductive behaviour, asymmetries of

the habenula show sex- and seasonal-dependent variations in some species.

4.2 Chondrichthyes

In all previous studies, in almost all species of cartilaginous fishes examined, the habenula is enlarged on the left side (Kemali & Miralto, 1979; Smeets et al. 1983). One exception to this rule is the dogfish *Scyliorhinus canicula* in which contradictory reports have placed the enlarged nucleus on either the left (Farner 1978; Smeets et al. 1983; Rodriguez-Moldes et al. 1990) or the right (Anadòn et al. 2000). Asymmetry of the habenula extends to neuronal organisation and fibre myelination (Kemali et al. 1980; Miralto & Kemali 1980; Smeets et al. 1983), and to the distribution of the calcium-binding protein calbindin-D28k (Rodriguez-Moldes et al. 1990). Whereas the habenula on the right contains small densely packed cells and lacks calbindin-D28k-immunoreactivity, on the left it is organised into a nucleus medialis, of densely packed cells similar to the right nucleus but showing calbindin-D28k-immunoreactivity, and a nucleus lateralis consisting of larger neurons associated with myelinated fibres.

4.3 Osteichthyes

The bony fishes constitute by far the largest class of extant vertebrates encompassing 2 subclasses: the Actinopterygii or ray-finned fishes, and the Sarcopterygii or fleshy-finned fishes (Meek & Nieuwenhuys, 1998). Actinopterygian fishes can be further subdivided into 5 major radiations: Cladistia, Chondrostei, Ginglymodi, Halecomorphi and Teleostei (Lauder & Liem, 1983). In the Cladistia, or Brachipterygii (arm-finned fishes), the habenula displays a marked asymmetry as the right side is larger and contains a wider layer of tightly packed neurons than the left (Nieuwenhuys & Bodenheimer, 1983). In most species of Chondrostean fishes the habenula is markedly enlarged

on the right, although the opposite is observed in *Polyodon* (Hocke Hoogenboom, 1929). In the siberian sturgeon *Acipenser baeri*, more choline-acetyltransferase (ChAT) immunoreactive (ir) fibres but less ChAT-ir cells are observed on the right than on the left side of the habenula. Furthermore, efferents coursing in the right fasciculus retroflexus are more abundant in number and larger in caliber than in the left fasciculus, and are also immunoreactive to ChAT (Adrio et al. 2000). In other ganoids (non-teleost actinopterygian fishes) like the longnose gar *Lepisosteus osseus* (Ginglymodi) (Braford & Northcutt, 1983) and the bowfin *Amia calva* (Halecomorphi)(Meek & Nieuwenhuys, 1998) the habenula is somewhat enlarged on the right, and this asymmetry is again reflected by an enlarged right fasciculus retroflexus (Braford & Northcutt, 1983).

Within the teleosts, studies of habenular cytoarchitecture have been done in species belonging to three of the major subdivisions (Lauder & Liem, 1983). The habenula is described as symmetric is Osteoglossomorpha (*Pantodon bucholzi*: Butler & Saidel, 1991), Clupeomorpha (*Clupea harengus*: Butler & Northcutt, 1993) and in most Euteleostei (*Fundulus heteroclitus*: Peter et al. 1975; *Carassius auratus*: Peter & Gill, 1975: Braford & Northcutt, 1983; *Haplochromis burtoni*: Fernald & Shelton, 1985; *Ictalurus punctatus*: Striedter, 1990; *Coris julis, Syngnathus acus, Gasterosteus aculeatus, Pleuronectes platessa, Gaidropsaurus mediterraneus*: Gòmez-Segade & Anadòn, 1988; *Apteronotus leptorhynchus*: Maler et al. 1991). A few exceptions to bilateral symmetry, however, have been reported within euteleosts.

The habenula in this group can be subdivided into a ventral nucleus of densely packed small cells, and a dorsal nucleus of larger, more loosely packed neurons arranged in strands (Meek & Nieuwenhuys, 1991). In the eel *Anguilla anguilla* (Braitenberg & Kemali, 1970), the coho salmon *Oncorhynchus kisutch* (Ekström & Ebbesson, 1988) and the

larval zebrafish *Danio rerio* (Concha et al. 2000), the habenula is enlarged on the left. Besides this difference in size, the left dorsal habenula is more lobate than the right dorsal nucleus in the eel (Braitenberg & Kemali, 1970), contains a distinct serotoninergic subnucleus in the coho salmon (Ekström & Ebbesson, 1988) and shows an enlarged neuropil in the larval zebrafish (Concha et al. 2000). Although the enlarged neuropil regions of zebrafish are likely to arise from afferent projections reaching the habenula through the stria medullaris, a possible involvement of local habenular circuits has not been discounted. A reversed direction of asymmetry has been decribed in some species of salmonids in which the habenula is described as "somewhat" larger on the right and having a looser arrangement of neurons than on the left (Holmgren, 1920; Yañez & Anadòn, 1996).

It is commonly believed that all terrestrial vertebrates have evolved from the sarcopterygian group, which is constituted by the Dipnoi, or lungfishes (Nieuwenhuys, 1998*d*) and the Crossopterygii, or tassel-finned fishes. In lungfishes, a slightly enlarged habenula on the right is described in some species (Schnitzlein & Crosby, 1968; Nieuwenhuys, 1998*d*) although in some others the habenula is reported as being symmetric (*Neoceratodus forsteri*: Holmgren & Van der Horst ,1925). In Crossopterigyan fishes, on the other hand, asymmetry of the habenula is clearly observed in the coelacanth *Latimeria chalumnae* where the left side is enlarged (Nieuwenhuys, *1998c*).

4.4 Amphibia

Asymmetry in the habenula of modern amphibians has been described in species belonging to the orders Urodela (newts and salamanders) and Anura (frogs and toads). The habenula in both orders can be subdivided into dorsal and ventral nuclei (ten Donkelaar, 1998*a,c*), but asymmetry is only observed in the dorsal nucleus. In

the newt *Triturus cristatus*, neurons of the left dorsal habenula organise into a layer that extends far more laterally than in the right dorsal nucleus thus defining an enclosed region poor in nuclei (Braitenberg & Kemali, 1970). In contrast to this single description of asymmetry in urodeles, the habenula of anuras and in particular that of the frog *Rana asculenta* is probably the most extensively studied example of epithalamic asymmetry in vertebrates. The left dorsal habenula of anuras is considerable larger than the right dorsal nucleus (Frontera, 1952; Braitenberg & Kemali, 1970; Morgan et al. 1973), a feature that shows both seasonal and sex-dependent variations. Furthermore, while neurons in the right dorsal habenula are distributed around a single region of neuropil, a more complex assemblage of subdivisions is observed in the left dorsal habenula (Gaupp et al. 1899; Röthing, 1923; Braitenberg & Kemali, 1970; Morgan et al. 1973; Guglielmotti & Fiorino, 1999). The left dorsal habenula is divided into a lateral subnuclues similar in structure to the right dorsal habenula, and medial subnucleus showing unique features (Braitenberg & Kemali, 1970). This medial subnucleus can be further compartimentalized into a medial and a lateral neuropil based on cytoarchitecture (Guglielmotti & Fiorino, 1999), ultrastructure (Kemali & Guglielmotti, 1977), histochemistry (NADPH-diaphorase: Guglielmotti & Fiorino, 1999), immunohistochemistry (substance-P: Kemali & Guglielmotti, 1984; melatonin receptor expression: Wiechmann & Wirsing-Wiechmann, 1993) and connectivity (Guglielmotti & Fiorino, 1998).

Importantly, habenular asymmetry appears to be established early in development and probably originates from afferents projections, as suggested by the correspondance between the increased nitric oxide synthase (NOS) activity found in neuropil of the left medial habenula at the same stage that NOS(+) cells are detected in areas of the forebrain known to project to the habenula (Guglielmotti & Fiorino, 1999).

4.5 Reptilia

The habenula has been reported as asymmetric in some species of lizards (e.g. *Lacerta sicula*: Kemali & Agrelli, 1972: *Uta transburiana*: Engbreston et al. 1981) (Fig. 1) but appears symmetric in others (e.g.*Tupinambis nigropunctatus*: Cruce, 1974), and in species of turtles (ten Donklaar, 1998b), ophidians (Nagasaki, 1954) and crocodiles (Huber & Crosby, 1926; Tamura et al. 1955). The habenula of the lizard *Uta transburiana* (Engbreston et al. 1981), as in other species of reptiles (Butler & Northcutt, 1973), can be subdivided into a lateral nucleus containing scattered cells, and a medial nucleus with linear arrays of cells arranged around cell-free regions. As in amphibians, asymmetry is restricted to one of these subdivisions, the medial nucleus, and involves a further compartimentalisation of the medial nucleus into 2 components, denominated pars dorsolateralis and pars ventromedialis. While the pars ventromedialis displays a cytoarchitecture similar to the right medial habenula, the pars dorsolateralis has unique cytoarchitectonic (Kemali & Agrelli, 1972; Engbretson et al. 1981), connectional (Engbretson et al. 1981; Korf & Wagner, 1981) and immunohistochemical (Substance-P: Engbretson et al. 1982) featrures. Volumetric studies have shown that the presence of the pars dorsolateralis in the left medial habenula of the lizard *Uta transburiana* accounts for the marked (60%) difference in size between the left and right nucleus (Engbretson et al. 1981).
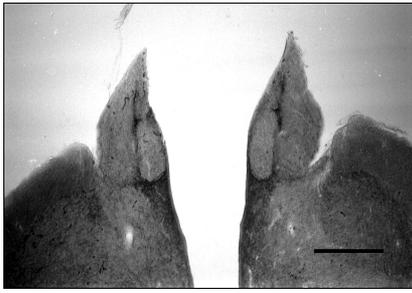
Fig. 1: habenular nuclei in *Lacerta sicula*

Fig. 3: habenular nuclei in *Rattus norvegicus*

4.6 Aves and Mammalia

Reports of habenular asymmetry in birds and mammals are scarce. Overall, the habenula in these classes appears symmetric. However, subtle differences between the right and the left habenula can be detected when using quantitative volumetric studies. In the chick, a sex-dependent asymmetry of the medial component of the habenula is observed.

On the other hand, the development of some encephalic regions should stop the extention of habenula (Fig. 2)

Fig. 2: brain of bird, longitudinal section.

In mammals, analyses of the developing and mature habenula in two different species reveal contrasting results. While in albino rats the medial habenula is slightly but significantly enlarged on the left (Wree et al. 1981), an enlarged right lateral habenula is observed in albino mice (Zilles et al. 1976) (Fig. 3). Asymmetry in neuronal organisation has also been detected in the macrosmatic mole in which a row of dark cells is seen lying along the lateral border of the habenula only on the left (Kemali, 1984).

4.7 Asymmetry of the pineal complex

The pineal complex is formed by either one or two evaginations from the roof the diencephalon, known as pineal and parapineal organs. The pineal organ has been described in almost all species of vertebrates and appears to show little sign of major asymmetry. The parapineal organ, on the other hand, is much less conserved in evolution but shows asymmetric connectivity and is sometimes also asymmetrically positioned within the epithalamus.

A parapineal organ is described in lampreys, the bowfin, teleosts, the coelacanth, and in some reptiles, and is absent (at least in adult stages) in other extant vertebrate groups such as hagfishes, cartilaginous fishes, amphibians, birds and mammals.

The pineal complex in the bowfin *Amia calva* (Halecomorphi) and in teleosts is generally situated beneath the roof of the skull, although in a few species of extant teleosts it emerges from a foramen in the skull to reach a position underneath the skin (Steyn & Webb, 1960). In addition to the prominent pineal organ, which contains photoreceptors, supporting and other neuronal cells and serves a photoneuroendocrine role (rewieved in Ekström & Meissl, 1997), an asymmetrically positioned parapineal organ has been described in the bowfin (Hill, 1894; Kingsbury, 1897) and in many teleost species (for a list of species see Borg et al. 1983; plus Vigh-Teichmann et al 1991, Concha et al. 2000). Both pineal and parapineal organs originate during

embryogenesis as evaginations of the diencephalic roof plate, the pineal developing earlier and in a more posterior position than the parapineal (Hill, 1891; Eycleshymer & Davis, 1897). While the pineal organ preserves its median connection with the diencephalon, the parapineal organ appears to move laterally (Holmgren, 1965) to become positioned caudal to the left habenula in the horizontal plane of the habenular commissure (Borg et al. 1983; Concha et al. 2000). A further movement of the parapineal organ often takes place to situate it posterior to the pineal stalk in the adult (Holmgren, 1965). Parapineal cells display ultrastructural features of rudimentary photoreceptors (Rüdeberg, 1969; van Veen, 1982; Ekström et al. 1983), and in some cases show immunoreactivity to the visual proteins opsin (Vigh-Teichmann et al 1980, 1983, 1991; Ekström et al. 1987; Concha et al. 2000), S-antigen (Ekström et al. 1987) and transducin (van Veen, 1986; Ekström et al. 1987).

Unmyelinated nerve fibres emanate from the parapineal organ and constitute the parapineal tract, which courses towards the left medial habenula (Rüdeberg, 1969; van Veen, 1980; van Veen, 1982; Concha et al. 2000) terminating in a well defined rostrodorsal field (*Oncorhynchus mykiss*: Yañez et al. 1996). This terminal field appears to correspond to the serotoninergic subnucleus described in the left medial habenula (*Oncorhynchus kisutch*: Ekström & Ebbesson, 1988). The arrangement of rudimentary photosensory cells and nerve tracts suggests a role, although perhaps rather rudimentary, in photoreception.

In the coelacanth *Latimeria chalumnae,* pineal and parapineal organs occupy a deep position in the head covered by adipose tissue and by the roof of the skull. They form a pair of saccular vescicles in open communication with each other and with the diencephalic ventricle. A photoreceptive function of both pineal and parapineal organs is suggested by the presence of photoreceptors, supporting and other neuronal cells (Hafeez & Merhige, 1977). In contrast to what is seen in teleosts, the parapineal in the coelacanth appears as the more substantial organ within the pineal complex (Hafeez & Merhige, 1977).

MATERIALS AND METHODS

1. Animals for experiments

Some animals were obtained from local commercial sources, some other animals were caught during cruises. The catch in the larger net often consisted of bigger specimens, which arrived on deck mostly moribund or dead; some species, as the deep demersal eel, *Synaphobranchus kaupi*, were collected at a depth of ~2000 m on a cruise aboard *RRS Discovery* (255) to the Porcupine Seabight and abyssal plan in the Eastern North Atlantic.

The sample considered in my analysis comprises specimens from 29 species, summarized inTable 1.

CLASS	ORDER	FAMILY	SPECIES
Chondrichthyes	Squaliformes	Squalidae	*Squalus acanthias*
			Centrophorus granulosus
	Lamniformes	Carcharhinidae	*Mustelus mustelus*
		Scyliorhinidae	*Scyliorhinus canicula*
			Scyliorhinus stellaris
			Galeus melastomus
	Orectolobiformes	Hemiscyllidae	*Chiloscyllium arabicum*
			Chiloscyllium griseum
	Raiformes	Rajidae	*Raja asterias*
			Raja miraletus
			Raja montagui
			Bathyraja sp.
		Myliobatidae	*Myliobatis aquila*
	Chimaeriformes	Chimaeridae	*Hydrolagus mirabilis*
Osteichthyes	Anguilliformes	Anguillidae	*Anguilla anguilla*
		Synaphobranchidae	*Synaphobranchus kaupii*
	Perciformes	Sparidae	*Diplodus sargus*
			Boops boops
		Mugilidae	*Liza aurata*
		Scombridae	*Scomber scombrus*
		Thunnidae	*Euthynnus alletteratus*
	Pleuronettiformes	Scophthalmidae	*Lepidorhombus bosci*
		Soleidae	*Solea vulgaris*
	Gadiformes	Gadidae	*Trisopterus minutus*
			Micromesistius potassou
		Macrouridae	*Coryphaenoides armatus*
	Cipriniformes	Cyprinidae	*Danio rerio*
	Osteoglossiformes	Notopteridae	*Xenomystus guentheri*
		Mormiridae	*Gnathonemus petersi*

Table 1: List of species used

After identification the heads were fixed in 4% formalin in phosphate buffered saline (2-12 hours, depending on the size of the specimen).

Under a dissection microscope, the heads were prepared in order to expose the diencephalic area, and photographs of the brain were taken on a Zeiss Stereomicroscope equipped with a digital camera (Figure 1).

The original research reported herein was performed under the guidlines established by Italian law, under the control of a local bioethical committee and the supervision of a veterinary commission for animal care and control.

a

b

c

d
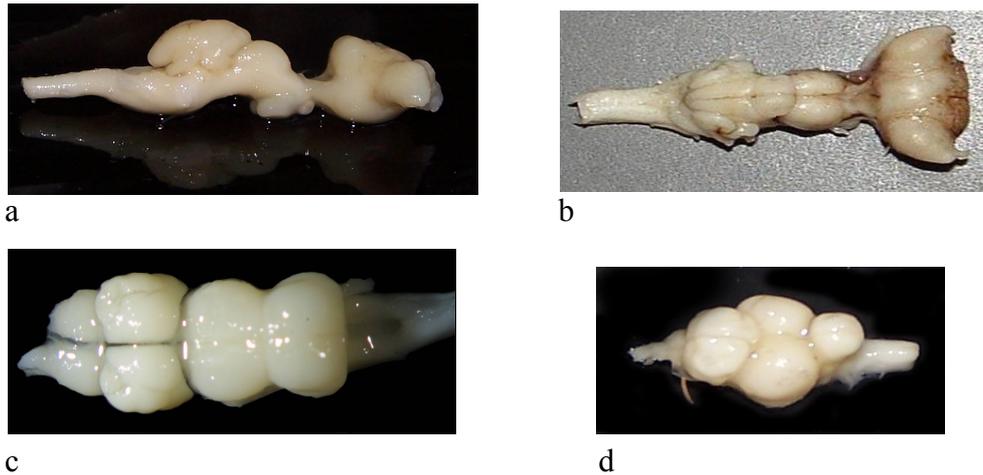
Figure 1: lateral view of *Scyliorhinus canicula*'s brain (a) and dorsal view ofbrains of *Raja montagui* (b), *Anguilla anguilla* and *Diplodus sargus* (d).

2. Histology

Brains removed from the animals have been fixed using 4% paraformaldehyde in phosphate buffer; subsequently the brains have been dehydrated with alchools and embedded in paraffin. Serial transverse sections were cut at 7-8 micron on a rotary microtome. The slides have been stained with the Giemsa method or with Ematoxilin-Eosin method.

The brain most symmetrically sectioned was chosen for documentation and representative sections were selected and photographed. The boundaries of cell masses and fiber tracts were drawn on photographs, copied into a transparent paper, and digitized.

3. Connections analysis: DiI and fluorescent tracers

Lipophilic carbocyanine dyes are useful for tracing neural connections because these dyes diffuse along cell membranes both in vivo and in fixed tissue (Godement et al., 1987; Holmqvist et al., 1992). In some studies about *A. baeri* (Huesa et al., 2000, 2003), has been demonstrated that DiI is a very sensitive

tracer and the results are both specific and reproducible. However, the diffusion of DiI along axonal branches is a drawback of this method, because it does not unequivocally reveal the origin of labeled fibers due to the mixed (anterograde/retrograde) labeling of axons via axon collaterals. Another feature of

this method is that labeling is done by dissolution of DiI in the lipidic bilayer, so that the effective area of labeling is limited by the size of the crystals applied. The small size of the DiI crystals used has the advantage that the point of application is well defined, the area of DiI uptake is small, and the results are highly reproducible; but the number of cells and processes actually labeled is limited by the crystal size (for further discussion, see Folgueira et al., 2004a).

The brain case was opened and the head was left in 4% paraformaldehyde in PB for 1–3 hours. Brains were then excised from skulls and stored in fresh fixative for 4 hours at 4°C. For tracing, the lipophilic tracer 1,1'-dioctadecyl 3,3,3',3' tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Eugene, OR) was applied to the dried brain, with the aid of a sharpened 000 insect pin: several small crystals of DiI were inserted into the left habenula (Fig. 2). After application to the structure, as described above, the brains were stored in fresh fixative for 20–60 days at 37°C in darkness. After this time, some brains were embedded in gelatin, cut with a cryostat, mounted on gelatinized slides with PB and coverslipped; some other brains were cryoprotected with 30% sucrose, embedded in OCT Compound (Tissue-Tek, Redding, CA), frozen with liquid-nitrogen-cooled isopentane and cut with a cryostat (35-40 μm). Sections were immediately observed.

A rhodamine-type filter set, applied to a Zeiss fluorescence microscope, was used to visualize the DiI distribution, and samples were photographed on Kodak TMAX 400 film.

Fig. 2: dorsal view of two brains after DiI insertion

4. Immunohistochemical analysis: Calbindin D 28k

Intracellular messengers mediate the effects of neurotransmitters on intracellular events. Targets of these messengers include certain intracellular calcium-binding proteins (CaBPs) (Cheung, 1980). These proteins are probably indirectly related to neurotransmission. CaBPs are a group of homologous proteins with a characteristic structure, consisting of pouches for the acceptance of Ca2+.

CaBPs are considered to be selective markers of nerve cells, and calretinin (CR), calbindin D-28k (CB) and parvalbumin (PV) are the most relevant and specific to the nervous tissue (Andressen et al., 1993). These proteins belong to the so-called ''EF-hand'' family of CaBPs, which has more than 150 different members. They are particularly

interesting from a morphological point of view, since they occur only in certain subpopulations of nerve cells in the CNS and peripheral nervous system (Garcia-Segura et al., 1984; Braun, 1990; Rogers et al., 1990).

Many Ca2+-dependent cellullar functions, such as neurotransmission and contraction, are known to be mediated by proteins able to bind calcium ions. However, their mechanisms of action are hardly understood. A calcium-binding protein whose synthesis is dependent on the availability of vitamin D was first demonstrated in the chick duodenal mucosa (Wasserman and Taylor, 1966) and later in other absorptive epithelia, suggesting its involvement in the membrane transport of calcium. Since its first demonstration, a plethora of names has been given to this vitamine-D-induced calcium-binding protein. However, the name of calbindin-D-28k is now generally applied (Wasserman, 1985). Calbindin-D-28k appears to be restricted to selective groups of neurons. Its neuronal distribution might indicate more specific functions than merely the membrane transport of Ca2+, such as the regulation of the intracellular calcium level in relation with neurotransmission (Jande et al., 1981a; Baimbridge and Miller, 1982; Celio and Norman, 1985) or with the production and maintenance of cytoskeletal elements (Rabiè et al., 1983; Enderlin et al., 1987). In birds and mammals, the synthesis of CB in the central nervous system is not necessarily mediated by vitamin D (Taylor, 1974; Christakos et al., 1979; Baimbridge and Parkes, 1981), and in fishes, no functional role for vitamin D in the calcium metabolism has yet been established (Feinblatt, 1982).

The fact that the presence of CB in fishes has so far only been demonstrated in the central nervous system may indicate that its appearance in other tissue could be a secondary acquisition related with the evolution of higher vertebrates (Parmentier et al., 1987).

After sacrifice by decapitation, brains were removed, fixed in 4% paraformaldehyde in PB, and postfixed in the same solution between 12 hours and 5 days. The brains were then cryoprotected with 30% sucrose, embedded in OCT Compound (Tissue-Tek, Redding, CA) and frozen with liquid-nitrogen-cooled isopentane. Serial transverse sections (20 μm thick) were cut on a cryostat and mounted on gelatin-coated slides. Cryosections were sequentially rinsed once in PBS pH 7.4 (10 minutes) and preincubated with pre-incubation serum (NGS; Sigma) in 0.3% Triton X-100 in PBS (PBS-T) for 60 minutes. The sections were then incubated with a monoclonal anti-Calbindin28kD antibody (Sigma; dilution 1:2,000) for 12 hours at 4°C. After 3 PBS washes, sections were incubated for 2 hours with goat antimouse serum (Sigma; dilution 1:1,000). Control sections in which the primary antibody was omitted showed no staining, confirming specificity.

Slides were coverslipped with Mowiol and the material was examined under a light microscope.

5. Electron microscopy

The dissected habenulae were fixed in a mixture of 4% paraformaldheyde, 2% glutaraldheyde in 0.1 mol 1^{-1} cacodylate buffer (Karnovsky, 1965). After 3 washes in buffer phosphate, they were post-fixed in 1% osmium tetroxide for 2 hours, blockstained with 1% uranyl acetate and embedded in Epon. Short series of 1-2 μm sections were cut alternating with ultrathin (80 nm) sections. Digital micrographs were obtained with a Zeiss Axioskop and a LEO EM912.

Fig.3: Dorsal view of two skulls, almost dissected for the electron microscopy

RESULTS

1. Histology

The diencephalon is probably the most complex region in the brain of fishes. It comprises centers for neural integration of gustatory information, olfaction, reproduction, and vision (Braford and Northcutt, 1983; Northcutt and Wullimann, 1988; Wullimann, 1988). An important contribution towards the understanding of diencephalic organization was achieved by Braford and Northcutt (1983). These authors studied the interspecific variations of the different cell groups recognized in both teleost and non-teleost forebrains and proposed a terminology for cell masses that has became the standard nomenclature for morpho-functional studies on the diencephalon of fishes (See below). As in other vertebrate radiations, the diencephalic organization varies considerably among ray-finned fishes (Braford and Northcutt, 1983; Northcutt and Wullimann, 1988; Wullimann, 1988). Furthermore, interspecific differences appear to occur preferably in some diencephalic regions, as the posterior tuberculum and pretectum, which represent the most variable areas among teleosts (Striedter, 1990a).

Abbreviations	
Cgl	Corpus geniculatum laterale
chab	habenular commissure
chopt	optic chiasma
cpo	postoptic commissure
epiph	epiphysis
fbt	fasciculus basalis telencephali
Hab	Habenular nuclei
lih	inferior lobe of hypothalamus
NCLI	Central Nucleus of the Inferior Lobe
ND	Diffuse Nucleus
Ndm	dorsomedial Nucleus
Nl	Lateral Nucleus
NM	Nucleus Mammillare
NPG	Preglomerular Nucleus
NRP	Nucleus of the Posterior Recess
NSG	Pseudoglomerular Nucleus
Nvm	ventromedial Nucleus
RL	Lateral Recessus
sdm	sulcus diencephalicus medius
ssh	subhabenular sulcus
sth	sulcus thalamus hypothalamicus
T	Telencephalon
TeO	Optic Tectum
thdm	pars medialis thalamus dorsalis

thvl	pars lateralis thalamus ventralis
thvm	pars medialis thalamus ventralis
TL	Torus Lateralis
topt	optic tract
tpal	tractus pallii
tpohyp	tractus preopticus hypophysarius

This analysis of the diencephalon allowed to recognize the subdivisions already described by some authors (Braford and Northcutt, 1983): the preoptic area, ventral thalamus, dorsal thalamus, epithalamus, hypothalamus, posterior tuberculum, synencephalon, pretectum, and accessory optic nuclei.

Several subnuclei of the habenular complex were identified in sections stained with Giemsa; Fig. 6 shows a series of sections through the habenular nuclei. The cut has been effectuated exactly at right angle to the long axis of the brain.

It is clear that the left habenula is lager and longer than the right habenula. When the two habenulae start to fuse together to form a bridge over the third ventricle, a midline nucleus appears while the peduncle of the epiphysis, which in the previous sections is lying over the habenular nuclei, fuses with them forming the shape of a triangle.

In the most caudal sections the two habenulae are again separated in a right and a left portion while on their top the peduncle of the epiphysis seems to continue with the commissural organ.

A quantitative analysis about diameters of the cell nuclei revealed that the largest cells are situated in the lateral portion of the left habenula; thickly myelinated bundles of fibers traverse the middle of the left habenula. In the right habenula a few myelinated fibers were observed in its lateral edge.

My results confirm data obtained studying some Elasmobranchs species (*Chiloscyllium arabicum*, *Myliobatis Aquila*) (Minelli D. et al., 2003), in which in general the left habenula is larger than the right one; in the course of the studies, a correlation between the habits of life and the diencephalic asymmetry seems to emerge: among the Teleostean analyzed, the species with benthic life (as *Lepidorhombus boscii, Platichthys flesus, Solea vulgaris*) (Fig. 7a) seem to possess a slight asymmetry, analogous to the one of the Elasmobranchs, while in other species (*Liza aurata*, *Anguilla anguilla, Trisopterus minutus*) (Fig. 7b) the habenulae are symmetrical.

However, various aspects of the neuroanatomical asymmetries of the epithalamus have not been deepened in order to obtain a complete picture of the evolution of this phenomenon, and new searches are needed to examine the species without clear asymmetry, in order to understand the spread and the diversity of the asymmetry among the habenulae between the Vertebrates.
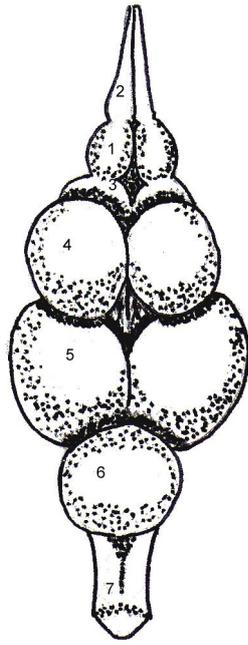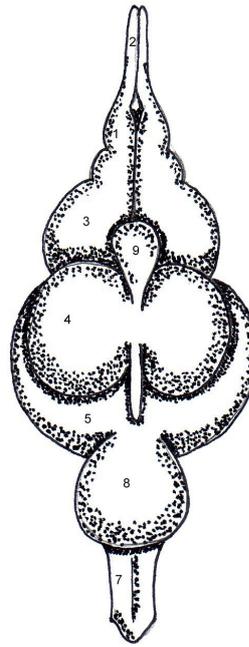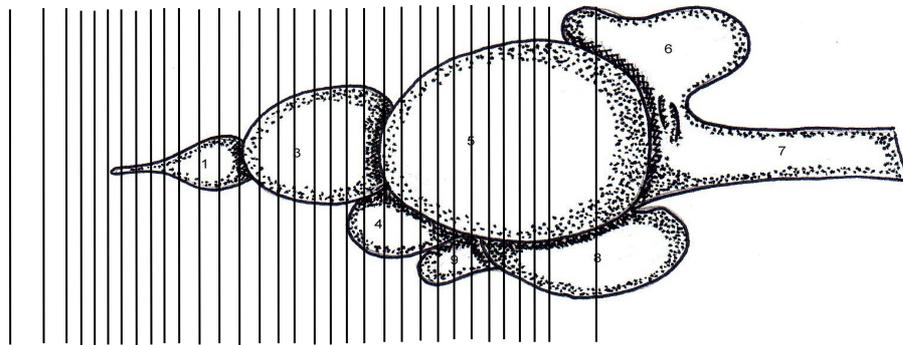
Fig.1 External vision of *Diplodus sargus*'s brain; dorsal, ventral and lateral.
　　　Notice in 1c lines indicating transversal serial sections.
　　　1. Olfactory lobe **2.** Olfactory tract **3.** Telencephalon **4.** Diencephalon **5.** Mesencephalon
　　　6. Cerebellum **7.** Brain stem **8.** Saccus vasculosus **9.** Hypophysis

+2400μm

0,5 mm
—

+2800μm
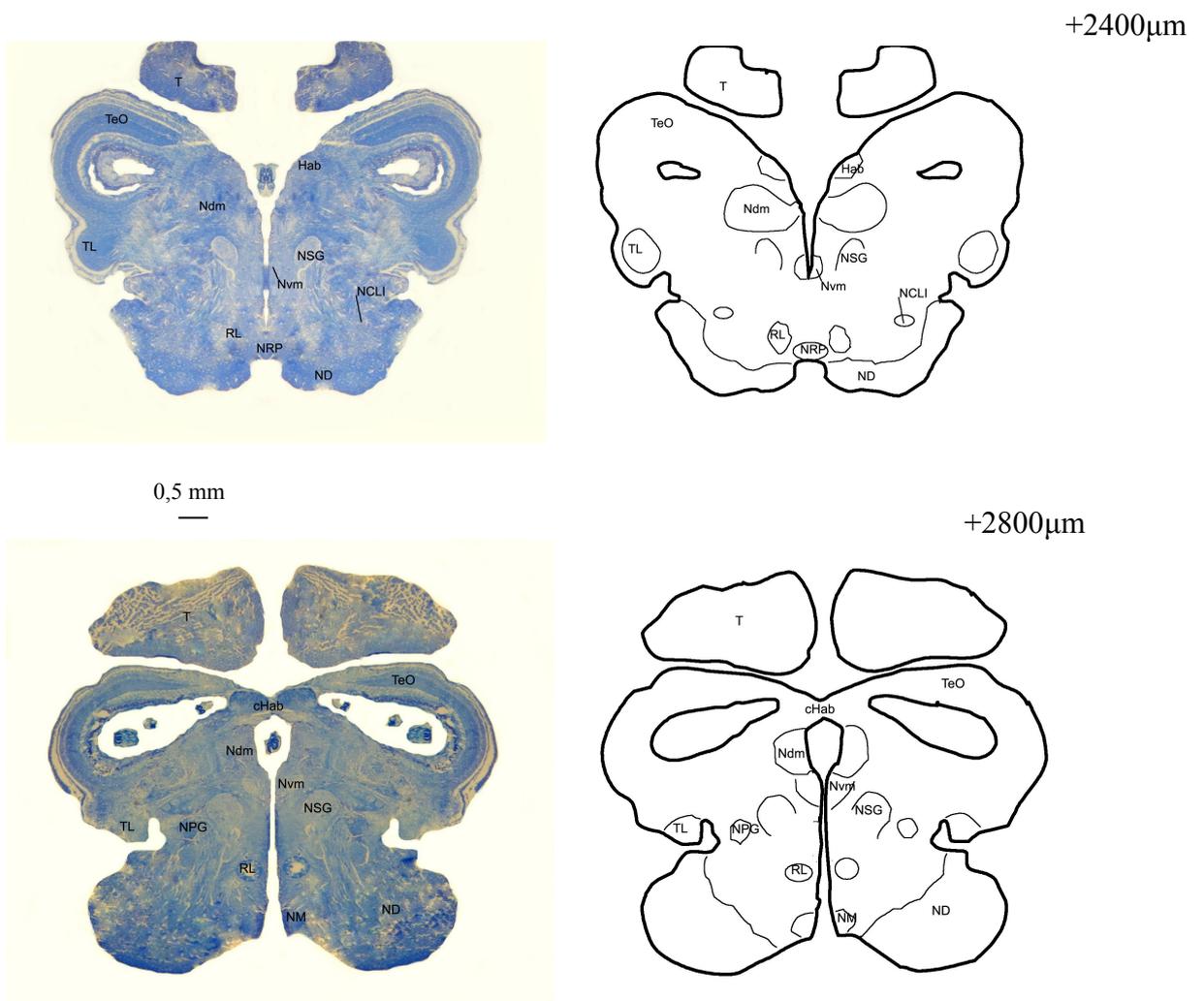
Fig.2 Trasversal sections of diencephalon of *Diplodus sargus*.
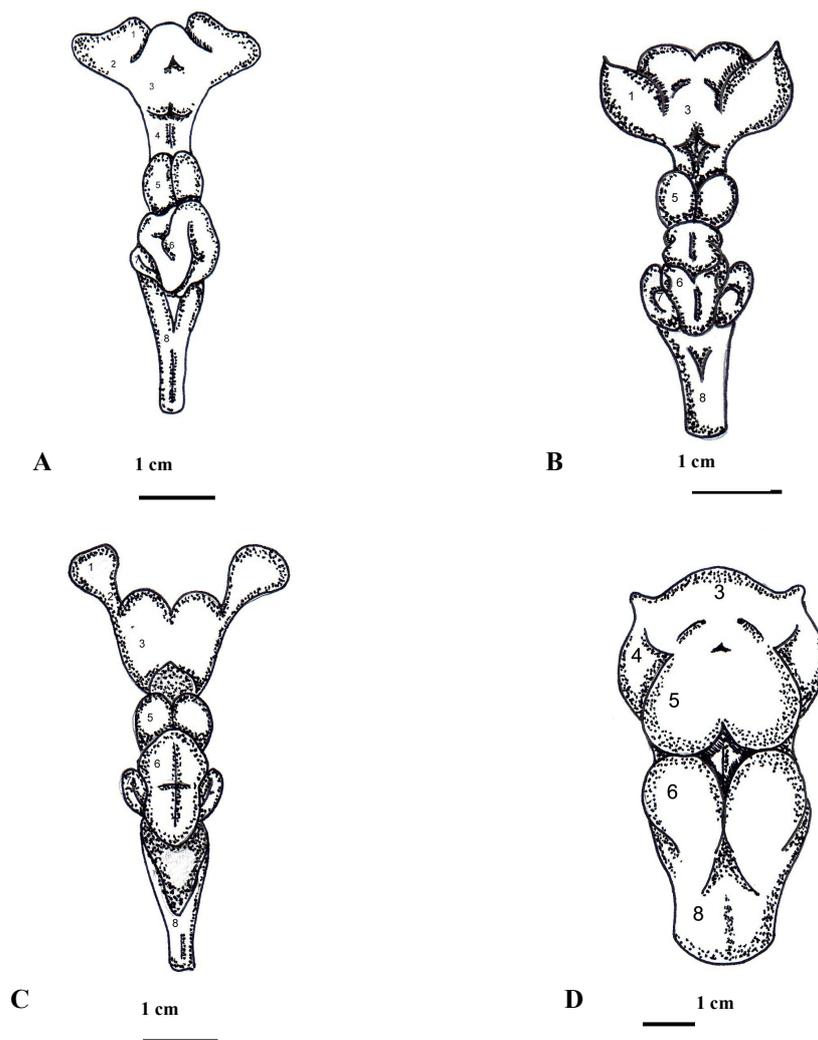
Fig.3 Dorsal vision of brain of some species studied: **A.** *Scyliorhinus canicula*; **B.** *Raja miraletus*;
C. *Squalus acanthias*; **D.** *Myliobatis aquila.*
1. Olfactory bulb **2.** Olfactory tract **3.** Telencephalon **4.** Diencephalon **5.** Mesencephalon
6. Cerebellum **7.** Auricola **8.** Brain stem
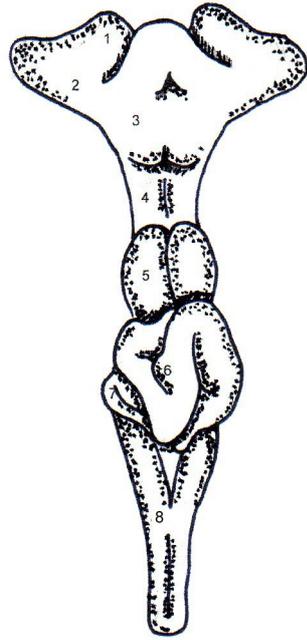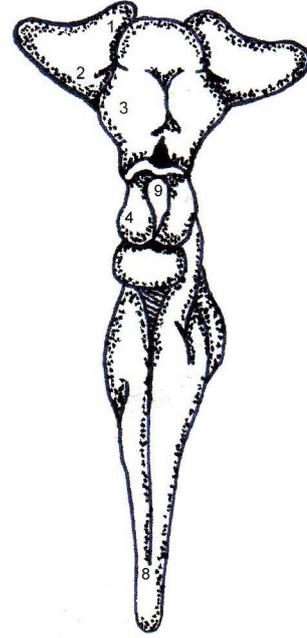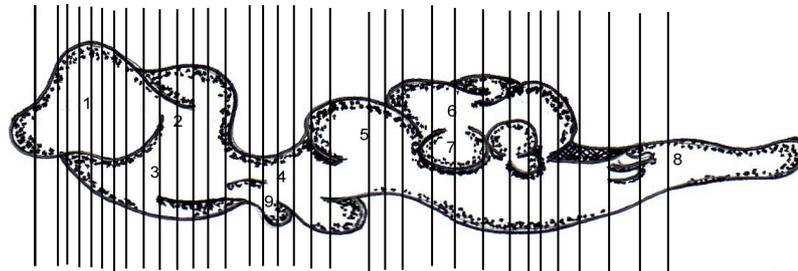
Fig. 4 External vision of *Scyliorhinus canicula*'s brain; dorsal, ventral and lateral.
Notice in 2c lines indicating transversal serial sections.
1. Olfactory lobe **2.** Olfactory tract **3.** Telencephalon **4.** Diencephalon **5.** Mesencephalon
6. Cerebellum **7.** Auricola **8.** Brain stem **9.** Hypophysis
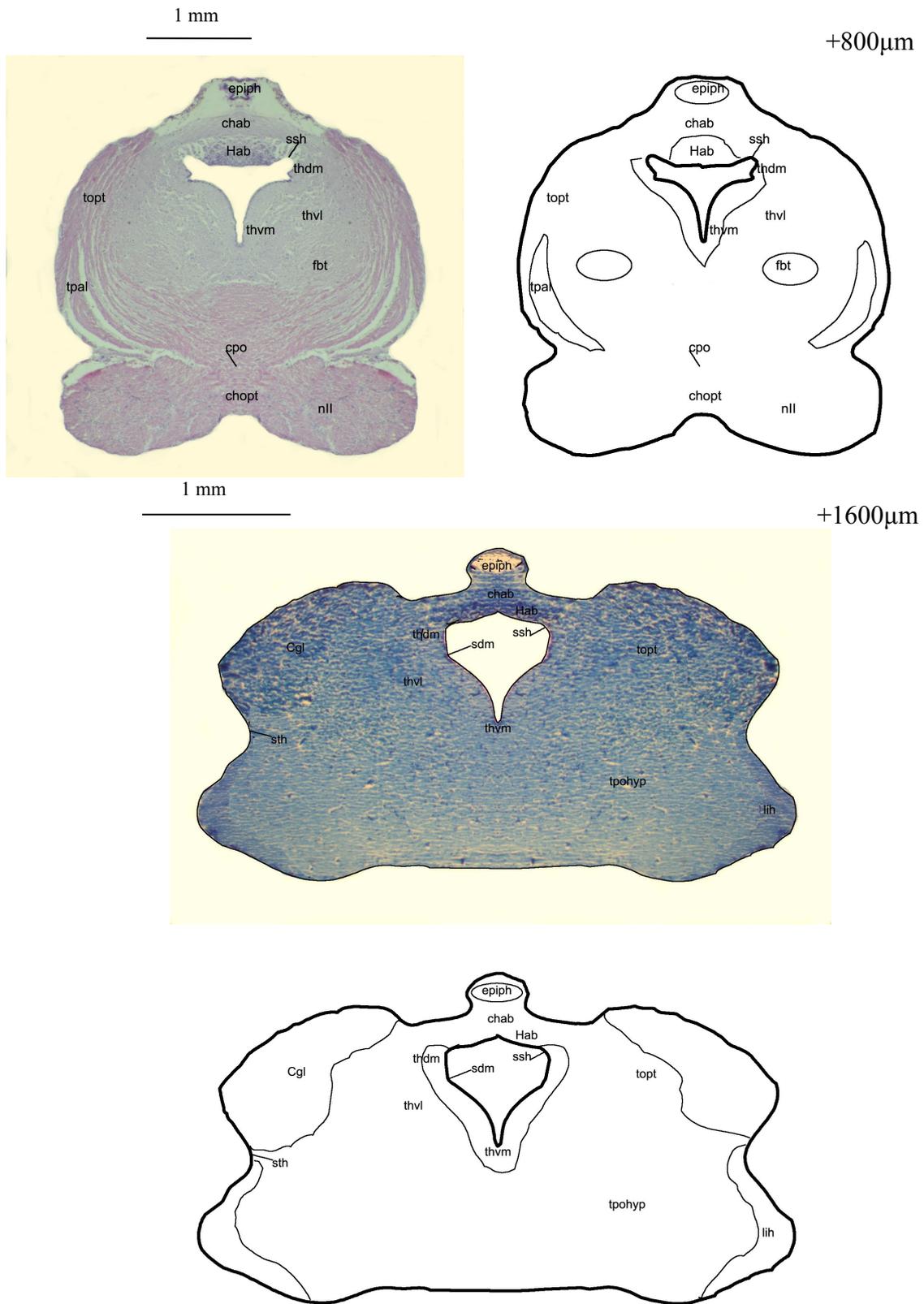
Fig. 5 Trasversal sections of diencephalon of *Scyliorhinus canicula.*
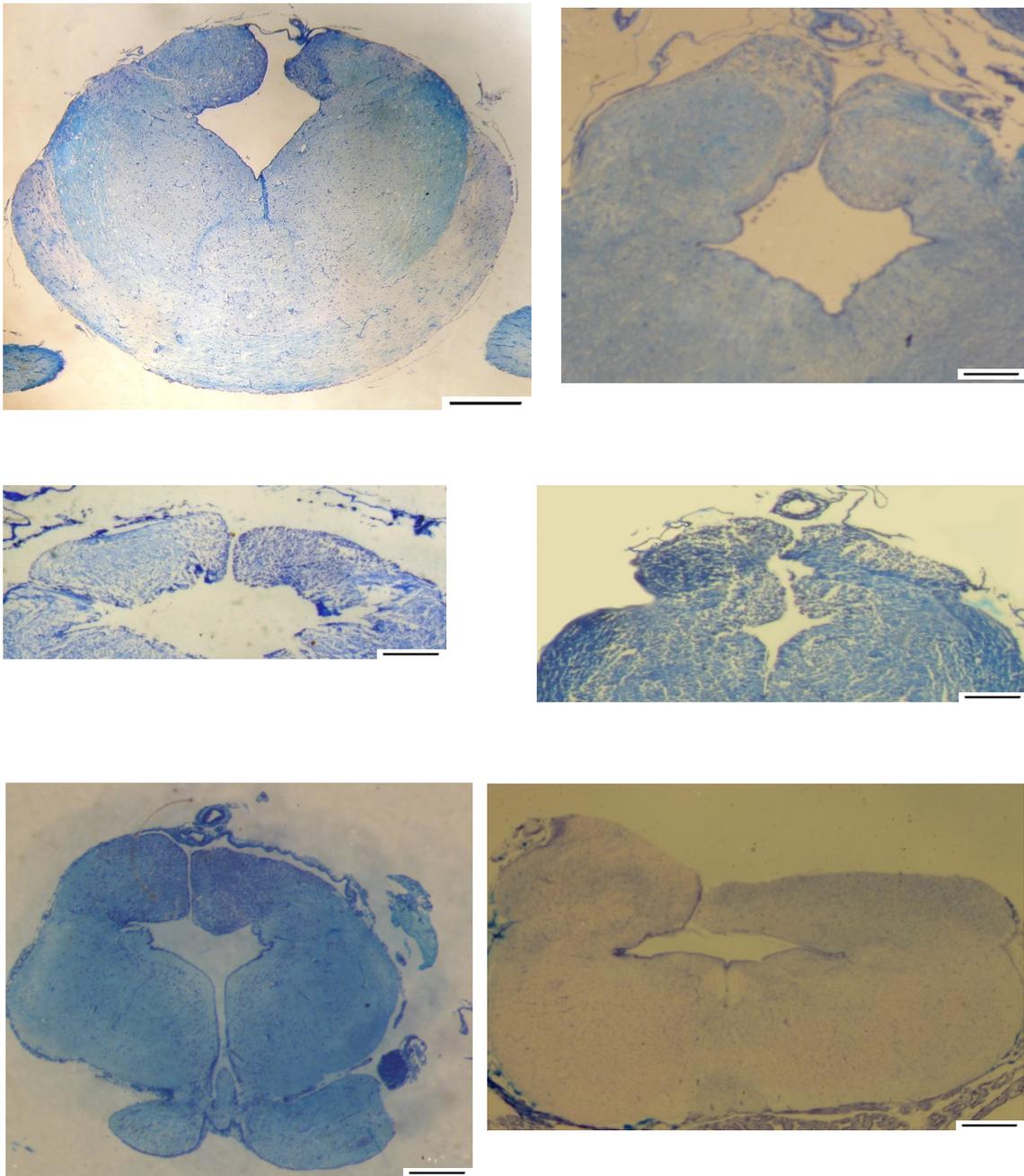
Fig. 6 Trasversal sections of diencephalon of some elasmobranch at habenulae level (*Chiloscyllium arabicum, Scyliorhinus canicula, Galeus melastomus, Centrophorus granulosus, Scyliorhinus stellaris* and *Squalus acanthias*).
Scale bar 10 mm = 100 micron
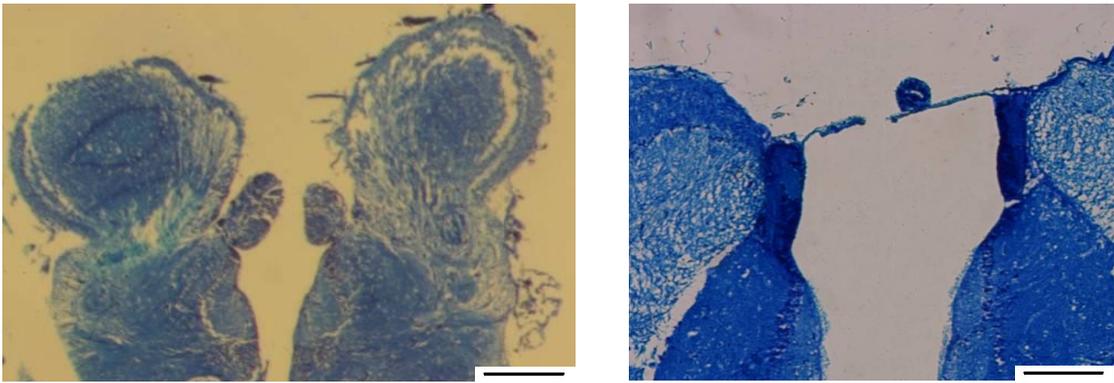
Fig. 7a Trasversal sections of diencephalon of *Solea vulgaris* and *Lepidorhombus boscii* at habenulae level.
Scale bar 10 mm = 100 micron
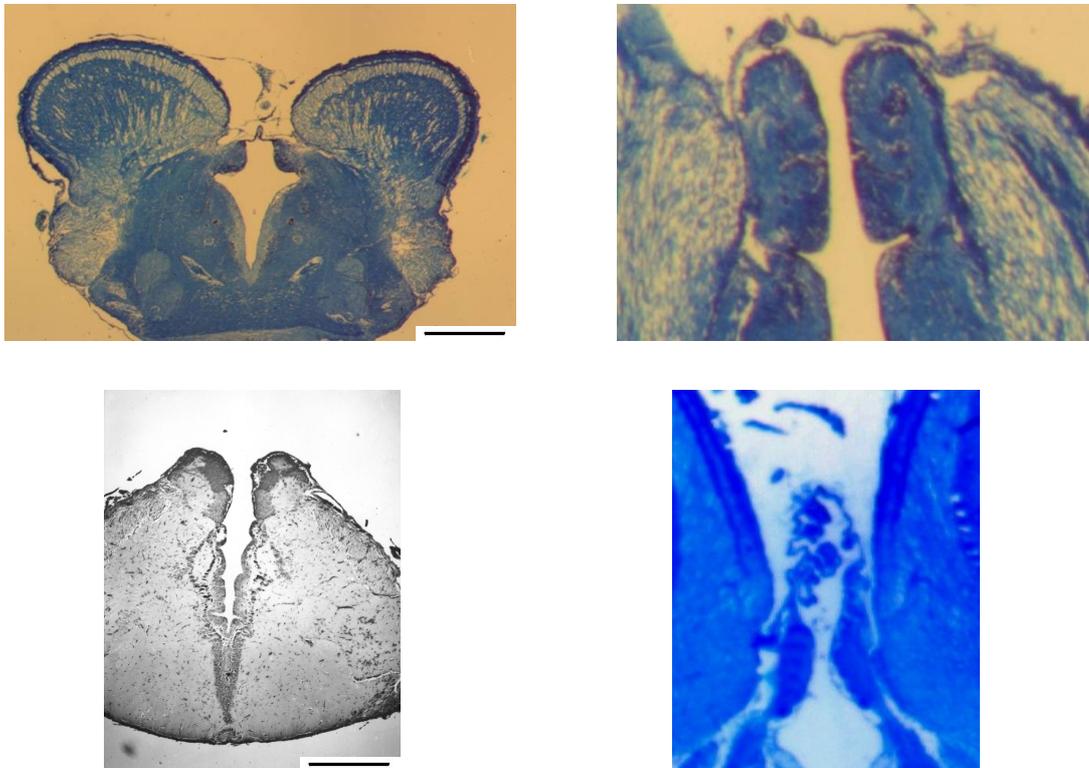
Fig. 7b Trasversal sections of diencephalon of *Trisopterus minutus*, *Anguilla anguilla, Liza aurata* and *Euthynnus alletteratus* at habenulae level.
Scale bar 10 mm = 100 micron

2. Connections analysis

The crystals of DiI were inserted into the left habenula by the dorsal surface of the fixed brain. Some small residual crystals of DiI were observed in the habenula after application, and the habenula was intensely labeled; the labeled habenular neurons were small-sized round cells with the fluorescent dye in the cytoplasm surrounding the nucleus. In the telencephalon, the retrogradely transported fluorescent dye was observed in a few fibres of the medial olfactory tract, and a very few cell bodies were faintly labeled. These neurons showed a round nucleus, a thin rim of cytoplasm and no dendritic labeling; they were localized in the anterior zone of the dorsal nucleus of area ventralis telencephali (Fig. 2).

The 1,1'-dioctadecyl-3,3,3',3' tetramethylcarbocyanine perchlorate application into the habenula resulted in anterograde diffusion of the dye to fibres and terminals in the interpeduncolar nucleus. Retrograde transport of DiI after application in the habenula resulted in labeled perikarya in some forebrain nuclei. After bilateral application of DiI into the habenula, the midbrain sections showed massive labeling of the fascicula retroflexa; their fibres entered the interpeduncular nucleus ipsilaterally, but ran within the ipsilateral and contralateral halves of the interpeduncolar nucleus. After unilateral habenular application of DiI, a uniform distribution of fluorescence was observed in the interpeduncolar nucleus.

Retrograde transport of the DiI inserted into the habenula involved the fibres of the medial and lateral olfactory tracts in the telencephalon. Unilateral habenular application of DiI retrogradely labeled different forebrain nuclei: scattered large pyriform neurons in the posterior zone of the dorsal nucleus of area ventralis, some round neurons in the anterior zone of the dorsal nucleus of area ventralis, and some neurons in the bed nucleus were bilaterally labeled, while bipolar neurons with labeled dendritic branches were seen ipsilaterally in the ventro-lateral area, dorsoal to the lateral preoptic area, and in the entopeduncolar nucleus.
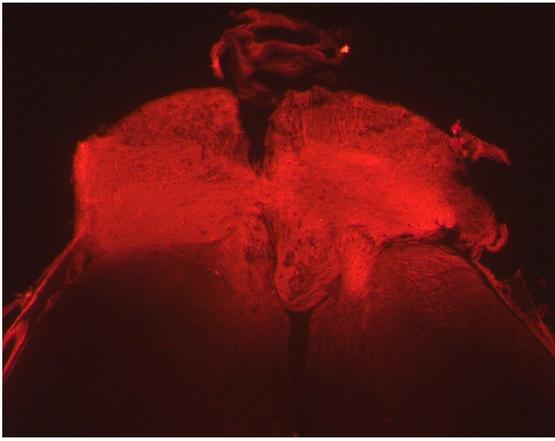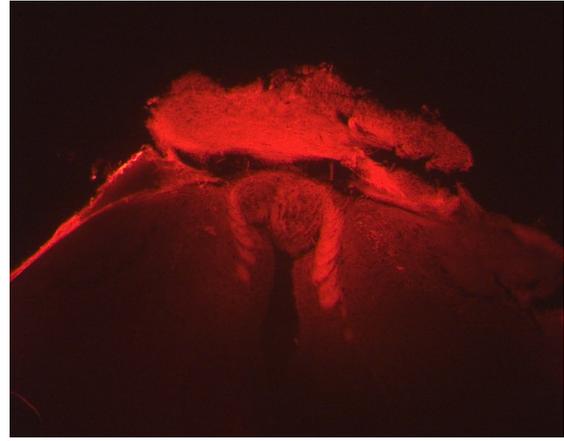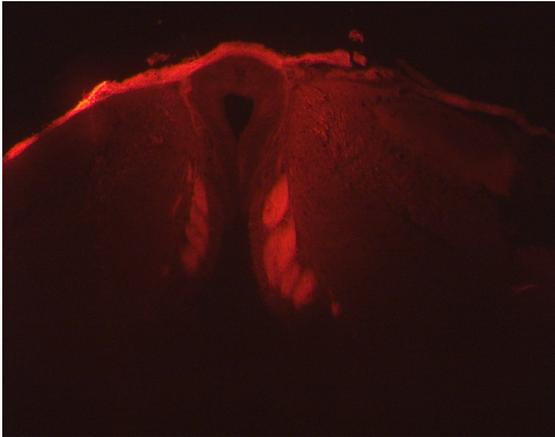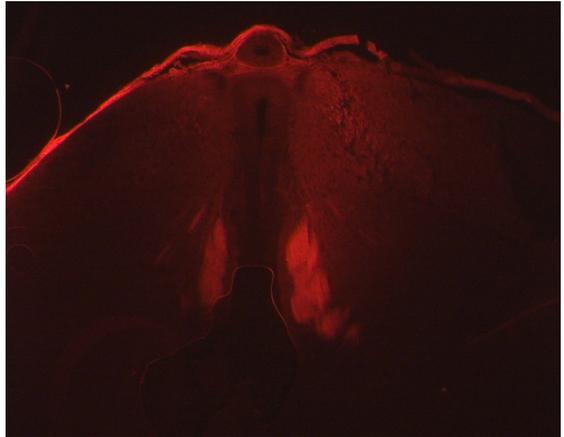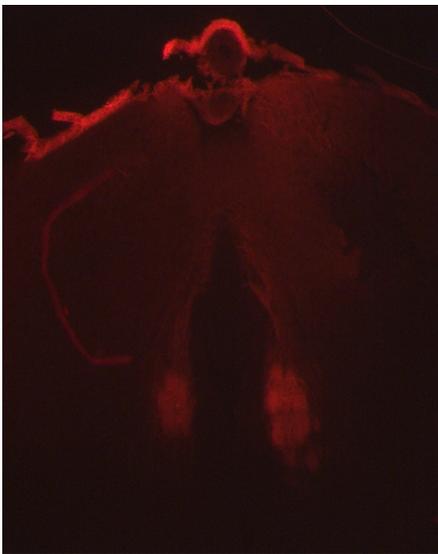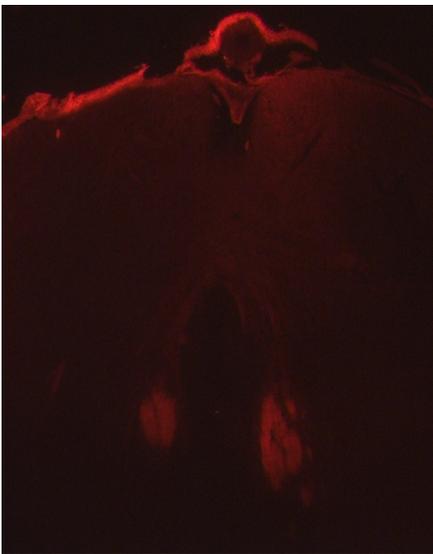
Fig. 1 (a-f) Photomicrographs illustrating the fluorescent labeling of the fascicula retroflexa in *Hydrolagus mirabilis*. Notice the left bundle (on the right side of the picture) larger than the right.
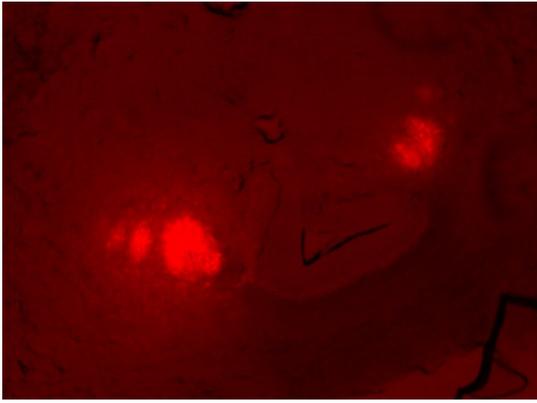
a
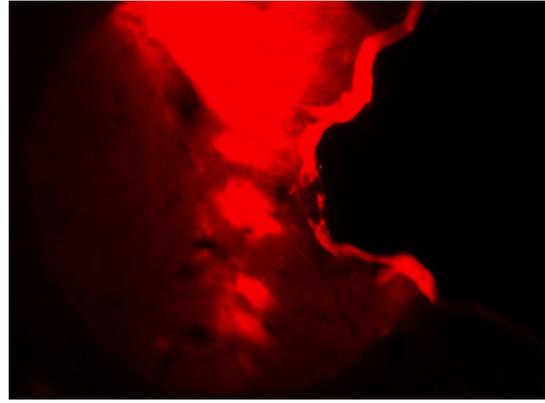
b
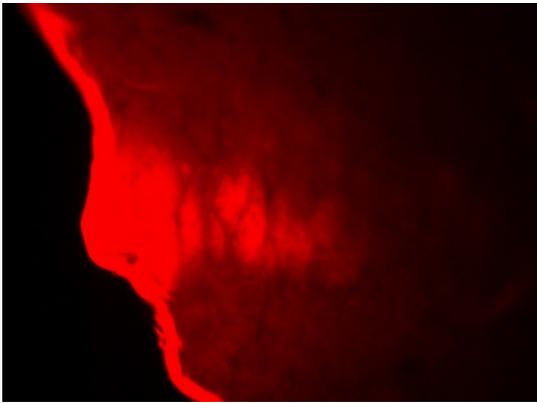
c
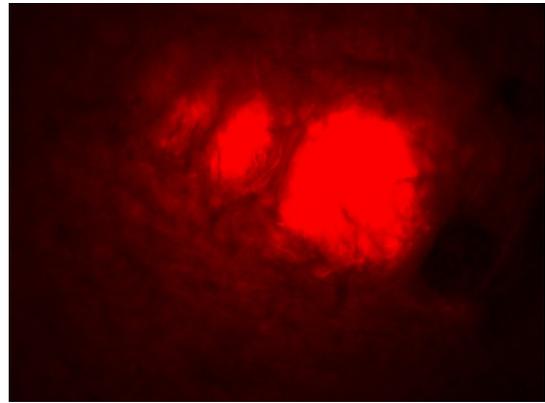
d

Fig. 2 (a-d) Photomicrographs illustrating the fluorescent labeling of the fascicula retroflexa in
Myliobatis aquila.
Notice the left bundle (d) larger than the right (c).
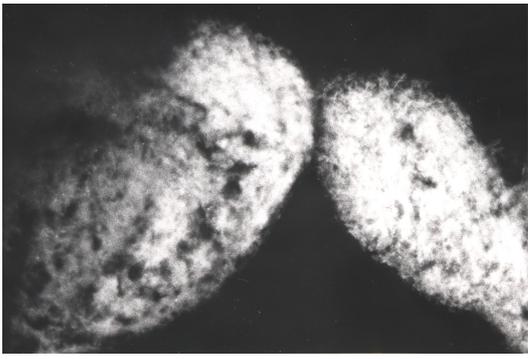
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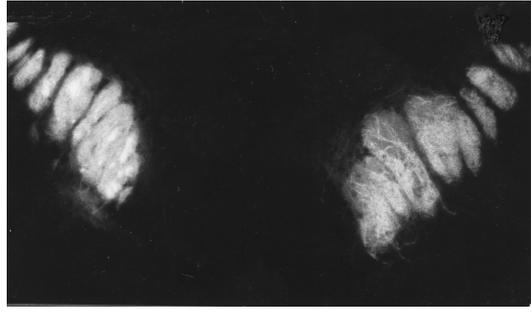
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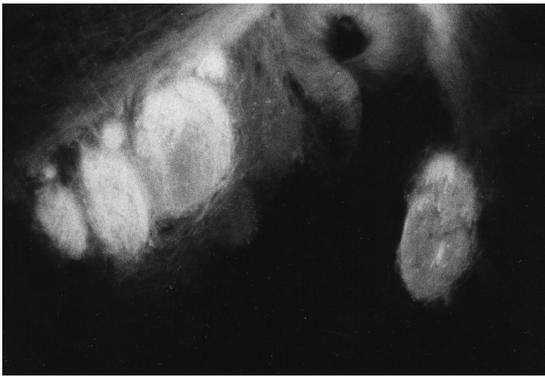
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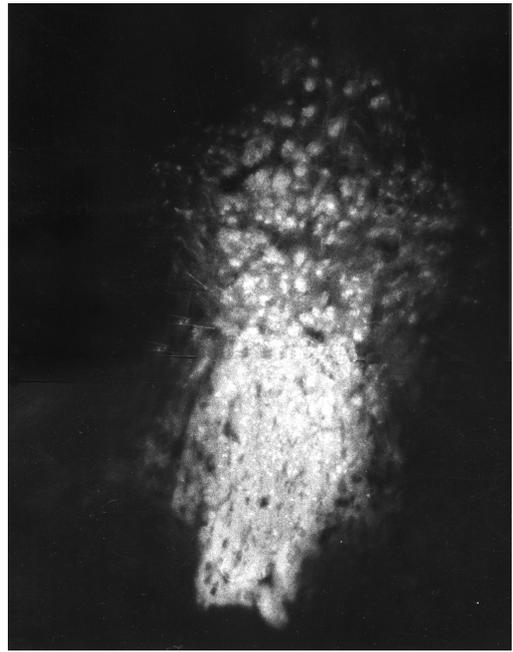
d

Fig. 3 (a-d) Photomicrographs illustrating the fluorescent labeling of the fascicula retroflexa in *Chiloscyllium arabicum.* Notice the left bundle (on the left side in the picture) larger than the right.
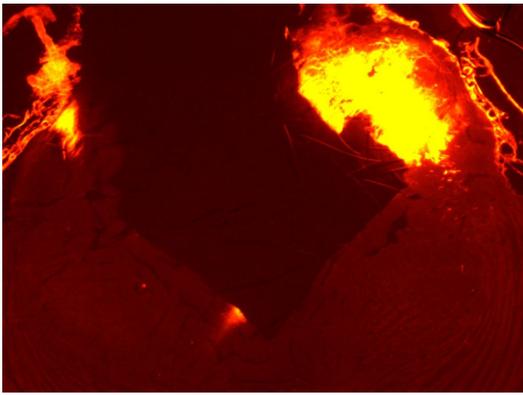
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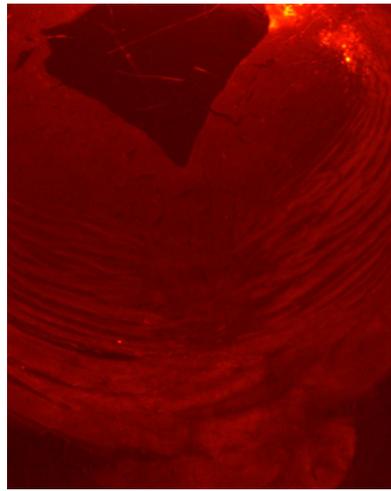
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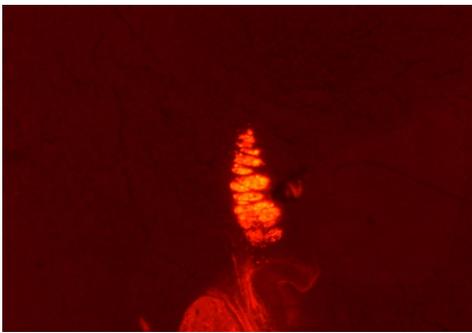
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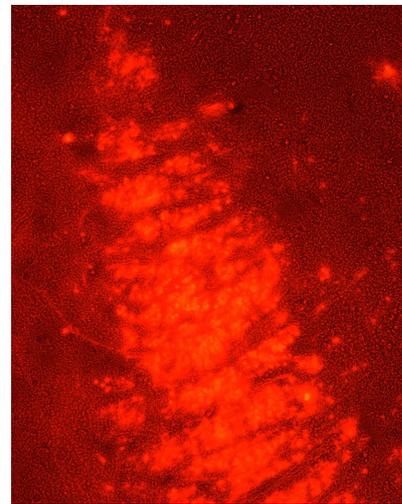
d

Fig. 4 (a-d) Photomicrographs illustrating the fluorescent labeling of the habenulae and of the fasciculus retroflexus in *Scyliorhinus canicula*. Notice the left nucleus (on the right side of the picture) more developed than the right.
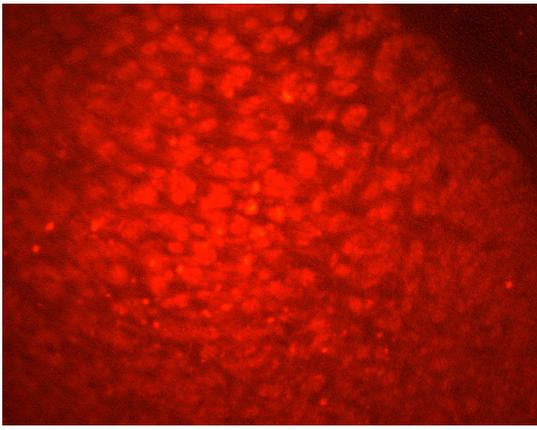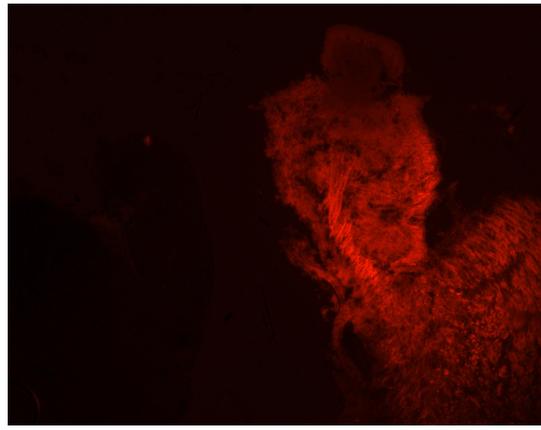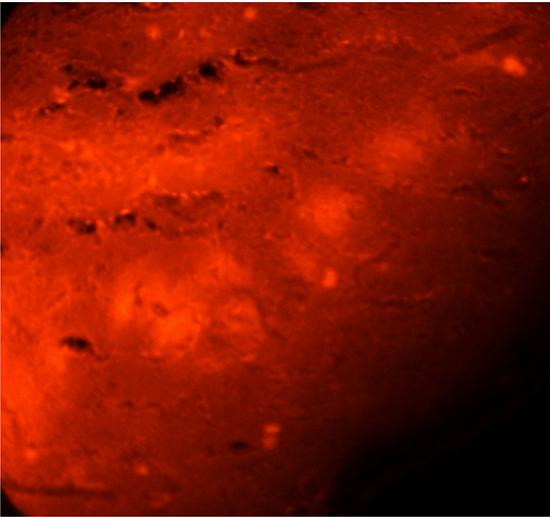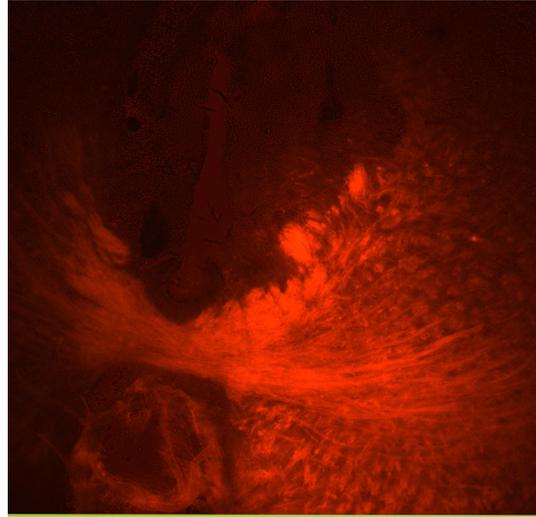
Fig. 5 (a-d) Photomicrographs illustrating the fluorescent labeling of some cell bodies in the area
ventralis of the telencephalon (a), habenular nuclei (b), left fasciculus retroflexus (c) and
ventral thalamus (d) in *Raja asterias.*
Left side on the right.
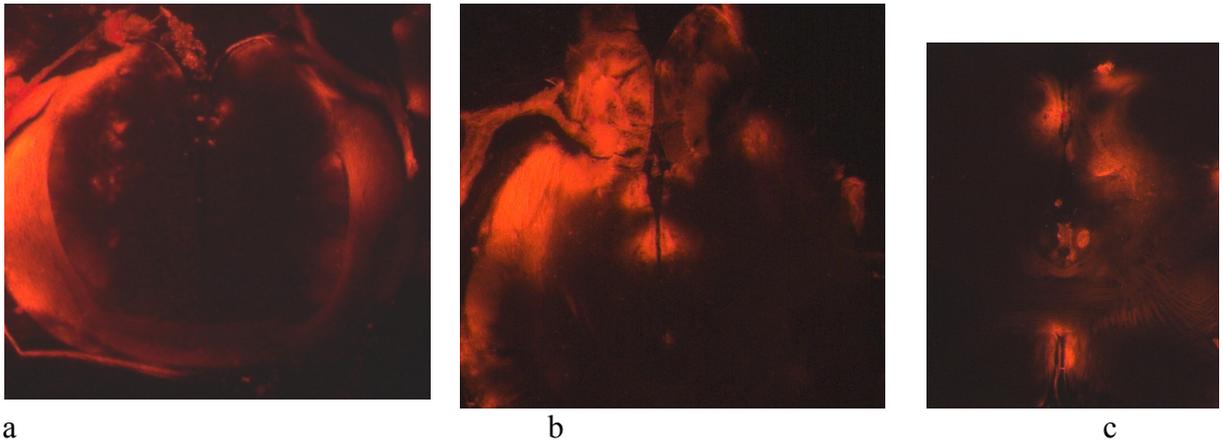
Fig. 6 (a-c) Photomicrographs illustrating the fluorescent labeling of some diencephalic areas in *Anguilla anguilla;* picture b shows the habenular nuclei.
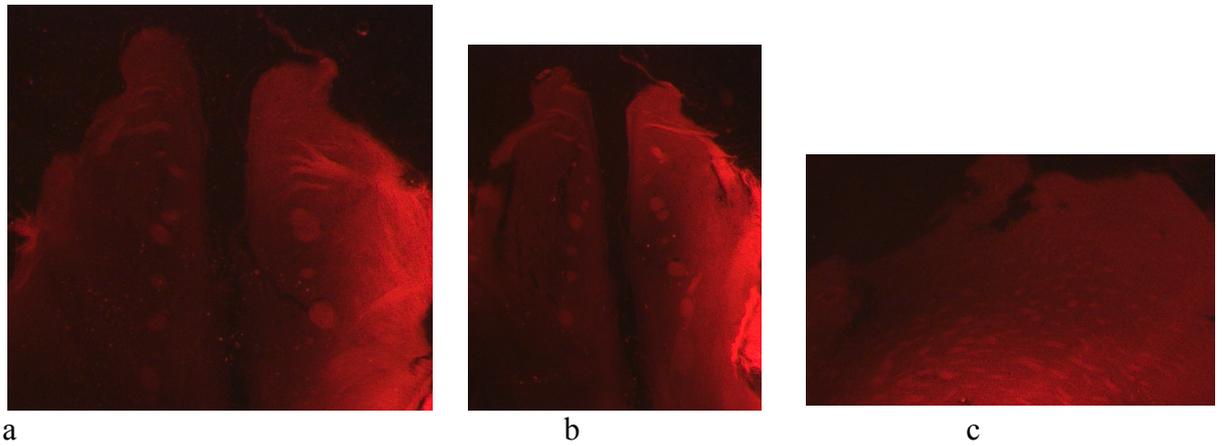
Fig. 7 (a-c) Photomicrographs illustrating the fluorescent labeling of different areas of the encephalon in *Diplodus sargus.* Telencephalic neurons are shown in picture c.
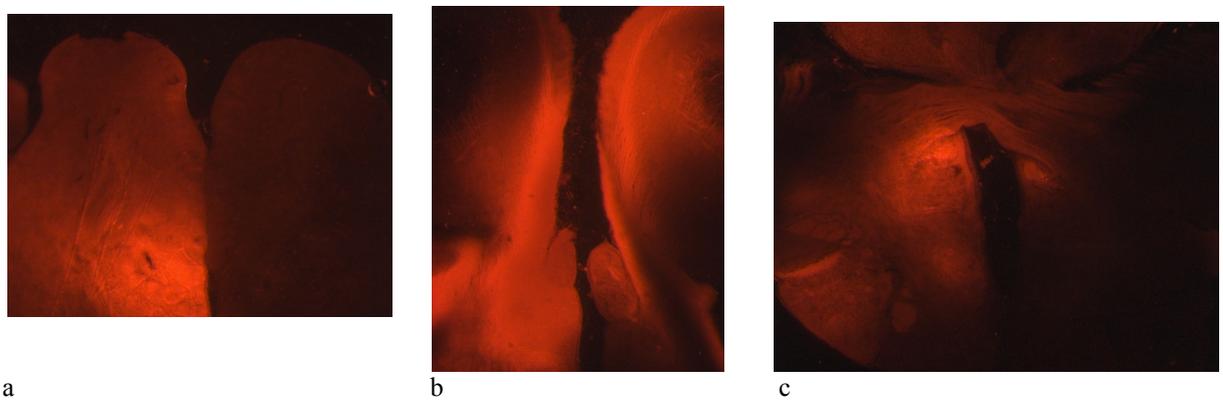
Fig. 8 (a-c) Photomicrographs illustrating the fluorescent labeling in the brain of *Xenomystis guentheri*.
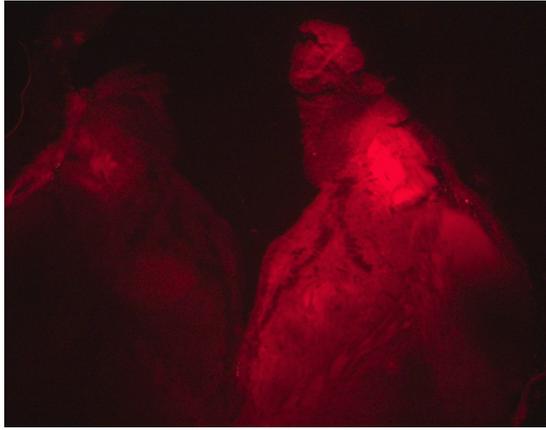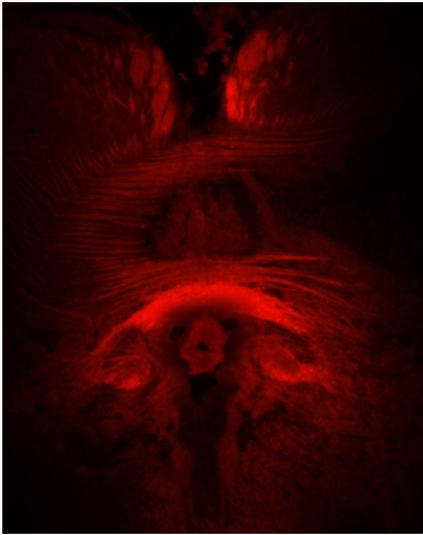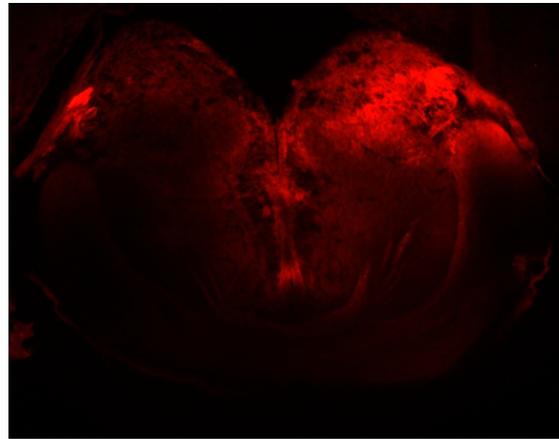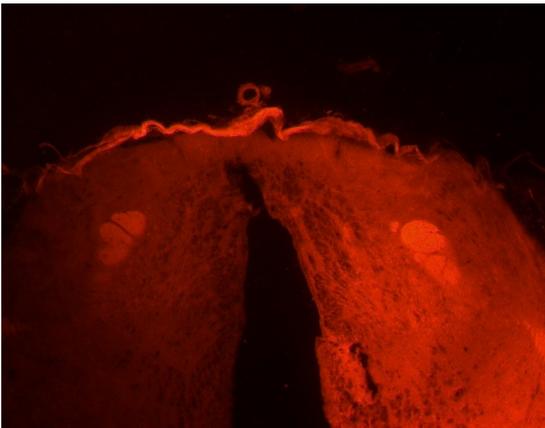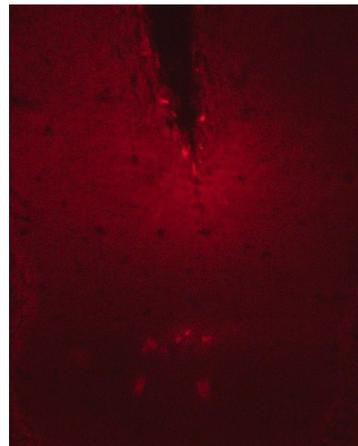
Fig. 9 (a-f) Photomicrographs illustrating the fluorescent labeling of different areas
of the diencephalon in the deep sea eel S*ynaphobranchus kaupi:* habenular nuclei (a-b), fascicula
retroflexa (e) and interpeduncolar nucleus (f).

3. Immunohistochemical analysis

Diencephalon: in the dogfish *Scyliorhinus canicula*, small strongly stained neurons and some scarce fibres were mainly found in the nucleus medialis of the left habenula; fibre tracts related to the ganglion habenulae, such as the stria medullaris, the commissura habenulae and the fasciculus retroflexus, carried positive fibres (Fig. 1). Fibres of the stria medullaris could be traced from the level of the impar telencephalon to the ganglion habenulae. At the level where both habenulae join, positive fibres were observed crossing the midline asymmetrically through the commissura habenulae, with more fibres appearing on the right than on the left side. In contrast, no positive reaction was found in the fibres of the commissura posterioris, located behind the commissura habenulae, nor in the subcommissural organ. Immunopositive fibre salso run through the habenular efferent tract, or fasciculus retroflexus, to the tegmentum mesencephali whereby the left fasciculus contains more immunopositive fibres than the right one. However, in both tracts, there are less positive than negative fibres. No positive reaction was observed in the epiphysis.

Thalamus: CaBP28k-immunopositive cells and fibres appeared mainly in periventricular nuclear groups of both the thalamus dorsalis and ventralis but also in lateral areas. Immunopositive fibres run in a rostrodorsal direction through a dorsolateral thalamic area forming a clear tract whose origin and ending could not be determined. This tract passed ventrolaterally to the corpus geniculatum laterale whose large neurons showed a weak positivity.

Hypothalamus. Here, the only well-defined CaBP28k-containing nuclear group was the nucleus lobi lateralis. Its cells, especiallly those of the pars ventralis, were heavily stained in the cytoplasmatic region facing the ventricle. Thick weakly stained dendrites emerging from the opposite cell pole are running towards the meningeal surface before joining the dense plexus of positive fibres near the external border of the hypothalamic lobes. In addiction to the neurons of the nucleus lobi lateralis, a few poorly stained nerve cells were observed in the nucleus suprachiasmaticus, the nucleus medium and the nucleus lateralis tuberis which are all located in the medial hypothalamic floor. In the latter nucleus, some positive cells appeared to be contacting the cerebrospinal fluid. The organon recessus preopticus and vasculosum hypothalami of the dogfish (Wilson and Dodd, 1973; Rodriguez-Moldes and Anadon, 1987) did not show any reaction with the CaBP28k antiserum.
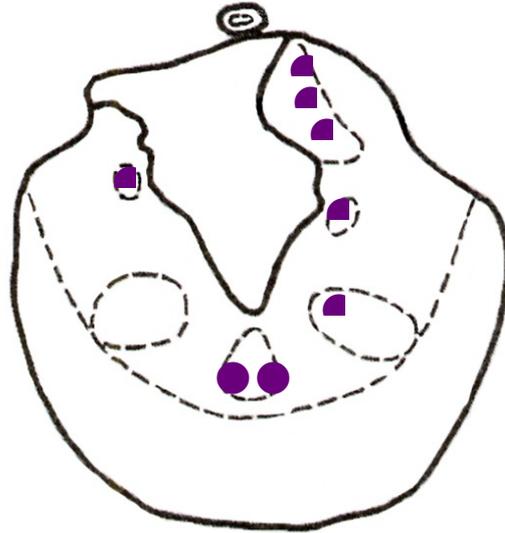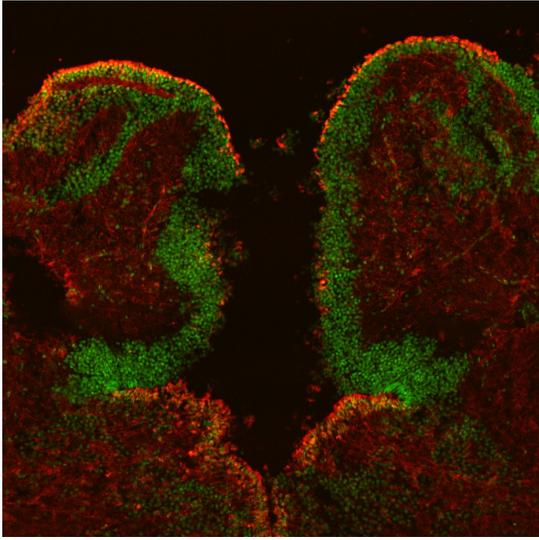
Fig. 1a. Schematic drawing of transverse section illustrating the distribution of CaBP28k immunopositive cells and fibres in the diencephalon of dogfish.
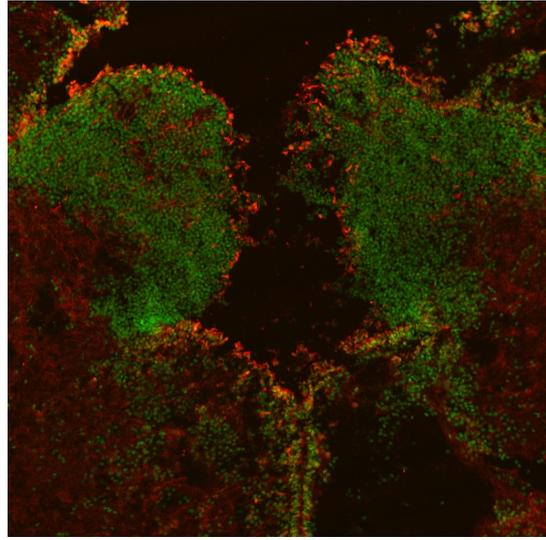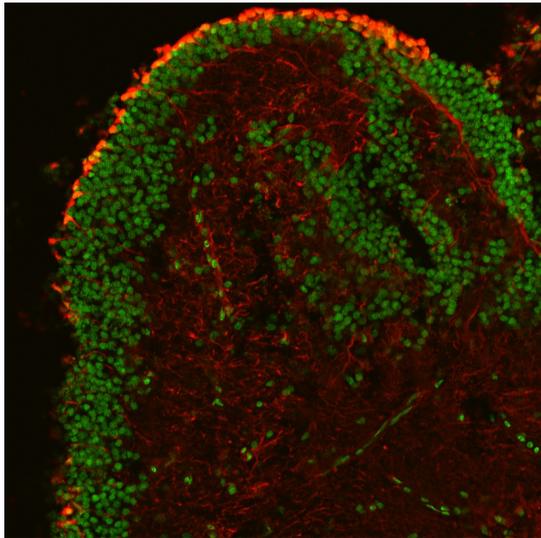
● Immunopositive cells and fibres
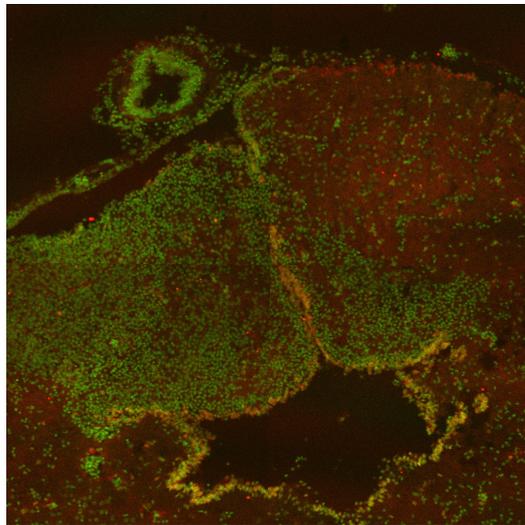
Fig. 1b. Transverse section through the diencephalon of dogfish, showing the CaBP28k distribution at the level of habenulae.5x.
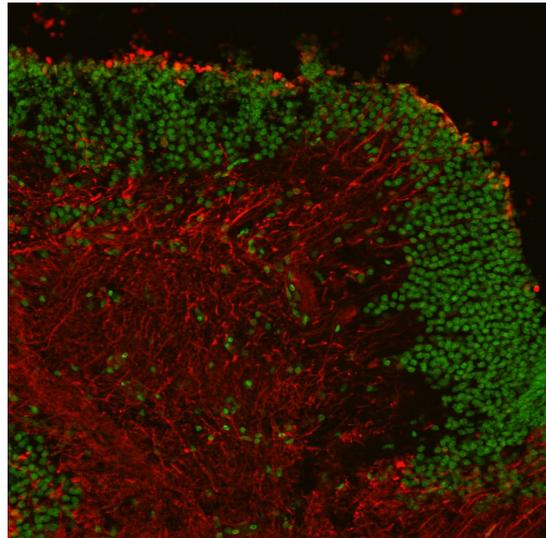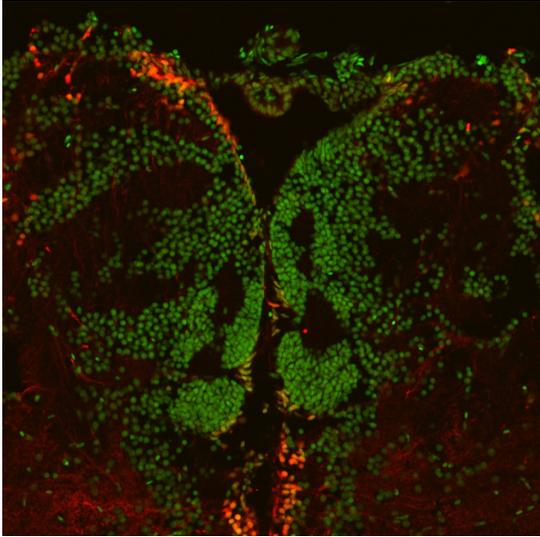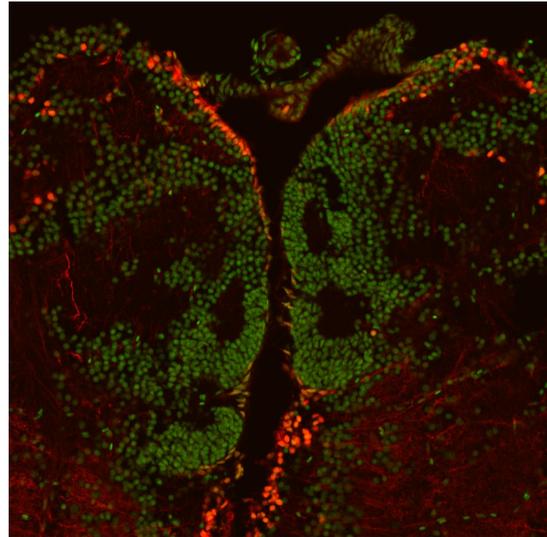
Fig. 2 (a-d): *Synaphobranchus kaupii;* cross sections showing CaBP28k-positive neurons and fibres in the habenular nuclei.
2a and 2b, 5x.
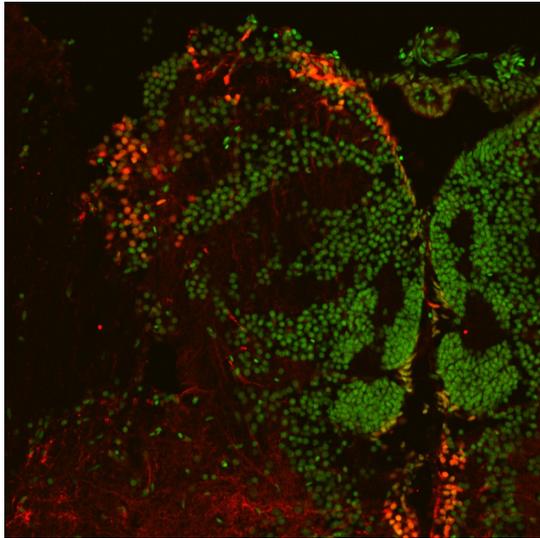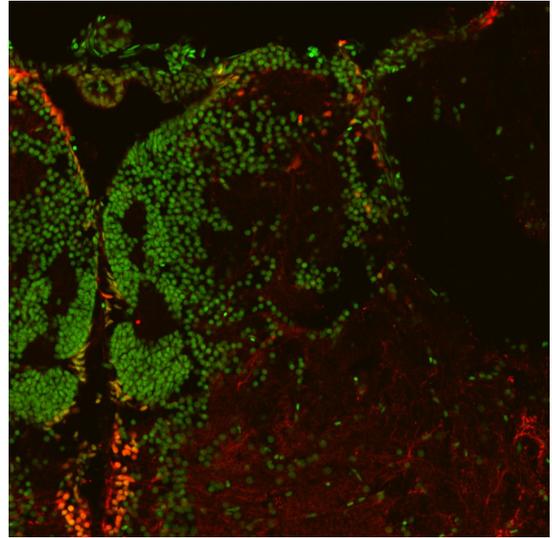Details of left and right side corresponding of figure 2c and 2d.10x.

Fig. 3: *Danio rerio;* cross sections showing CaBP28k-positive neurons and fibres in the habenular nuclei. 5x.

In two teleosts, *Danio rerio* and the deep sea eel *Synaphobranchus kaupii,* the distribution of CaBP28k seems to be different: Fig.2 and Fig. 3 show trasversal sections of diencephalic regions, where the presence of calbindin is put in evidence. The difference between the two nuclei is less clear than in the dogfish sections.

4. Transmission Electron Microscopy

Few ultrastructural studies have been carried out on the habenular nuclei of vertebrates (Kumar and Kumar, 1975; Cupedo, 1977; Kemali and Guglielmotti, 1977; Tokunaga and Otani, 1978), and none on the habenulae of fishes, except for the shark *"Scyllium stellare"* (Miralto and Kemali, 1980) and for the goldfish (Villani et al., 1993).

In this study I have examined, at the electron microscopic level, the various areas in the habenular nuclei of some species, in a special way about *Coryphaenoides armatus* and *Danio rerio.* My first aim is to astablish the fine morphological differences between right and left side of the habenular complex.

The most prominent type of neuron appearing in the left habenula is that of the lateral portion which has the largest diameter. This type of cell has a round nucleus with some indentations and a very prominent nucleolus located eccentrically. Cisternae of rough endoplasmatic reticulum, isolated Golgi apparatus and elongated mitochondria are visible against a pale cytoplasmatic background. Synapses have been observed on their cell surface, particularly at the site of emergence of their process and on the spines which may protrude from them. Often their cell surface is covered by stacks of glial lamellae.

Myelinated axons may occur around these cells and sometimes their cell body is completely enwrapped by myelin which, close to the emergence of a process, may look like a node of Ranvier.

Another cell type found in the left habenulae has a dark cytoplasm and large granules.

The cells of the right habenula have very large nuclei which have prominent eccentric nucleoli and poor cytoplasm. Often these cells, as for the left habenula, are enveloped by several glial lamellae which separate the cells from each other.

Another type of cell of the right habenula has cytoplasm rich in rough endoplasmatic reticulum, Golgi apparatus with a number of coated vesicles as well as a quantity of organelles, probably lysosomes; the Golgi apparatus shows, besides the cisternal stalks, also spherical vesicles. Also present are lysosomal like inclusions; glial cell, with a typical dark nucleus, is in close contact with two myelinated axons.

A large variety of synaptic vesicles occur in the habenulae of both sides. They vary with regards to their size, their shape and the electron density of their content. The type of vesicles observed in the left habenula are small, spherical and translucent; often the synapses are enveloped by a multi-lamellar glial formation. Glycogen granules are also visible within the synaptic terminals.

On the right habenula we also have synapses with spherical or flat vesicles, which may be translucent or with a dense core. The synapses vary in the density of their vesicle population.

In the midline portion of the habenulae, we may have synaptic terminals with spherical translucent vesicles alone or with dense core vesicles; sometimes this type of synapses is very complex, and it appears to be enveloped by a system of multi-lamellar glia. Also oval vesicles have been observed in terminals which occur together with terminals where only spherical translucent vesicles occur.

With reference to the myelin pattern, I have already observed at the light microscope that the left habenula is more myelinated than the right; in addition, serial semithin sections, 1 μm thick, of

specimens prepared for the electron microscope, have shown that in the left habenula there is heavily myelinated bundle of fibers which meanders within the middle of the nucleus occupying a prominent position within it. On the contrary, a modest bundle of myelinated fibers runs along the lateral edge of the right habenula.
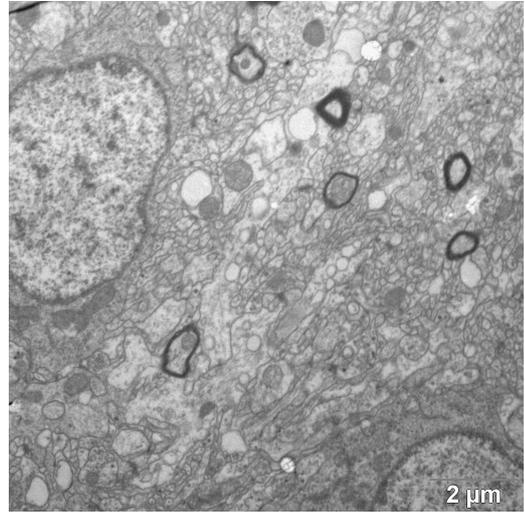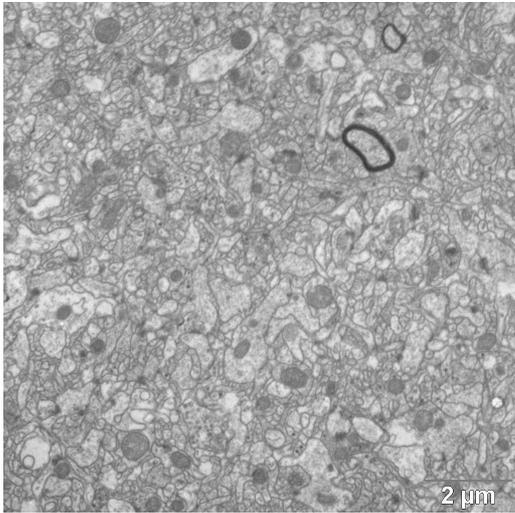
The different pattern of myelination of the two habenulae is stressed at the electron microscope, by the presence in the left habenula only, of unusually myelinated perikarya as reported elsewhere (Kemali and Miralto, 1979).
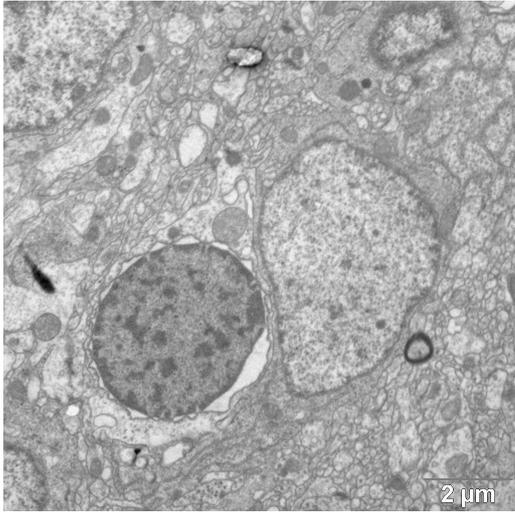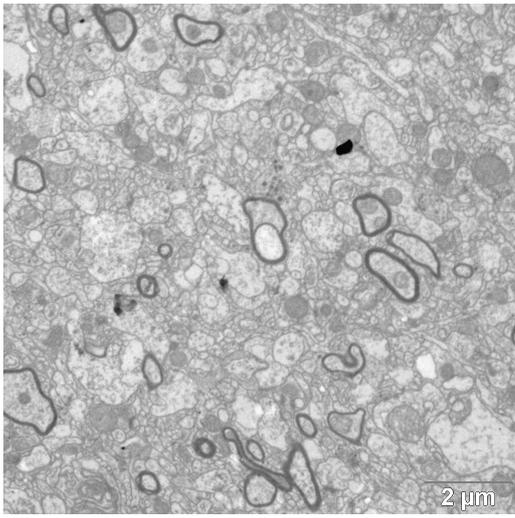
a
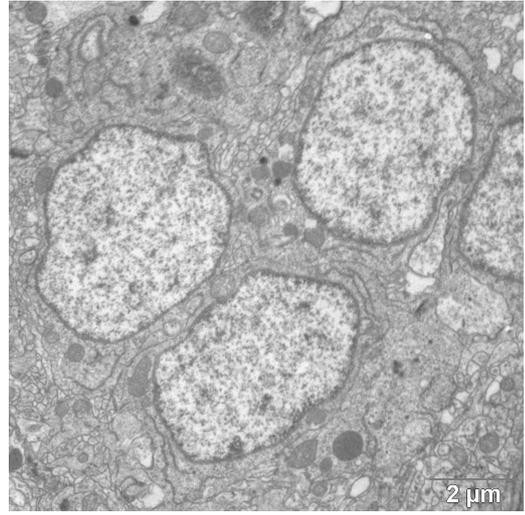
b

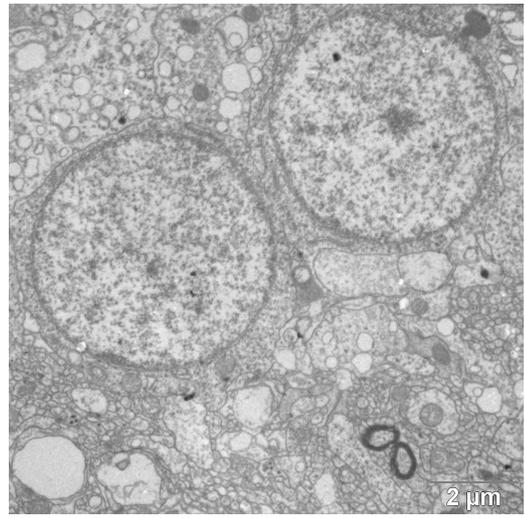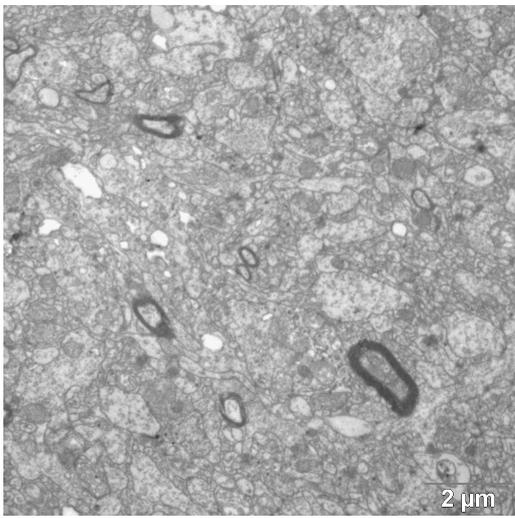**Fig. 1 (a-d)**: *Coryphaenoides armatus,* right habenula.
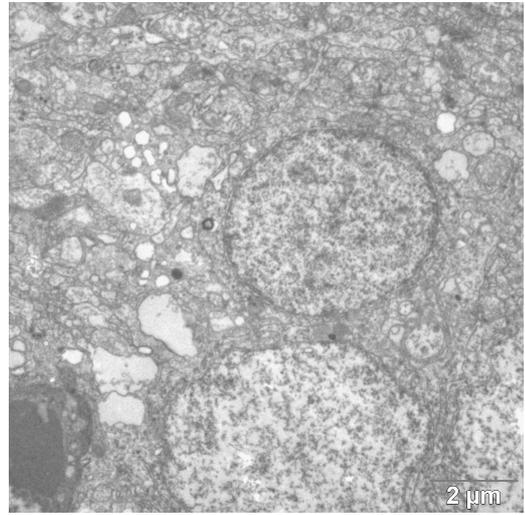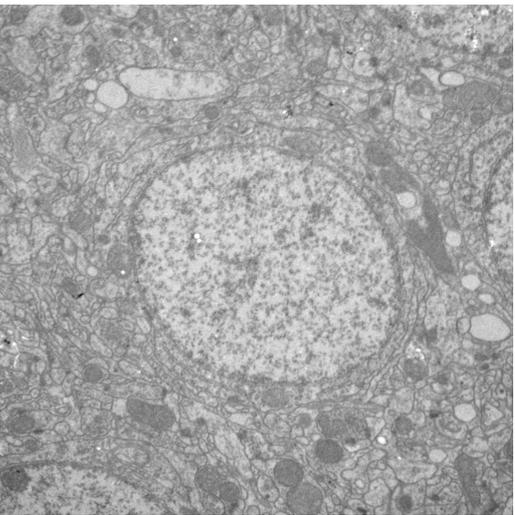
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b
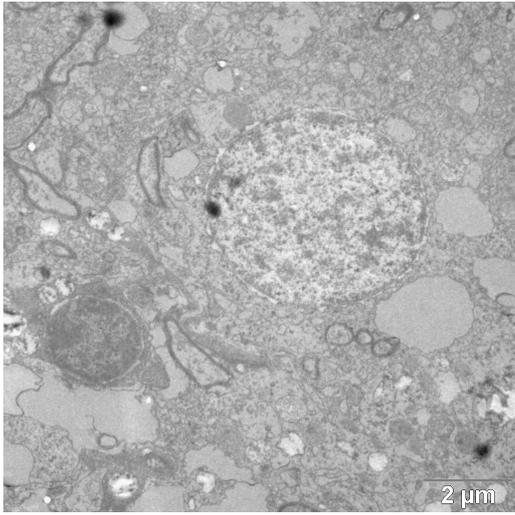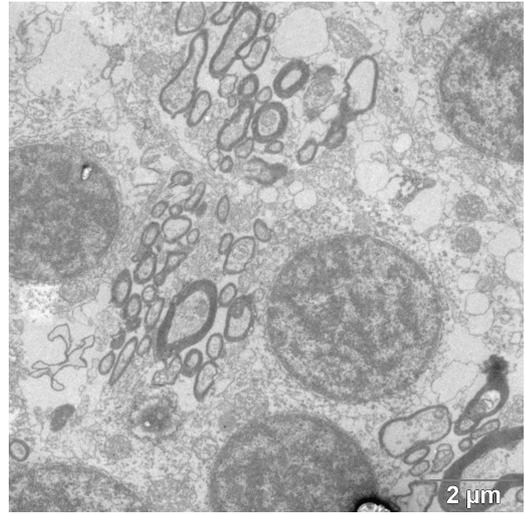

c

d

e
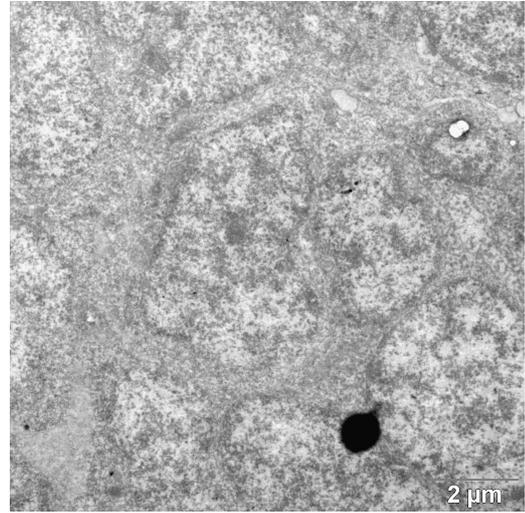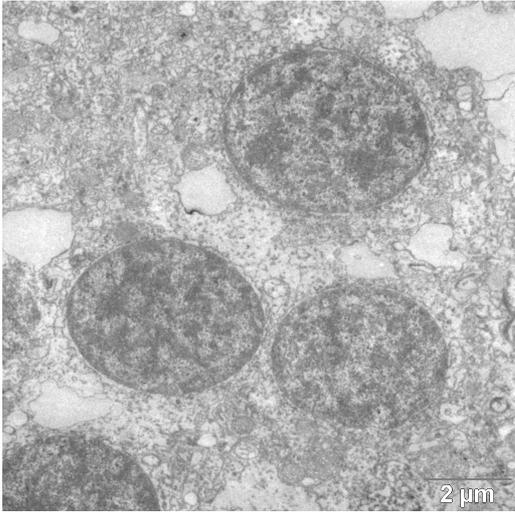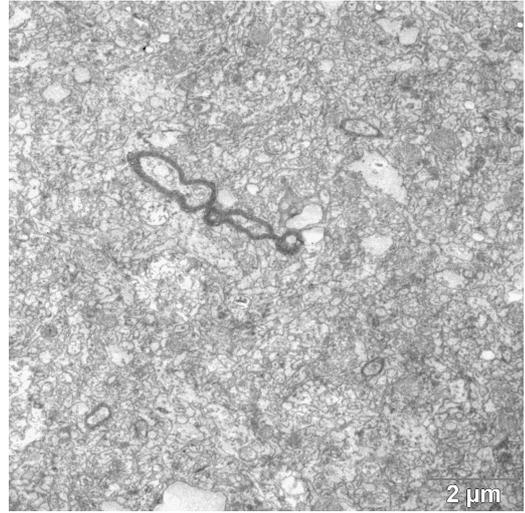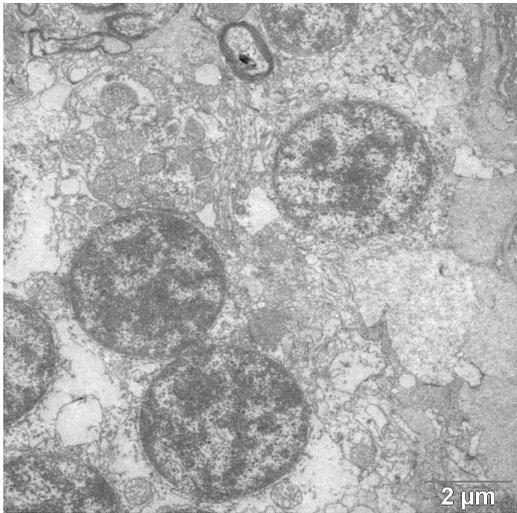
Fig. 2 (a-e): *Coryphaenoides armatus,* left habenula.
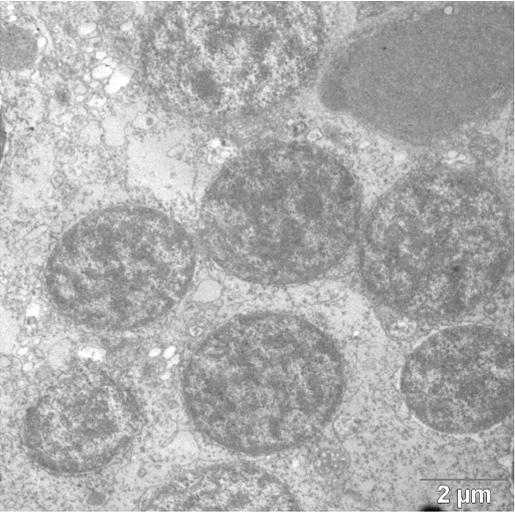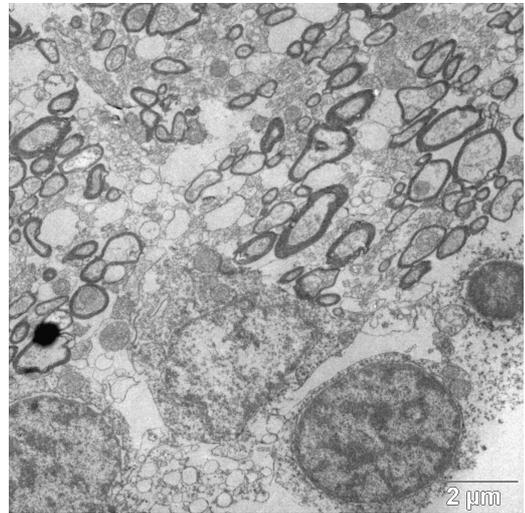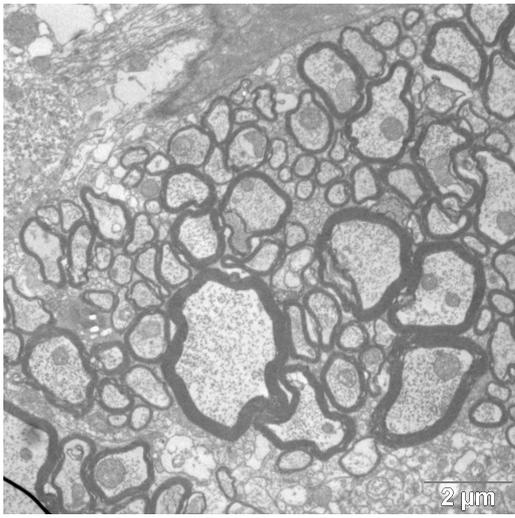
Fig. 3 (a-d): *Danio rerio,* right habenula.

e

f

g
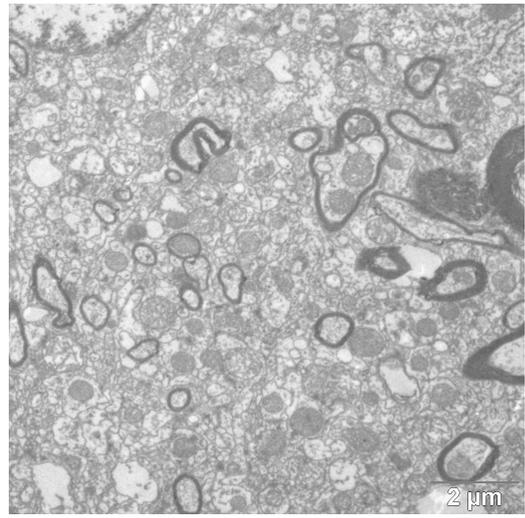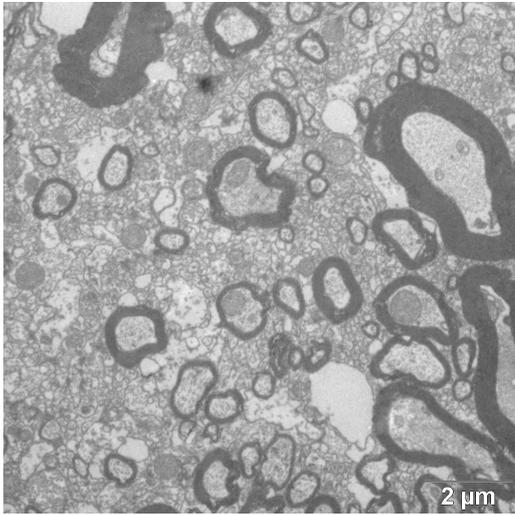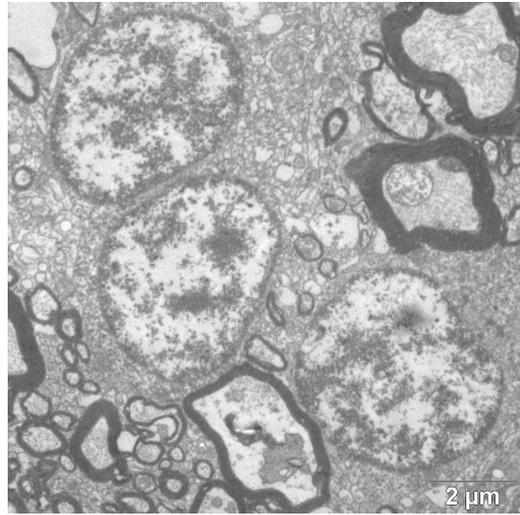

h

Fig. 3 (e-h): *Danio rerio,* right habenula.

Fig. 4 (a-d): *Danio rerio,* left habenula.
Notice the quantity of myelinated fibers.

DISCUSSION AND CONCLUSIONS

1. Histology

My results show that the left and right habenular nuclei of fishes are substantially different; the two habenulae differ not only in their size, but also in their cytological organization. The left habenula in the elasmobranchs is always the larger and the longer; these observations confirm previous description of the asymmetry in other species, as *Mustelus canis* (Houser, 1901).

The difference between the two habenulae is further accentuated especially by the presence of a larger number of myelinated fibers on the left side than on the right side. Some authors have also expressed the opinion that the asymmetry of the habenulae is due to the asymmetrical development of the parietal organ during phylogeny (Ariens Kapper et al., 1965; Beccari, 1943); the fact that in a large number of vertebrate the asymmetry – if present – always favoured the left side, gives some support to the hypothesis of Morgan (1977) that "there is a general factor operative in vertebrates, as well as in their ancestors, favoring more rapid development of the left side of the embryo".

However, the meaning of this striking morphological asymmetry remains obscure. In addition, the role of the habenular nuclei, without considering their asymmetry, has not yet been settled.

It is known that these structures belong to the limbic system. By means of histochemical techniques, it has been demonstrated that the habenulae are interconnected with the nucleus of origin of the mesolimbic and mesocortical dopaminergic systems (Cuello et al., 1978).

In addition, the habenulae have been found to be the site of the central nervous system where the neurons contain substance P (Hökfelt et al., 1975). All these observations point to an interesting role of these structures; their striking asymmetry is of general interest in the lateralization of structures and functions of the nervous system.

The morphologic asymmetry of the habenular nuclei reflects a different functional specialization of the two sides of the epithalamus as postulated for the frog (Kemali, 1977).

Finally, among the species of teleostean fishes, the habenulae of *Liza aurata, Anguilla anguilla* and *Trisopterus minutes* are quite symmetrical, while in *Lepidorhombus boscii, Platichthys flesus* and *Solea vulgaris* the habenulae are slightly asymmetrical. This data could suggest a relationship, to be examined thoroughly, among benthic life and habenular asymmetry.

2. Connections analysis

The results obtained after DiI application into the habenulae demonstrated the presence of a massive habenulointerpeduncolar projection in all species examined. This evidence confirms previous ultrastructural studies which showed a large number of degenerating terminals in the goldfish interpeduncolar nucleus after habenular ablation (Villani et al., 1994). The presence of a massive habenulointerpeduncolar connection was also demonstrated by HRP-tract-tracing technique in frogs (Kemali and Guglielmotti, 1982), lizards (Diaz and Puelles, 1987) and mammals (Contestabile and Flumerfelt, 1981).

Furthermore, labeling of some telencephalic neurons in the anterior zone of the dorsal nucleus of area ventralis, also demonstrated the presence of a minor direct telencephalointerpeduncolar projection in fishes. In comparsion to the few telencephalic neurons labeled after DiI application into the interpeduncolar nucleus, several pools labeled neurons were observed in different telencephalic areas after DiI application into the habenular nuclei. These results suggest a

massive innervation of habenulae from telencephalic nuclei; these results are comparable to those from an investigation of the afferents to the habenula in frogs (Kemali et al., 1980) showing connections from the septal area, the bed nucleus and the entopeduncolar nucleus. In lizard (Diaz and Puelles, 1987) HRP injection in the habenula produced labeling of the nucleus of the posterior pallial commissure and additional massive projections to the habenulae coming from the nucleus septalis, the lateral preoptic area and the nucleus of the stria medullaris. The pattern of telencephalic-habenular-interpeduncolar connections in fish is in agreement with the well established connections observed in mammals: in particular, the demonstration of a direct telencephalointerpeduncolar connection in these species, which had been demonstrated in mammals with the use of selective neurotoxins (Contestabile and Villani, 1983; Contestabile and Villani, 1984), suggest some speculations on telencephalic homologies in fishes and other vertebrates (Echteler and Saidel, 1987; Murakami et al., 1987; Northcutt and Davis, 1987).

The dorsal nucleus of area ventralis was considered homologous to the septum of mammals by Northcutt and Braford (1987); this hypothesis is corroborated by present observations, since this area projects to the interpeduncolar nucleus as does the septum in mammals. Furthermore, the presence labeled neurons in the forebrain ventrolateral area, dorsal to the preoptic area, observed after habenular application of DiI, suggests the homology of this area with the entopeduncolar nucleus of mammals, which massively projects the the habenulae (Herkenham and Nauta, 1987). The neuroanatomical pattern of connectivity of the telencephalic mesencephalic projections in fishes supported by neurochemical data that reveal interesting homologies, based on the localization of neurotransmitters/neuromodulators, such as substance P (Villani et al., 1991; Villani et al., 1994), acetylcholine and nitric oxide (Villani et al., 1987; Villani et al., 1994).

Finally, present results on the cells of the telencephalointerpeduncolar pathway add further information to that obtained by conventional and electron microscopy, and suggest that, during the course of vertebrate evolution, no major changes have occurred in the neuroanatomical pattern of connections of these telencephalic-mesencephalic projections.

3. Immunohistochemical analysis

CaBP28k has been demonstrated in the brain and other nervous tissue of many vertebrates including fishes, indicating that is a neuronal protein. However, there were no reports about its presence in the nervous system of the more primitive cartilaginous fishes.

This investigation not only shows that a protein similar to the chick duodenal CaBP28k is present also in the brain of the shark, but also that it is located in particular neuronal groups.

Compared to tetrapods, the distribution of CaBP28k in fishes shows many particularities. In the latter, this protein appears to be restricted to the nervous system, since it has not been detected in the kidney, intestine or gills of teleosts (Parmentier et al., 1987).

In tetrapods, CaBP28k is always found in certain well-defined neurons such as the cerebellar Purkinje cells and sensory nerve cells. Studies concerning the distribution of CaBP28k in the nervous system of teleosts (Maler et al., 1984; Parmentier et al., 1987; Dechesne et al., 1988; Denizot et al., 1988; Roman et al., 1988) and the present investigation reveal that the tetrapod pattern of CaBP28k distribution cannot be generalized to fishes.

While CaBP28k has been demonstrated in the peripheral vestibular system of mammals, birds and amphibians (Rabiè et al., 1983; Sans et al., 1986; Dechense et al., 1988; Legrand et al., 1988), in the

teleost fish, only some vestibular ganglion neurons and fibres of the sensory epithelium showed a faint immunoreaction (Dechense et al., 1988). Although sensory structures were not investigated in my work, I have observed that the primary sensory centres of the dogfish brain related to olfaction (bulbus olfactorius) and vision (tectum mesencephali) or to the innervation of the mechanoreceptors of the lateral-line and vestibular organs (projecting to the vestibulolateral area), showed a faint or negative immunoreaction. In contrast, positivity was observed in the olfactory bulb of the rat and chick (Jande et al., 1981b; Baimbridge and Miller, 1982; Garcia-Segura et al., 1984; Enderlin et al., 1987). Compared to other vertebrates, CaBP28k has a different distribution in fish sensory structures. Immunopositivity was observed in pineal transducers and retinal photoreceptors of amniote vertebrates (Verstappen et al., 1986; Roman et al., 1988) but not in cyclostomes, teleosts and amphibians (Roman et al., 1988).

Roman et al (1988) have suggested that the variations in the presence of CaBP28k between amniotes and anamniotes could indicate differences in the pineal calcium metabolism between the two groups.

It has been demonstrated biochemically that in the chick and rat brain, the largest amount of CaBP28k is located in the cerebellum (Taylor, 1974; Baimbridge and Parkes, 1981; Baimbridge et al., 1982; Thomasset et al., 1982). Moreover, the Purkinje cells are immunoreactive to CaBP28k in mammals (Jande et al., 1981b; Fournet et al., 1986), birds (Jande et al., 1981a; Roth et al., 1981), amphibians (Gona et al., 1986) and also fishes, although with a less intense stain than the one observed in higher vertebrates (Maler et al., 1984). The absence of CaBP28k immunoreactivity in the Purkinje cells of the dogfish is therefore a significant difference and suggests that these neurons have a distinct calcium metabolism from those of other vertebrates. It is very improbable that

these observations are due to an artefact, because some positive fibres could clearly be seen in the fibrous layer located between the Purkinje cell layer and the ependyma.

CaBP28k appears to be a useful marker to study the habenulo-interpeduncolar system. In the habenular ganglion, immunopositive cells have been observed in amniotes (Jande et al., 1981b; Roth et al., 1981; Baimbridge et al., 1982; Garcia-Segura et al., 1984; Enderlin et al., 1987) and in fishes (Maler et al, 1984). The fasciculus retroflexus interconnecting the ganglion habenulae and the nucleus interpeduncularis also harbours immunopositive fibres. The relation of this system with other brain regions is less clear. The stria medullaris connects different telencephalic areas (Smeets et al., 1983). with the ganglion habenulae after crossing the commissura habenulae. Smeets (1983) has demonstrated that part of these fibres originate from the striatum but this telencephalic area lacks CaBP28k immunoreactivity. In other telencephalic areas, some positive elements have been observed, but their relationship to the stria medullaris cannot be confirmed. On the other hand, the connections between the nucleus interpeduncularis and the caudal brain regions are not well known.

The function of CaBP28k in the different groups of neurons is not yet known and neither are the common causal factors that might explain the CaBP28k content. It should however be noted that most of the dogfish brain regions that contain CaBP28k-immunopositive neurons, such as the ganglion habenulae, the dorsal thalamus and the lobi lateralis hypothalami, have been considered as relay centres between the telencephalon and the brainstem (Smeets et al., 1983; Smeets and Boord, 1985). In addition, primary viscerosensory centres, such as the lobi vagi and substantia gelatinosa, also showed immunoreactivity. Jande et al (1981b) postulated that the presence of CaBP28k in a neuron could indicate voltage-dependent Ca^{2+} currents in that

cell. The presence of CaBP28k in the nervous centres might be related with the control of free Ca^{2+} availability necessary for neurotransmission. In addition, CaBP28k might be related to other cellular activities, since it was demonstrated in neurons long before the beginning of synapse formation (Enderlin et al., 1987).

However, as far as we know, there are no electrophysiological studies which can help us to understand the calcium activity in these neurons or the processes in which calcium-binding proteins are involved.

4. Transmission Electron Microscopy

In the previous data I have shown at the light microscope that the habenular complex is composed of several distinct zones; at the electron microscopy the areas differ with respect to their cell types and myelin distribution.

The right, left and middle portions of the habenular complex contain different cell types: these cells, especially in the Elasmobranchs, vary in their cytoplasmatic organelle content, electron density, the presence of large cytoplasmatic granules or coated vesicles and the presence of unusual features such as perikaryal myelin.

The fact that the habenulae have several cell types has been documented. In fatc, Iwanhori (1977), in a Golgi study of the habenulae of the cat, classified the neurons of the medial habenular nucleus into two types and those of the lateral habenular nucleus into four types. This author's observations were compiled from soma size, dendrite morphology and the axonal course.

The presence in only the left habenula of cells bearing large granules - probably secretory - and of cells with myelinated soma suggest a functional diversification of the habenulae. However, there is a common characteristic of the cell types occurring in the left, right and middle portions of the habenulae in Elasmobranchs: that is the presence of multilamellar astroglial formations wrapping these cell bodies. This glia ensheating is similar with the arrangement of astrocytes surrounding neurons observed by Klatzo (1967).

Such a lamellar wrapping has been also observed in synapses, particularly in the left habenula and sometimes in the right and middle habenula. Synapses ensheathed by onion-like formations have been described by Tokunaga and Otani (1978) in the medial habenular nucleus in the rat, whilst Robert and Ryan (1976) demonstrated synapse-bearing perikarya enclosed by layers of compact myelin in the anterior laeral-line lobe of *Scyliorhinus canicula.*

Similarly as reported for the frog (Kemali, 1976), I observed a large variety of synaptic vesicles. This is in accordance with the fact that several putative neurotransmitters and substances related to neurotransmission have been demonstrated in habenulae by several techniques: serotonin (Björklund et al., 1972; Saavedra et al., 1974; Trueman and Herbert, 1970), catecholamines (Björklund et al., 1972; Jacobowitz and Palkovits, 1974; Björklund, 1978), acetylcholinesterase (Trueman and Herbert, 1970; Jacobowitz and Palkovits, 1974), glutamic acid and decarboxylase (Gottesfeld et al., 1977; Gottesfeld and Jacobowitz, 1978), substance P (Hökfelt et al., 1975), luteizining hormone releasing factor (Barry, 1978), vasopressin and oxytocin (Bujis, 1978). The difference between right and left habenula is stressed by the different myelination pattern of the two habenulae; the functional meaning of this anomalies is obscure.

In conclusion, also the ultrastructural differences occurring in the right and left habenulae might reflect different functional specializations, and this is an interesting observation in the light of the functional asymmetry of higher vertebrates.

References

Adrio F, Anadon R, Rodriguez-Moldes I (2000) Distribution of choline acetyltransferase (ChAT) immunoreactivity in the central nervous system of a chondrostean, the siberian sturgeon (*Acipenser baeri*) -

Journal of Comparative Neurology 426, 602-621

Anadon R, Molist P, Rodriguez-Moldes I, Lopez JM, Quintela I, Cervino MC et al. (2000) Distribution of choline acetyltransferase (ChAT) immunoreactivity in the central nervous system of an elasmobranch, the lesser spotted dogfish (*Scyliorhinus canicula*) -

Journal of Comparative Neurology 420, 139-170

Andressen, C., Blümcke, I., Celio, M.R., 1993. Calcium-binding proteins:

selective markers of nerve cells. Cell Tissue Res. 271, 181–208.

Andrew, R.J., ed. Neural and behavioural plasticity. The use of the chick as a model. Oxford: Oxford University Press; 1991:536–554

Andrew, R.J.; Mench, J.; Rainey, C. Right-left asymmetry of response to visual stimuli in the domestic chick. In: Ingle, D.J.; Goodale, M.A.; Mansfield, R.J.; eds. Analysis of visual behaviour. Cambridge: MIT press; 1982: 197-209

Andrew, R.J. and Brennan, A., The lateralization of fear behaviour in the male domestic chick: A developmental study. *Anim. Behav.,* 1983, 31, 1166-1176

Baimbridge, K.G.; Miller, J.J.; Parkers, C.O.: Calcium-binding protein distribution in the rat brain. Brain Res. 239: 519-525 (1982)

Bianki, V.L., The right and left hemispheres: Cerebral lateralization of function. Monographs in neuroscience. Vol. 3. New York: Gordon and Breach; 1988

Bisazza A, Rogers LJ, Vallortigara G "The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians" –

Neurosci Biobehav Rev. 1998 May; 22 (3): 411-26

Bisgrove BW, Essner JJ, Yost HJ (2000) Multiple pathways in the midline regulate concordant brain, heart and gut left-right asymmetry -
Development 127, 3567-3579

Borg B, Ekström P, Van Veen T (1983) The parapineal organ of teleosts -
Acta zoologica (Stockholm) 64, 211- 218

Bradshaw, J.L. Hemispheric specialization and psychological function. Chichester: Wiley; 1989

Bradshaw J.L.; Rogers L.J. The evolution of lateral asymmetries, production in

language, tool use, and intellect. New York: Academic Press; 1993

Braford MRJ, Northcutt RG (1983) Organization of the diencephalon and pretectum of ray-finned fishes -
In *Fish Neurobiology. 2. Higher Brain Areas and Functions* (ed. Davis RE, Northcutt RG), pp. 117-163. Ann Arbor: University of Michigan Press

Baimbridge, K.G.; Parkes, C.O.: Vitamin D dependent calcium-binding protein in the chick brain. Cell Calcium 2: 65-67 (1981)

Braitenberg V, Kemali M (1970) Exceptions to bilateral symmetry in the epithalamus of lower vertebrates -
Journal of Comparative Neurology 138, 137-146

Braun, K., 1990. Calcium-binding proteins in avian and mammalian central

nervous system: localization, development and possible functions. Prog.

Histochem. Cytochem. 21, 1–64.

Burdine RD, Schier AF (2000) Conserved and divergent mechanisms in left-right axis formation - Genes Dev 14, 763-776

Butler AB, Northcutt RG (1993) The diencephalon of the Pacific herring, *Clupea harengus*: cytoarchitectonic analysis - Journal of Comparative Neurology 328, 527-546

Butler AB, Hodos W (1996) Comparative Vertebrate Neuroanatomy: Evolution and Adaptation - Chichester: John Wiley

Capdevila J, Vogan KJ, Tabin CJ, Izpisua Belmonte JC (2000) Mechanisms of left-right determination in vertebrates - Cells 101, 9-21

Celio, M.R.; Norman A.W.: Nucleus basalis Meynert neurons contain the vitamin D-induced calcium-binding protein (Calbindin-D 28k). Anat. Embryol. 173: 143-148 (1985)

Cheung, W.Y., 1980. Calmodulin plays a pivotal role in cellular regulation. Science 207, 19–27.

Christakos, S.; Friedlander, E.J.; Frandsen, B.R.; Norman, A.W.: Studies on the mode of action of calciferol. 13. Development of a radioimmunoassay for vitamin D-dependent chick intestinal calcium-binding protein and tissue distribution. Endocrinology 104: 1495-1503 (1979)

Cole WC, Youson JH (1982) Morphology of the pineal complex of the anadromous sea lamprey, *Petromyzon marinus* L.- American Journal of Anatomy 165, 131-163

Concha ML, Burdine RD, Russel C, Schier AF, Wilson SW (2000) A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain - Neuron 28, 399-409

Concha ML et al. "Local tissue interactions across the dorsal midline of the forebrain establish CNS laterality" – Neuron. 2003 Jul 31; 39 (3): 423-38

Concha ML, Wilson SW "Asymmetry in the epithalamus of vertebrates" – J Anat. 2001; 199 (Pt 1-2): 63-84

Corballis MC (1998) Cerebral asymmetry: motoring on - Trends in Cognitive Sciences 2, 152-157

Cupedo, R.N.J.: The surface ultrastructure of the habenular complex of the rat. Anat. Embryol. 152 (1977) 43-64

Denenberg, V.H., Hemispheric laterality in animals and the effect of early experience. Behav. Brain Sci., 1981, 4, 1-49

De Renzi, E., Disorders of space, exploration and cognition. New York: Wiley; 1982

Diaz C, Puelles L (1992a) Afferent connections of the habenular complex in lizard *Gallotia galloti* - Brain behaviour and Evolution 39, 312-324

Ekström P, Ebbesson SO (1988) The left habenular nucleus contains a discrete serotonin-immunoreactive subnucleus in the coho salmon (*Oncorhynchus kisutch*) Neurosci letters 91, 121-125

Ekström P, Meissl H (1997) The pineal organ of teleost fishes - Review in Fish Biology and Fisheries 7, 199-284

Enderlin, S.; Norman, A.W.; Celio, M.R.: Ontogeny of the calcium binding protein calbindin-D28k in the rat nervous system. Anat.

Embryol. 177: 15-28 (1987)

Engbretson GA, Brechan N, Reiner A (1981) Habenular asymmetry and the central connections of the parietal eye of the lizard - Journal of Comparative Neurology 198, 155-165

Fekete DM "Left-right brain asymmetry: lessons from the heart" –

Trends Neurosci. 2001 Jan; 24 (1): 6

Feinblatt, J.D.: The comparative physiology of calcium regulation in submammalian vertebrates. Adv. Compar. Physiol. Biochem. 8: 73-110 (1982)

Fernald RD, Shelton LC (1985) The organization of the diencephalon and the pretectum in the cichlid fish, *Haplochromis burtoni* - Journal of Comparative Neurology 238, 202-217

Folgueira M, Anadon R, Yaňez J. 2004a. An experimental study of the
connections of the telencephalon in the rainbow trout (*Oncorhynchus
mykiss*). I. Olfactory bulb and ventral area. J Comp Neurol 480:180 –

203.

Gamse JT, Thisse C, Thisse B, Halpern ME "The parapineal mediates left-right asymmetry in the zebrafish diencephalon" –

Development. 2003 Mar; 130 (6): 1059-68

Garcia-Segura, L.M.,Baetens,D., Roth,J., Norman,A.W., Orci,L.,

1984. Immunohistochemical mapping of calcium-binding protein

immunoreactivity in the rat central nervous system. Brain Res. 296,

75–86

Godement P, Vanselow J, Thanos S, Bonhoeffer F.

1987. A study in developing visual systems with a new method of staining neurones and their

processes in fixed tissue. Development 101:697–713.

Gomez-Segade P, Anadon R (1988) Specialization in the diencephalon of advanced teleosts -

Journal of Morphology 197, 71-103

Goodale, M.A.; Mansfield, R.J., eds. Analysis of visual behaviour. cues in food-storing and non-storing birds. J. Comp. Physiol. A, 1994, Cambridge: MIT Press; 1982:197–209. 174, 371–379.

Guglielmotti V, Fiorino L (1998) Asymmetry in the left and right habenulo-interpeduncolar tracts in frog -

Brain Research Bulletin 45, 105-110

Guglielmotti V, Fiorino L (1999) Nitric Oxide Synthase activity reveals an asymmetrical organization of the frog habenulae during development: a histochemical and cytoarchitectonic study from tadpoles to the mature *Rana esculenta,* with notes on the pineal complex -
Journal of Comparative Neurology 411, 441-454

Güntürkün, O., Lateralization of visually controlled behavior in
pigeons. Physiol. Behav., 1985, 34, 575–577

Jande, S.S.; Maler, L.; Lawson, D.E.M.; Immunohistochemical mapping of vitamin D-dependent calcium-binding protein in brain. Nature 294: 765-767 (1981a)

Halpern ME, Liang JO, Gamse JT "Leaning to the left: laterality in the zebrafish forebrain" –

Trends Neurosci. 2003 Jun; 26 (6): 308-13

Harris JA, Guglielmotti V, Bentivoglio M "Diencephalic asymmetries" –

Neurosci Biobehav Rev. 1996 Winter; 20 (4): 637-43

Hauser, M.D., Right hemisphere dominance for the production of facial expression in monkeys. Science, 1993, 261, 475-477

Herkenham M, Nauta WJH (1977) Afferent connections of the habenular nuclei in the rat: a horseradish peroxidase study, with a note on the fiber-passage problem -

Journal of Comparative Neurology 173, 123-146

Herkenham M, Nauta WJH (1979) Efferent connections of the habenular nuclei in the rat -

Journal of Comparative Neurology 187, 19-48

Holloway, R.L. and de LaCoste-Lareymondie, M.C., Some preliminary

findings on the paleontology of cerebral dominance. Am. J.

Phys. Ant., 1982, 58, 101–110

Holmqvist BI, Östholm T, Ekström P. 1992. DiI tracing in combination

with immunocytochemistry for analysis of connectivities and chemoarchitectonics of specific neural systems in a teleost, the Atlantic salmon. J Neurosci Meth 42:45–63.

Hook-Costigan, M.A., and Rogers, L.J., Each hemisphere produces differents vocalizations: Contact and fear calls of marmosets. Xxxx, 1997, Neuropsychologia, submitted

Huesa G, Anadon R, Yaňez J. 2000. Olfactory projections in a Chondrostean fish, Acipenser baeri: an experimental study. J Comp Neurol

428:145–158.

Huesa G, Anadon R, Yaňez J. 2003. Afferent and efferent connections of

the cerebellum of the chondrostean Acipenser baeri: a carbocyanine dye

(DiI) tracing study. J Comp Neurol 460:327–344.

Ifune, C.K., Vermeire, B. and Hamilton, C.R., Hemispheric differences

in split-brain monkeys viewing and responding to videotape

recordings. Behav. Neur. Biol., 1984, 41, 231–235

Izraeli S et al. "The SIL gene is required for mouse embryonic axial development and left-right specification" –

Nature. 1999 Jun 17; 399 (6737): 691-4

Kemali (1984) Morphological asymmetry of the habenulae of a macrosmatic mammal, the mole -

Zeitschrift für Mikroskopisch Anatomische Forschung 98, 951-954

Kemali M, Agrelli I (1972) The habenulo-interpeduncolar nuclear system of a reptilian representative *Lacerta sicula* -

Zeitschrift für Mikroskopisch Anatomische Forschung 85, 325-333

Kemali M, Guglielmotti V (1977) An electron microscope observation of the right and the two left portions of the habenular nuclei of the frog -

Journal of Comparative Neurology 176, 133-148

Kemali M, Miralto A (1979) The habenular nuclei of the elasmobranch *Scyllium stellare:* myelinated perikarya -

American Journal of Anatomy 155, 147-152

Kemali M, Guglielmotti V, Fiorino L (1990) The asymmetry of the habenular nuclei of female and male frogs in spring and in winter -

Brain Research 517, 251-255

Kemali M et al. "Asymmetry of the habenulae in the elasmobranch *Scyllium stellare.* Light microscopy"-
Z Mikrosk Anat Forsch. 1980; 94 (5): 801-813

King, V.R. and Corwin, J.V., Spatial deficits and hemispheric
asymmetries in the rat following unilateral and bilateral lesions of
posterior parietal or medial agranular cortex. Behav. Brain Res.,
1992, 50, 53–68

Kumar. K., and T.C. Anand Kumar: The habenular ependyma: a neuroendocrine component of the epithalamus in the resus monkey. In: Anatomical Neuroendocrinology. Eds.: W. E. Stumpe and L.D. Grant. Basel: Karger 1975, 40-51

Liang JO et al. "Asymmetric nodal signaling in the zebrafish diencephalon positions the pineal organ" –
Development. 2000 Dec; 127 (23): 5101-12

Long S, Ahmand N, Rebagliati M "The zebrafish nodal-related gene southpaw is required for visceral and diencephalic left-right asymmetry" –
Development. 2003 Jun; 130 (11): 2303-16

Maler L, Sas E, Johnston S, Ellis W (1991) An atlas of the brain of the electric fish *Apteronotus leptorhynchus* -
Journal of Chemistry and Neuroanatomy 4,1-38

Masai I, Heisenberg CP, Barth KA, MacDonald R, Adamek S, Wilson SW (1997) *floating head* and *masterblind* regulate neuronal patterning in the roof of the forebrain -
Neuron 18, 43-57

Miralto A, Kemali M "Asymmetry of the habenulae in the elasmobranch *Scyllium stellare.*

Electron microscopy"-
Z Mikrosk Anat Forsch. 1980; 94 (5): 794-800

Morgan MJ, O'Donnel JM, Oliver RF (1973) Development of left-right asymmetry in the habenular nuclei of *Rana temporaria* -
Journal of Comparative Neurology 149, 203-214

Morris, R.M. and Hopkins, W.D., Perception of human chimeric
faces by chimpanzees: evidence for a right hemisphere advantage.
Brain Cognit., 1993, 21, 111–122

Nottebohm, F., Neural lateralization of vocal control in a Passerine
bird. I. Song. J. Exp. Zool., 1971, 177, 229–261

Nottebohm, F. Asymmetries in neural control of vocalization in the
canary. In: Harnad, S., ed. Lateralization of the nervous system. New York: Academic Press; 1977:23–44

Nottebohm, F., Alvarez-Buylla, A., Cinx, J., Chang-Ying, L., Nottebohm,
M., Suter, R., Tolles, A. and Williams, H., Song learning in
birds: the relation between perception and production. Phil. Trans. R.
Soc. Lond., 1990, 329, 115–124.

Oksche A (1965) Survey of the development and comparative morphology of the pineal organ -
Progress in Brain Research 10, 3-29

Parmentier, M.; Ghysens, M.; Rypens, F.; Lawson, D.E.M.; Pasteels, J.L.; Pochet, R.: Calbindin in vertebrate classes: immunohistochemical localization and Western blot analysis. Gen. Compar. Endocr. 65: 399-407 (1987)

Parsons, C.H. and Rogers, L.J., Role of the tectal and posterior
commissures in lateralization of the avian brain. Behav. Brain Res.,
1993, 54, 153–164

Petersen, M.R., Beecher, M.D., Zoloth, S.R., Moody, D.B. and
Stebbins, W.C., Neural lateralization of species-specific vocalizations
by Japanese macaques (*Macaca fuscata*). Science, 1978, 202,
324–327

Petersen, M.R., Beecher, M.D., Zoloth, S.R., Green, S., Marler, P.R.,
Moody, D.B. and Stebbins, W.C., Neural lateralization of vocalizations
by Japanese macaques: communicative significance is more
important than acoustic structure. Behav. Neurosci., 1984, 98, 779–
790

Proudfoot, R.E., Hemiretinal differences in face recognition: Accuracy
versus reaction time. Brain Cognit., 1983, 2, 25–31

Rabiè, A.; Thomasset, M.; Legrand, C.: Immunohistochemical detection of calcium-binding protein in the cochlear and vestibular hair cells of the rat. Cell Tiss. Res. 232: 691-696 (1983)

Rashid, N.Y. and Andrew, R.J., Right hemisphere advantage for
topographical orientation in the domestic chick. Neuropsychologia,
1989, 27, 937–948

Regolin, L. and Vallortigara, G., Lateral asymmetries in the response

to novel-coloured objects in the domestic chick: A developmental
study. Behav. Process., 1996, 37, 67–74

Rodriguez-Moldes I, Timmermans JP, Adriaensen D, De Groodt-Lasseel MH, Scheuermann DW, Anadon R (1990) Asymmetric distribution of calbindin-D28K in the ganglia habenulae of an elasmobranch fish -
Anatomy and Embriology 181, 389-391

Rogers, J.H., Khan, M., Ellis, J., 1990. Calretinin and other CaBPs in the
nervous system. Adv. Exp. Med. Biol. 269, 195–203.

Rogers LJ (2000) Evolution of hemispheric specialisation: advantages and disavantages -
Brain and Language 73, 236-253

Rogers, L.J. Development of lateralization. In: Andrew, R.J., ed.
Neural and behavioural plasticity: The use of the domestic chick as a
model. Oxford: Oxford University Press; 1991:507–535

Sackeim, H.A., Gur, R.C. and Saucy, M.C., Emotions are expressed
more intensely on the left side of the face. Science, 1978, 202, 434–
436.

Schier AF, Shen MM "Nodal signalling in vertebrate development"
Nature. 2000 Jan 27; 403 (6768): 385-9

Smeets et al. "The central nervous system of cartilaginous fishes. Structure and functional

correlations"

1983, New York SPRINGER

Striedter GF (1990) The diencephalon of the channel catfish, *Ictalurus punctatus*. I. Nuclear organization -

Brain behaviour and Evolution 36, 329-354

Sutherland RJ (1982) The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex -

Neuroscience Biobehavioural Review 6, 1-13

Taylor, A.N.: Chick brain calcium-binding protein: comparison with intestinal vitamin D-induced calcium-binding protein. Archs Biochem. Biophys. 161: 100-108 (1974)

Tokunaga, A., and K. Otani: Fine structure of the medial habenular nucleus in the rat. Brain Res. 150 (1978) 600-606

Vallortigara G, Rogers LJ, Bisazza A (1999) Possible evolutionary origins of cognitive brain lateralisation -

Brain Research Review 30, 164-175

Vallortigara, G. and Andrew, R.J., Olfactory lateralization in the
chick. Neuropsychologia, 1994, 32, 417–423

Vallortigara, G. and Andrew, R.J., Differential involvement of right
and left cerebral hemisphere in individual recognition in the domestic
chick. Behav. Process., 1994, 33, 41–58

Vallortigara, G., Regolin, L., Bortolomiol, G. and Tommasi, L.,
Lateral asymmetries due to preferences in eye use

during visual
discrimination learning in chicks. Behav. Brain Res., 1996, 74, 135–
143

Vallortigara, G., Right hemisphere advantage for social recognition
in the chick. Neuropsychologia, 1992, 30, 761–768.

Vallortigara, G. and Andrew, R.J., Lateralization of response by
chicks to change in a model partner. Anim. Behav., 1991, 4, 187–194

Villani, L., Dipietrangelo, L., Pallotti, C., Pettazzoni, P., Zironi, I. And Guarnieri, T. Ultrastructural and immunohistochemical study of the telencephalo-habenulo-interpeduncolar connections of the goldfish. Brain Res. Bull., Vol. 34, No. 1, pp. 1-5 (1994) Elsevier Science Lld

Wallman, J. and Pettigrew, J.D., Conjunctive and disjunctive saccades
in two avian species with contrasting oculomotor strategies.
J. Neurosci., 1985, 5, 1418–1428

Wang RY, Aghajanian GK (1977) Physiological evidence for habenula as major link between forebrain and midbrain raphe -

Science 197, 89-91

Ward, J.P.; Hopkins, W.D. Primate laterality: Current behavioural
evidence of primate asymmetries. New York: Springer Verlag.; 1993

Wasserman, R.H., Nomenclature of the vitamin D-induced calcium-binding proteins; in Norman, Vitamin D. A chemical, biochemical and clinical

update, pp. 321-322 (de Gruyter, Berlin 1985)

Wasserman, R.H.; Taylor, A.N.; Vitamin D3-induced calcium-binding protein in chick intestinal mucosa. Science 152: 791-793 (1966)

Wolf, M.E. and Goodale, M.A., Oral asymmetries during verbal and
nonverbal movements of the mouth. Neuropsychologia, 1987, 25,
375–396

Wilson, J.F.; Dodd, J.M.: Distribution of monoamines in the diencephalon and pituitary of the dogfish, Scyliorhinus canicula. L.Z. Zellforsch. Mikrosk. Anat. 137: 451-469 (1973)

Wullimann MF. 1988. The tertiary gustatory center in sunfishes is not nucleus glomerulosus. Neurosci Lett. 86: 6-10

Yanez J, Anadon R (1994) Afferent and efferent connections of the habenula in the larval sea lamprey (*Petromyzon marinus* L.): an experimental study -
Journal of Comparative Neurology 345, 148-160

Yanez J, Anadon R (1996) Afferent and efferent connections of the habenula in the rainbow trout (*Oncorhynchus mykiss*): an indocarbocyanine dye (DiI) study -
Journal of Comparative Neurology 372, 529-543

Yanez J, Meissl H, Anadon R (1996) Central projections of the parapineal organ of the adult rainbow trout (*Oncorhynchus mykiss*) -
Cell and Tissue Research 285, 69-74

Yanez J, Pombal MA, Anadon R (1999) Afferent and efferent connections of the parapineal organ in lampreys: a tract tracing and immunocytochemical study -
Journal of Comparative Neurology 403, 171-189

Zhu L et al. "Cerberus regulates left-right asymmetry of the embryonic head and heart"
Curr Biol. 1999 Sep 9; 9 (17): 931-8