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RETINOFUGAL PATHWAYS IN FETAL AND ADULT SPINY DOGFISH, *Squalus acanthias*

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SUMMARY

Retinofugal pathways in fetal and adult spiny dogfish were determined by intraocular injection of [^{3}H]proline for autoradiography. Distribution and termination of the primary retinal efferents were identical in pups and adults. The retinal fibers decussate completely, except for a sparse ipsilateral projection to the caudal preoptic area. The decussating optic fibers terminate ventrally in the preoptic area and in two rostral thalamic areas, a lateral neuropil area of the dorsal thalamus and more ventrally in the lateral half of the ventral thalamus. At this same rostral thalamic level, a second optic pathway, the medial optic tract, splits from the lateral marginal optic tract and courses dorsomedially to terminate in the rostral tectum and the central and periventricular pretectal nuclei. The marginal optic tract continues caudally to terminate in a superficial pretectal nucleus and also innervates the superficial zone of the optic tectum. A basal optic tract arises from the ventral edge of the marginal optic tract and courses medially into the central pretectal nucleus, as well as continuing more caudally to terminate in a dorsal neuropil adjacent to nucleus interstitialis and in a more ventrally and medially located basal optic nucleus.

Comparison of the retinofugal projections of Squalus with those of other sharks reveals two grades of neural organization with respect to primary visual projections. Squalomorph sharks possess a rostral dorsal thalamic nucleus whose visual input is primarily, if not solely, axodendritic, and an optic tectum in which the majority of the cell bodies are located deep to the visual terminal zone. In contrast, galeomorph sharks are characterized by an enlarged and migrated rostrodorsal thalamic visual nucleus, and an optic tectum in which the majority of the cell bodies are located within the visual terminal zone. These data suggest that evolution of primary visual pathways in sharks occurs by migration and an increase in neuronal number, rather than by the occurrence of new visual pathways.

INTRODUCTION

Small sharks of the genus Squalus are distributed world-wide and belong to the superorder Squalomorphii. This group of sharks retain far more primitive neoselachian features than any other group of living elasmobranchs[2]. Unlike more advanced sharks (Galeomorphii), Squalus and its allies retain low brain:body ratios, poorly developed pallial areas of the telencephalon, a diencephalon characterized by prominent periventricular laminae, a relatively undifferentiated optic tectum and a cerebellum lacking foliation[11].

There is considerable information on the neural organization of specific galeomorph sharks[3], but little information on the range of variation and evolutionary trends characterizing the nervous systems of sharks. Such information would be useful in adding neurological detail to data on a major group of vertebrates. More importantly, because elasmobranchs have an independent evolutionary history for the last 400 million years, they offer an opportunity to discover whether similar neural solutions have evolved in response to common biological problems confronting many vertebrate species.

Examination of the retinofugal pathways in Squalus, reported here, was undertaken as part of a general program whose goal is the analysis of variation in elasmobranch neural organization.

During the course of this study, I discovered that Squalus pups can survive removal from the mother's uteri some 4–6 months prior to normal birth. This discovery allowed me to determine experimentally the state of development of the retinofugal pathways in these pups, in addition to obtaining information on subsequent development of the visually related neural centers. A preliminary report of this work appeared previously[10].

MATERIALS AND METHODS

Eight fetal spiny dogfish (total length, 13–16 cm) were removed from the uteri of females under MS222 anesthesia, and placed in natural sea water at 9–11 °C. Each pup received an intraocular injection of 60 μCi of [^{3}H]proline (20 μCi/μl) and pairs were allowed to survive 12, 34, 48 and 72 h respectively. The brains were cut in either transverse or horizontal plane and the sections coated with NTB-3 emulsion. After an exposure of 20–30 days at 7 °C, the sections were developed in Dektol and stained with cresyl violet.

Nine adult specimens of *Squalus acanthias* (total length, 76–83 cm) received intraocular injections of 100–200 μCi of [^{3}H]proline (20 μCi/μl) under MS222 anesthesia and were allowed to survive 24, 48, 54, 72, 124 and 216 h at 10–13 °C. The adults were perfused with AFA (90 ml of 80 % ethanol, 5 ml formalin, and 5 ml glacial acetic acid), and the brains stored in AFA for at least one week prior to dehydration and embedding in paraffin. Subsequent autoradiographic processing was identical to that of the fetal material.

Selected sections from the autoradiographic cases were photographed on

Kodalith Ortho film, type 3, with a Leitz large-format camera. The distribution of the retinofugal pathways and their terminal fields was charted on these photographs. Additional series of sections processed with Bodian, Golgi–Cox and Klüver–Barrera methods were available for study of nuclear groups and fiber tracts in Squalus and a number of other elasmobranch species.

RESULTS

There are marked differences in the survival times necessary to label retinofugal pathways in Squalus pups and adults. Pups with survival times as short as 24 h showed moderate label over the same diencephalic nuclei and superficial portions of the optic tectum that receive retinal terminals in the adults. However, survival times of two days revealed incomplete transport throughout the retinofugal pathways of adults. These animals revealed label over the diencephalic retinorecipient nuclei but not over the optic tract or the superficial layers of the optic tectum. With a survival time of 3 days, both diencephalic and mesencephalic targets exhibited high grain densities, while the optic tract was more sparsely labeled. With a survival time of 6 days, both the optic tract and terminal targets exhibited high grain densities. These survival times are relatively long for demonstrating retinofugal projections in vertebrates but are most likely due to the long length of the optic nerve and tract (approximately 5 cm in Squalus adults) and a low metabolic rate indicated by the water temperatures of 10–13 °C. (These are not unusual temperatures for Squalus, a cold water shark whose upper temperature tolerance is approximately 15 °C[1].)

Two criteria were used to determine fibers of passage from terminal fields. Grain densities higher than those over the optic tract — particularly when they occurred in areas of neuropil or over cell bodies medial to the optic tract — were judged to represent terminal fields. Labeled proteins are believed to accumulate in the terminals of retinal ganglion axons prior to substantial labeling of the axons, thus providing a second criterion for distinguishing terminal fields from axons of passage in cases with shorter survival times. The pattern of distribution and termination of the primary retinal efferents was identical for pups and adults. I have chosen to illustrate the visual pathways of the fetal material primarily, as the cell groups and their boundaries are not obscured by further brain enlargement (some 24-fold) that occurs with subsequent development. The levels of the illustrated sections (Fig. 1–4) are shown in Fig. 1A.

Normal anatomy

At present experimental studies on the diencephalon of elasmobranchs are difficult to communicate because of the absence of detailed descriptions of this region. The older descriptions[7] are of little help, as they give no details regarding nuclei and merely describe major subdivisions of the diencephalon. More recent studies[4,5] are more detailed with regard to the nuclei that receive retinal efferents, but few criteria for recognizing these groups are given.

In this description, the thalamus is defined as a region lying ventral to the habenula and dorsal to the preoptic area and hypothalamus (Fig. 1–4A). It extends

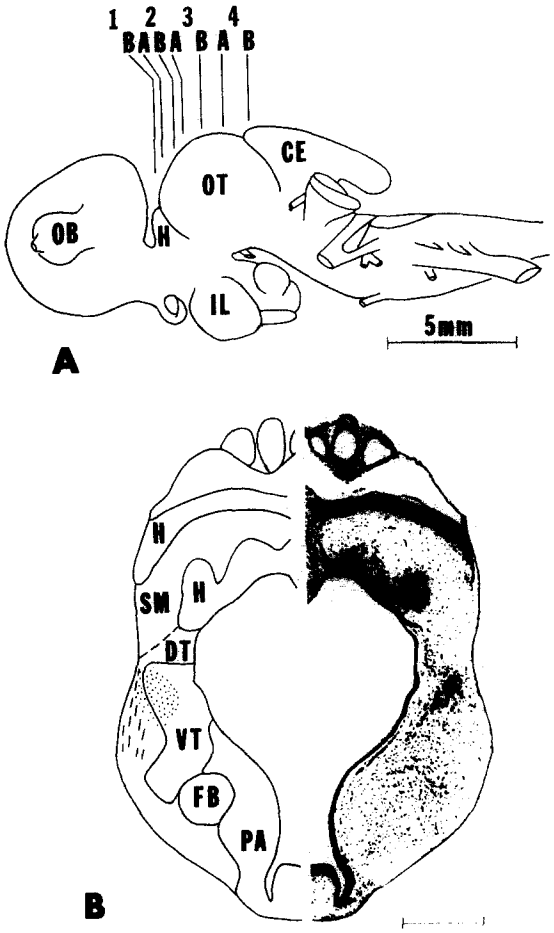

Fig. 1. A: lateral view of the brain of a Squalus pup. Numbered lines indicate the levels of the transverse sections in Figs. 1B–4. B: transverse section through the level of the rostral thalamus illustrating the most anterior retinofugal terminal field in the ventral thalamus. In this figure and Figs. 2–4, a Nissl preparation is shown on the right. To the left is a drawing of the side of the brain contralateral to the injected eye. Retinal fibers of passage are represented by dashed lines and terminal fields by stippling. The chartings are based on an autoradiographic case that survived for two days. Bar scales in Figs. 1B and 2–4 equal 500 μm. Abbreviations: BON, basal optic nucleus; C, central tectal zone; CE, cerebellum; CP, central pretectal nucleus; DT, dorsal thalamus; FB, forebrain bundles; FR, fasciculus retroflexus; H, habenular nuclei; HC, habenular commissure; HY, hypothalamus; IC, intercollicular nucleus; IL, inferior lobe; IN, nucleus interstitialis; IP, interpeduncular nucleus; MLF, medial longitudinal fasciculus; NP, nucleus profundus mesencephali; OB, olfactory bulb; OC, optic chiasm; ON, optic nerve; OT, optic tectum; P, periventricular tectal zone; PA, preoptic area; PC, posterior commissure; PP, periventricular pretectal nucleus; S, superficial tectal zone; SC, subcommissural organ; SM, stria medullaris; SP, superficial pretectal nucleus; T, tegmentum; TS, torus semicircularis; VT, ventral thalamus; III, oculomotor nucleus.

from the telencephalon medium (composed of the forebrain bundles, stria medullaris and rostral preoptic area) caudally to the level of the midbrain tegmentum and nucleus interstitialis (Fig. 4A). In most descriptions[6,7] the thalamus is divided into dorsal and ventral divisions. The ventral thalamus of anamniotes is usually defined as a series of

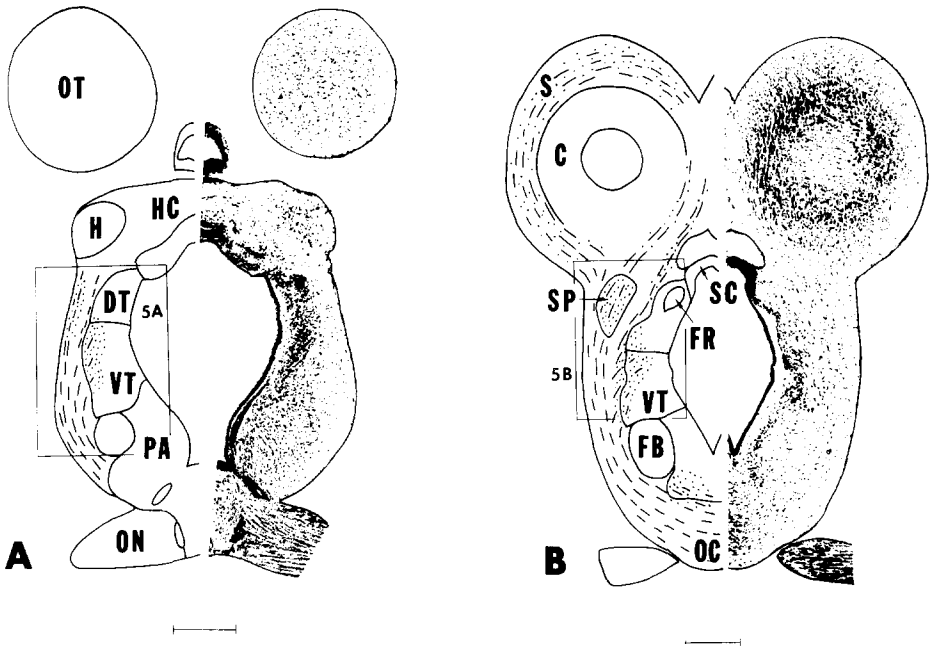

Fig. 2. A: transverse section through diencephalon illustrating rostral dorsal thalamic neuropil that receives retinal terminals. B: transverse section through pretectal area and suprachiasmatic retinal target. Abbreviations as in Fig. 1.

parallel laminae of compact cells lying adjacent to the sulcus medius thalami[9,11,13]. However, this sulcus cannot be used to identify an exact boundary between the dorsal and ventral thalamus, because its position varies rostrocaudally with reference to cell groups that run the length of the thalamus[9,11]. Several criteria allow recognition of thalamic nuclei in Squalus: differences in cell size, differences in packing density, cell-free boundaries, and similarities with diencephalic nuclei in other vertebrates. Finally, nuclei defined by these criteria can be confirmed by experimentally determined connections.

Rostrally the ventral thalamus of Squalus can be distinguished from the dorsal thalamus by differences in the thickness of the periventricular cellular plates and a cell-free boundary (Fig. 1B). A similar condition exists in other fishes[9,13]. Rostrally the dorsal thalamus consists of two major laminae: a thin medial lamina and a larger densely packed lateral lamina with a sparsely celled neuropil that receives retinal input (Fig. 2A and 5A). More caudally the lateral lamina drops out and is replaced by a sparse population of neurons embedded in the optic tract and here termed the superficial pretectal nucleus (Figs. 2B and 5B). Unlike the lateral lamina, the rostral medial lamina of the dorsal thalamus continues caudally and expands ventrally, assuming the shape of an inverted V (Fig. 2B). Further caudally the lamina is replaced by nucleus interstitialis at midbrain levels (Fig. 4A). The medial lamina of the dorsal thalamus forms the bulk of the thalamus in adults and is not a homogeneous plate, but can be divided into 4 or 5 subdivisions on the basis of differences in cell size and

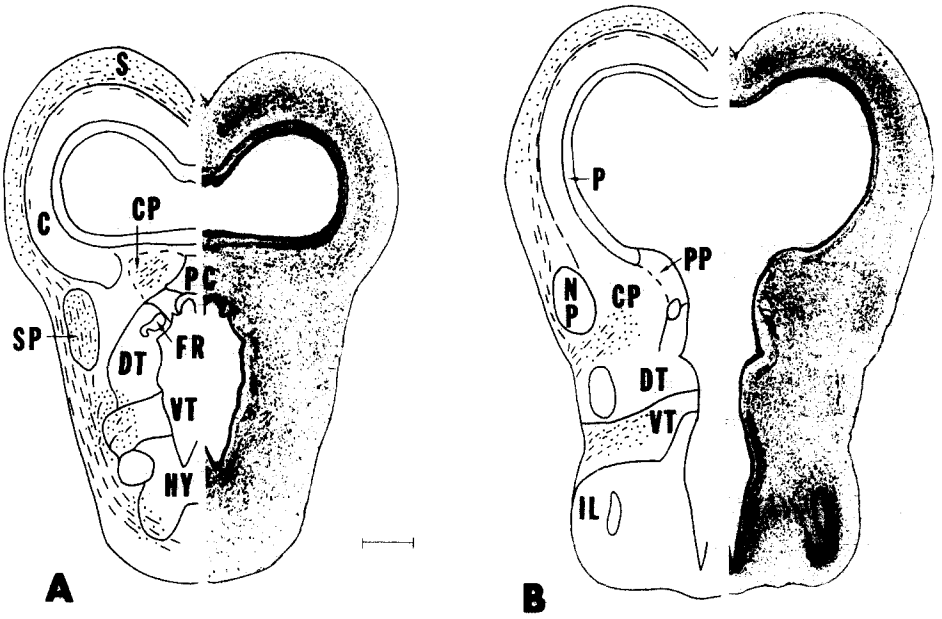

Fig. 3. A: transverse section through pretectal area at level of subcommissural organ. B: transverse section through caudal pretectal area. Abbreviations as in Fig. 1.

packing densities. However, analysis of the exact number and extent of these nuclei requires further experimental information.

Rostrally the ventral thalamus also consists of two cell groups: a dorsal circular nucleus and a ventral, more oblong, nucleus with more densely packed cells (Figs. 1B–3A). The ventrolateral edge of the ventral nucleus juts laterally around the edge of the forebrain bundles (Figs. 1 and 2) and may represent an entopeduncular nucleus as in many other non-mammalian vertebrates. Caudally the ventral thalamus is replaced by the midbrain tegmentum, which is first recognized as a medial and dorsal continuation of the hypothalamus (Fig. 3B and 4).

The caudal dorsal thalamus or pretectum consists of 3 cellular groups lying dorsal and lateral to the subcommissural organ and posterior commissure (Figs. 2B and 3). These pretectal nuclei correspond topologically to the rostral continuation of the superficial, central and deep or periventricular tectal cell layers (Fig. 3), and have therefore been named accordingly.

Three major cellular zones comprise the optic tectum of Squalus (Figs. 2–4). From deep to superficial, they are (1) periventricular gray zone, (2) central gray zone, and (3) superficial gray zone (Fig. 3B). The cells of the periventricular zone are piriform-shaped, with apical dendrites that branch in the central and superficial zones[8]. The majority of the tectal cells of Squalus lie in the central tectal zone, rather than in the periventricular zone as in bony fishes; however, the central zone consists of pyramidal and bipolar cells as in bony fishes[8]. Optic fibers do not project onto the surface of the optic tectum but enter more deeply, forming the ventral border of the superficial tectal zone (Figs. 3,4 and 5C). The bulk of the optic fibers course in the

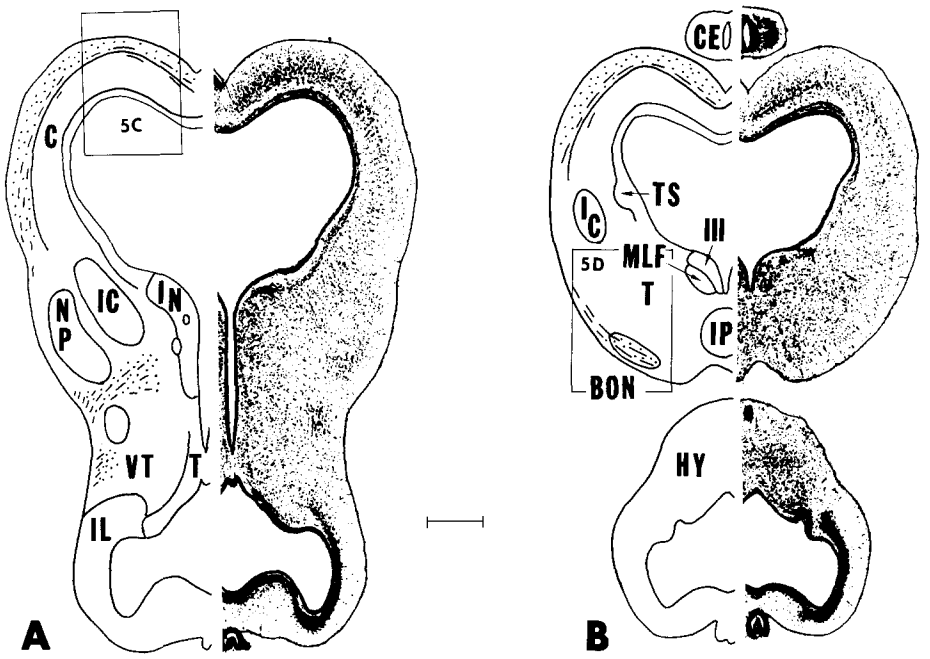

Fig. 4. A: transverse section through caudal ventral thalamic region illustrating dorsal fascicle of basal optic tract terminating in neuropil lateral to interstitial nucleus. B: transverse section through mesencephalon illustrating ventral fascicle of basal optic tract terminating in basal optic nucleus. Abbreviations as in Fig. 1.

ventral half of the superficial zone and all optic terminals appear confined to this tectal zone. Unlike bony fishes the superficial tectal zone of Squalus cannot be divided into sharply defined alternating layers of optic fibers and their terminal neuropils.

Retinal projections

The labeled optic nerve fibers decussate completely with the exception of a small ipsilateral projection to the caudal preoptic area. The decussating optic fibers also appear to terminate in the contralateral preoptic area (Fig. 2B) where caudally cells migrate ventrally among the decussating optic fibers (Fig. 3A). The crossed optic fibers form a lateral or marginal optic tract which courses both dorsally and rostrally (Figs. 1 and 2). The most rostral thalamic targets reached by the optic fibers are the dorsal and ventral nuclei of the ventral thalamus (Fig. 1B). At this level the optic fibers turn caudally and terminate more dorsally in the neuropil of the lateral lamina of the rostral dorsal thalamus (Figs. 2A and 5A). At this same level a second optic pathway the medial optic tract, forms by splitting from the marginal optic tract and courses dorsomedially, where it divides into dorsal and ventral fascicles. The dorsal fascicle courses over the intertectal commissure and enters the rostral tectum (Fig. 2B). The ventral fascicle continues caudally through the pretectal area and terminates among the cells of the central pretectal nucleus (Fig. 3A). Cells of both the periventricular

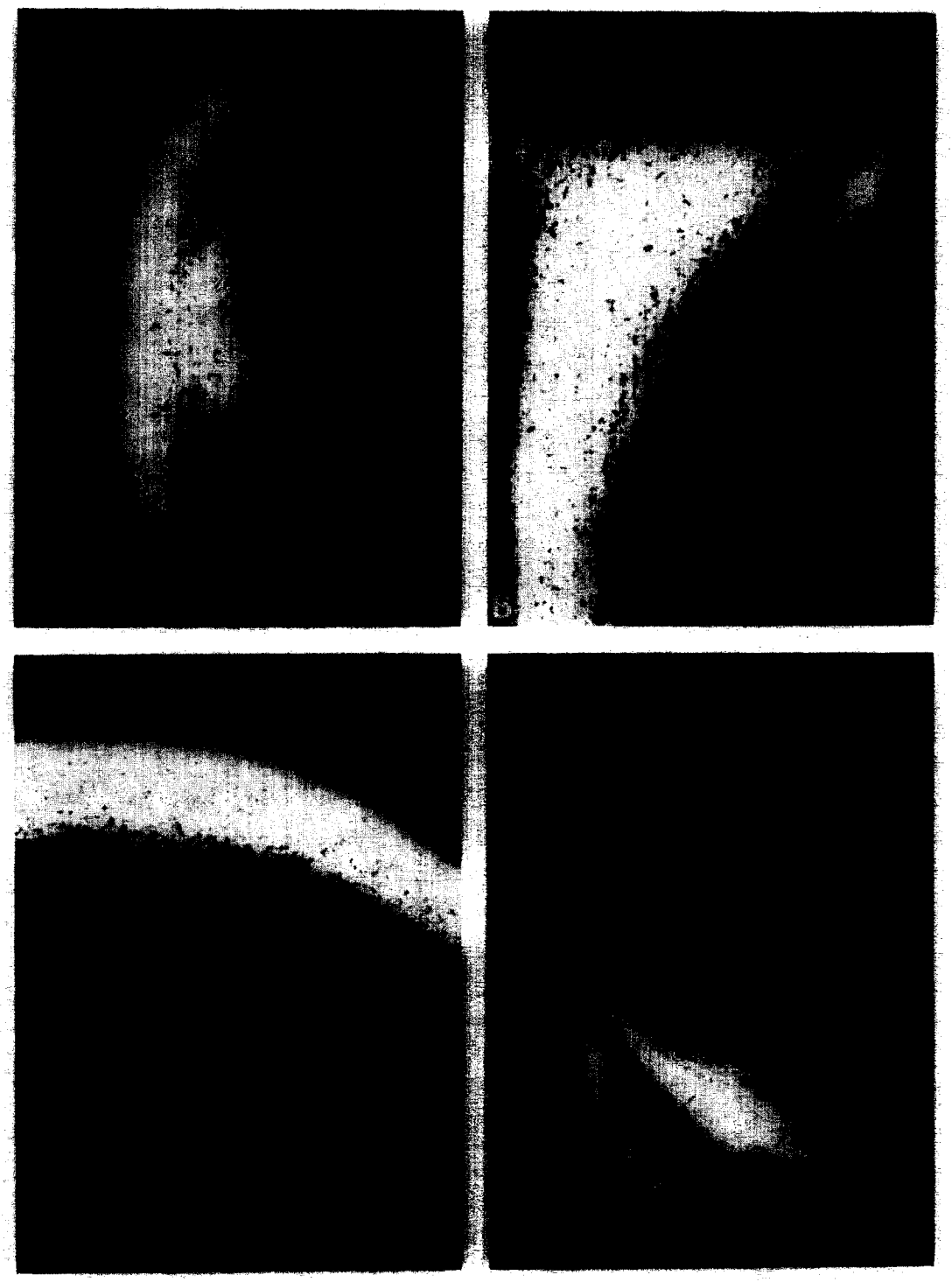

Fig. 5. Dark-field photomicrographs of the retinofugal pathways and terminal fields in a Squalus pup after intraocular injection of tritiated proline. A: contralateral optic tract and rostral thalamic terminal fields. Orientation and extent of photographed field illustrated in Fig. 2A. Bar scale equals 250 μm for A–D. Line at right of figure indicates boundary between dorsal (dt) and ventral (vt) thalamus. B: contralateral optic tract passing through superficial pretectal nucleus (cells are seen as black gaps among the white grains). The medial optic tract is seen to the right of the figure as it enters the medial margin of the tectum. Orientation and extent of photographed field illustrated in Fig. 2B. C: contralateral optic tectum illustrating optic fibers and terminals in superficial zone. Orientation and extent of photographed field illustrated in Fig. 4A. D: contralateral basal optic tract and terminal field in basal optic nucleus. Orientation and extent of photographed field illustrated in Fig. 4B.

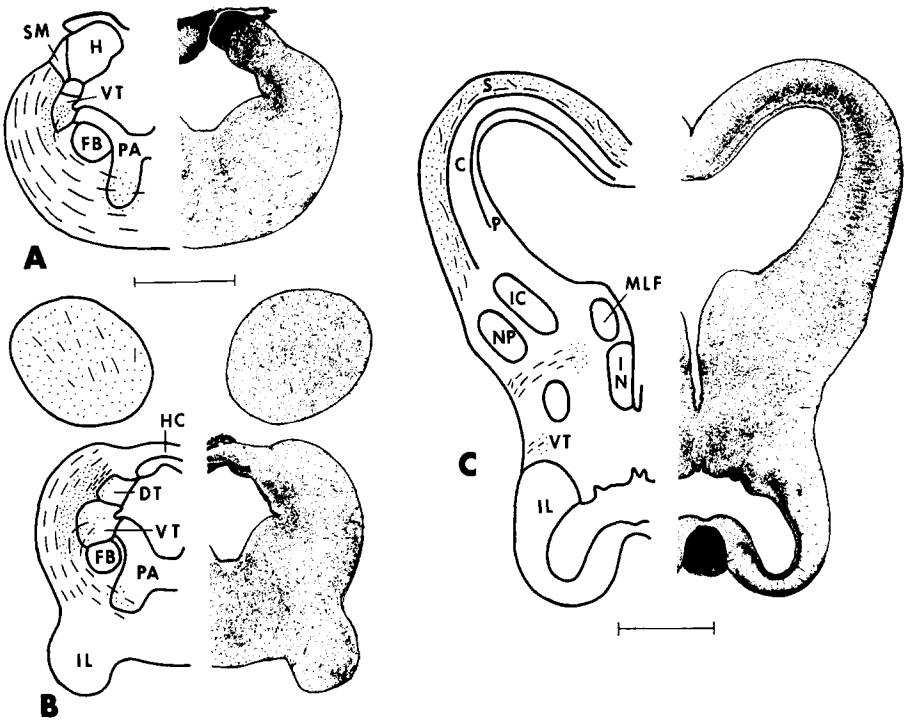

Fig. 6. Transverse sections through rostral thalamic levels (A and B) and more caudal midbrain level (C) in adult Squalus. Retinal fibers of passage indicated by dashed lines and terminal fields by stippling. Chartings based on SA-R-8, which survived 6 days postoperatively. Bar scales equal 2 mm. Magnification of A and B identical. Abbreviations as in Fig. 1.

pretectum and tectum may receive retinal input via this ventral fascicle of the medial optic pathway, as both possess apical dendrites that enter the central pretectal terminal zone.

Whereas only two distinct dorsal thalamic nuclei (rostral thalamic and superficial pretectal nuclei) receive visual input, the ventral thalamus receives retinal fibers throughout its entire lateral rostrocaudal extent (Figs. 1–4). The ventral thalamus extends far caudally beneath the optic tectum (Fig. 4A) and as its caudal border is approached (Fig. 3B) retinal fibers terminate medially almost in contact with the periventricular cell bodies.

At the same ventral caudal level a basal optic tract arises from the ventral optic tract and courses medially into the central pretectal nucleus (Fig. 3B), as well as continuing caudally. As it courses caudally, it divides into a dorsal fascicle that terminates in a neuropil just lateral to nucleus interstitialis (Fig. 4A) and a ventral fascicle that projects ventrally and medially along the pial surface to terminate in a basal optic nucleus of the midbrain tegmentum (Figs. 4B and 5D).

DISCUSSION

The gestation period of Squalus is approximately 20 months[1]. These sharks are

ovoviviparous, and there are no placental analogues as exist in many carcharhiniform sharks. Thus although the pups are retained within the uterus, their nutrient source remains an extensive yolk sac. They swim about in the uterine fluid and respire by pumping uterine fluid over their pharyngeal gills. Thus, the locomotive and respiratory movements of pups several months before birth are similar to those of the adults.

The present study reveals that the central nervous system of the unborn pups is well differentiated some 4–6 months prior to birth. As regards their visual system, the same diencephalic and mesencephalic nuclei can be recognized as in the adults (Fig. 6), and the pups already possess the retinal pathways that characterize the adults. It is not known whether the visual system of the pups is functional at this stage of development.

The pups offer an exciting opportunity to explore the parameters of neural development in an additional class of large-brained vertebrates; they may also prove extremely useful in further experimental study of elasmobranch central nervous system organization. There have been few experimental neuroanatomical studies on elasmobranchs due, in part, to the problems of maintaining large marine animals in a laboratory setting. Utilization of the pups could greatly facilitate study of elasmobranchs and — if additional experiments reveal identical neural connections in the pups and adults — analysis of their central nervous system organization.

The retinal projections of Squalus, revealed by the newer autoradiographic tracing method, agree closely with the retinal projections revealed by silver impregnation stains in other sharks[4,5]. A retinohypothalamic projection has been recognized in lemon sharks[5], nurse sharks[4] and tiger sharks[4]. In these species the retinorecipient hypothalamic nucleus was termed nucleus chiasmaticus. A similar retinorecipient cell group is recognizable in Squalus (Figs. 2B and 3A) as a distinct migrated tongue of cells projecting into and caudal to the optic chiasm. The position of this nucleus suggests that it should be considered a caudal preoptic cell group.

The rostral thalamus of Squalus, like that of other sharks[4,5], is characterized by dorsal and ventral retinorecipient nuclei (Figs. 1B and 2A). These nuclei were termed lateral geniculate nucleus and ventrolateral optic nucleus, respectively, by Graeber and Ebbesson[5]. These authors suggested that their lateral geniculate nucleus is homologous to the pars dorsalis of the lateral geniculate nucleus, and their ventrolateral optic nucleus homologous to the pars ventralis of the lateral geniculate nucleus of other vertebrates. This interpretation is reasonable in terms of topography and retinal input, but the efferent projections of these nuclei should be determined before the homologies are accepted.

The caudal thalamic or pretectal area of Squalus is characterized by 3 retinorecipient nuclei: superficial, central and periventricular pretectal nuclei (Figs. 2B and 3). The superficial pretectal nucleus of Squalus has been described previously as the posterior optic nucleus in other sharks, and separate central and periventricular pretectal nuclei have not been recognized[4,5]. These deeper pretectal nuclei have been collectively referred to as the pretectal area[4,5].

A distinct basal optic tract and nucleus have not been uniformly reported in sharks. In Squalus a distinct tract can be traced caudally into the tegmentum where it splits into dorsal and ventral fascicles, terminating in a vaguely defined dorsal

tegmental area and a more circumscribed ventral optic nucleus (Fig. 4). The dorsal tegmental field has not been previously reported, and a ventral optic nucleus has been reported only in tiger sharks[4]. These differences are probably not species differences; they are more likely due to variation in the application of silver impregnation methods, as the autoradiographic tracing method reveals a distinct basal optic system in both Squalus and Mustelus[12].

The present study demonstrates that squalomorph and galeomorph sharks possess the same number of retinorecipient nuclei. However, the retinofugal terminal sites are considerably different in squalomorph and galeomorph sharks. These differences now allow us to characterize evolution of the primary visual pathways in sharks. In Squalus, cell bodies of the retinorecipient rostral dorsal thalamic nucleus lie medial to the terminal field, suggesting that the bulk of the retinal terminals are axodendritic (Fig. 2A). In galeomorph sharks[4,5,12], the rostral dorsal thalamic nucleus has hypertrophied and the majority of its cells have migrated among the fibers of the optic tract; thus, synapses may be axosomatic. A similar trend characterizes the tecta of sharks. In Squalus (Fig. 4) and other squalomorphs[12], there is a distinct periventricular tectal zone, and the bulk of the tectal cell bodies lie ventral to the retinal terminal zone. In galeomorph sharks[12,14], the periventricular tectal zone is poorly developed, and the bulk of the tectal cell bodies have migrated into the superficial tectal zone; thus, they lie within the retinotectal terminal field.

Finally, the overall pattern of retinal projections in sharks suggests that these forms also possess a pattern of diencephalic organization similar to that of other vertebrates. Sharks, like other vertebrates, possess rostral and caudal superficial retinorecipient thalamic zones separated by a middle zone that receives other afferents. Preliminary observations[3] suggest that the middle zone receives tectal input, and that the deeper periventricular portions of the remaining zones receive such widely divergent inputs as spinal and cerebellar projections. Clearly, considerable work is necessary if we are to characterize the extensive variation in forebrain organization among sharks and the selective pressures responsible for this variation. However, existing comparative studies suggest that brain evolution in all classes of vertebrates occurs primarily by increase in neuronal number and by morphological changes in dendritic configuration, rather than by the emergence of numerous new pathways.

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