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Volumetric Analysis of the Telencephalon and Tectum During Metamorphosis in a Flatfish, the Turbot *Scophthalmus maximus*

Key Words
Asymmetry
Flatfish
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Telencephalon
Teleosts
Turbot
Volumetric
Analysis

Abstract
The telencephalic hemispheres and the optic tectum of the turbot *Scophthalmus maximus* were analysed volumetrically during metamorphosis. Both brain regions develop an asymmetry coordinated chronologically with metamorphic events that produce asymmetry in the rest of the animal. Furthermore, the brain asymmetries are correlated with, and may be determined by, the side of origin of their primary afferent inputs, such that the more voluminous side of the brain receives these inputs from the zenithal side. After metamorphosis, the telencephalon remains asymmetric, whereas the optic tectum recovers its bilateral symmetry.

Introduction

The Pleuronectiforms are excellent experimental models for the analysis of asymmetry because of the arrangement of certain of their exteroceptors, particularly those of smell [Prasada Rao and Finger, 1984] and sight [Gulley et al., 1975; Luckenbill-Edds and Sharma, 1977; Médina et al., 1987; Lara et al., 1990]. Studies of the olfactory bulb in flatfishes confirm the extension of asymmetry to this primary integration system [Prasada Rao and Finger, 1984]. Morphological studies of the optic pathway have disclosed certain particularities in the organization of the optic nerves and the chiasma [Parker, 1903; Gulley et al., 1975; Médina et al., 1987] but no asymmetry has been observed in the post-chiasmatic optic pathway in adult specimens [Luckenbill-Edds and Sharma, 1977; Médina et al., 1987].

Asymmetry in flatfishes is characteristic of adult animals, since during the embryonic period and in the early larval stages the Pleuronectiforms are symmetric. The development of asymmetry in the adults involves a complex metamorphic process which, in short, consists of a somatic eversion that entails migration of the eyes and nares towards the zenithal face and modifications of the mouth, the opercular orifices, the fins and pigmentation [Parker, 1903; Chabanaud, 1948; Grassé, 1958; Policansky and Sieswerda, 1979].

In spite of the striking metamorphosis occurring in the Pleuronectiforms [Chabanaud, 1948; Amaoka, 1971; Policansky and Sieswerda, 1979; Policansky, 1982], no study has been made of the effects of this process on the central nervous system. As a first approach in this direction, we analysed two neural structures: the telencephalic hemi-

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spheres, which are markedly asymmetric in adult specimens [Prasada Rao and Finger, 1984], and the optic tectum, on which there are no existing data to suggest asymmetry. Both brain regions are directly related to markedly asymmetric exteroceptive organs, although the association is ipsilateral in the telencephalon and contralateral in the tectum.

Material and Methods

We analysed a total of fifteeen specimens of the turbot *Scophthalmus maximus* L. (sinistral monomorphic Pleuronectiform) [Parker, 1903] of 2, 3, 5, 7 and 15 weeks post-hatching. Once anaesthetized with MS-222 (0.03%), the animals were sacrified, and their heads were removed and fixed by immersion in buffered (pH 7.3) formaldehyde (10%), embedded in Paraplast, and serially sectioned at 10 μm. The sections were stained with Hematoxylin-Eosin or Nissl. All animals were handled according to the guidelines for animal research established by the Canadian Council on animal care.

All sections containing the structures under study were drawn with the use of a camera lucida (×260 for animals of two and three weeks, and ×105 for the remaining ones), measured, and statistically analysed with a MOP-Videoplan (Kontron Bildanalyse) semi-automatic image analyser using the Videoplan-2000 version 5.41 program. Volumes were calculated, via the analyser by multiplying the total sum of all areas by the section thickness.

The entrance of the olfactory tract and the beginning of the preoptic area were considered the rostral and caudal boundaries, respectively, for the telencephalic hemispheres. These boundaries could be clearly distinguished in all stages examined. Measurements of the optic tectum, which did not include the torus longitudinalis, were easily carried out since it is clearly distinct from adjacent structures.

Results

Table 1 shows the volumetric changes in the telencephalon and optic tectum over the five stages studied. The degree of telencephalic asymmetry is almost insignificant in the two-week post-hatching group (fig. 1, 3A); however, during the third week, with the metamorphic process in full course [Chabanaud, 1948], the volume of the left telencephalon is clearly greater (fig. 1, 3B). At five weeks, the asymmetry continues to be notably in favour of the left hemisphere, although the degree of difference has declined and continues to do so through the seven- and 15-week stages. At 15 weeks after hatching the specimens display a degree of telencephalic asymmetry that is slightly less pronounced than at seven weeks and comparable to that described for adult specimens of *Pseudopleuronectes americanus* [Prasada Rao and Finger, 1984] (fig. 1, 3C). This suggests that three months after hatching the brain meta-

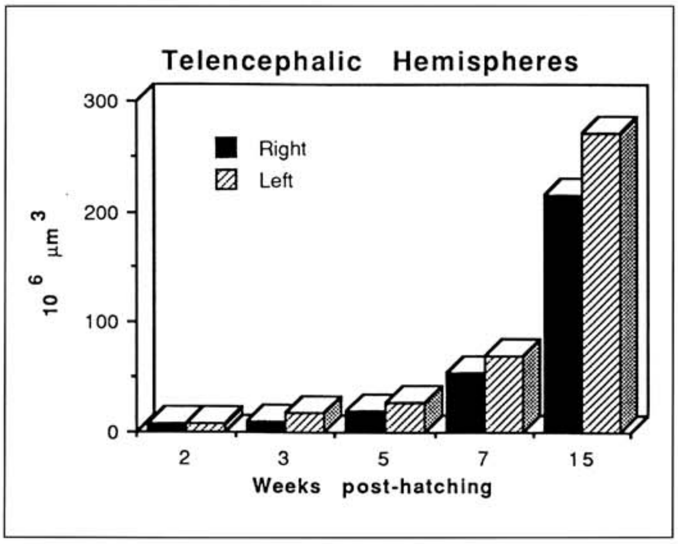

Fig. 1. Volumetric relationships between right and left telencephalic hemispheres during metamorphosis.

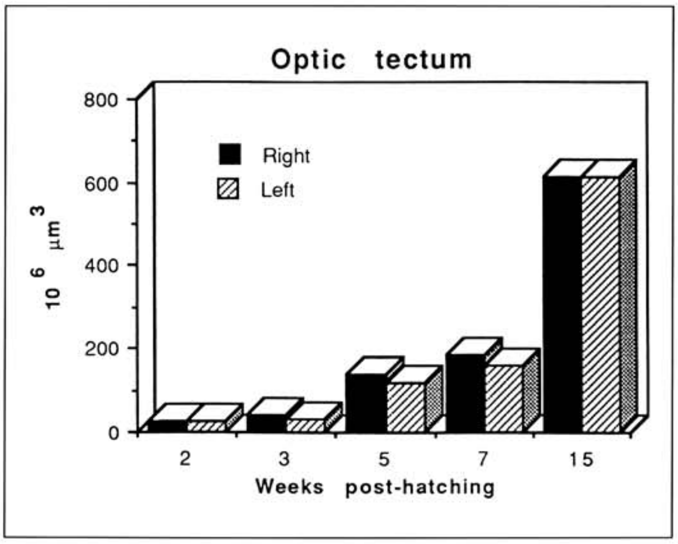

Fig. 2. Volumetric relationships between right and left hemitecta during metamorphosis.

Fig. 3. Hematoxylin-eosin stained transverse sections through the telencephalon (**A, B, C**) and optic tectum (**D, E, F**) of a turbot at two weeks (**A, D**), three weeks (**B, E**), and 15 weeks (**C, F**) after hatching. Bar scales in all frames = 100 μm. L = Left side; ndtl = nucleus diffusus tori lateralis; ng = nucleus glomerulosus; npt = nucleus posterior thalami; oc = optic chiasm; pc = posterior commissure; r = retina; R = right side.

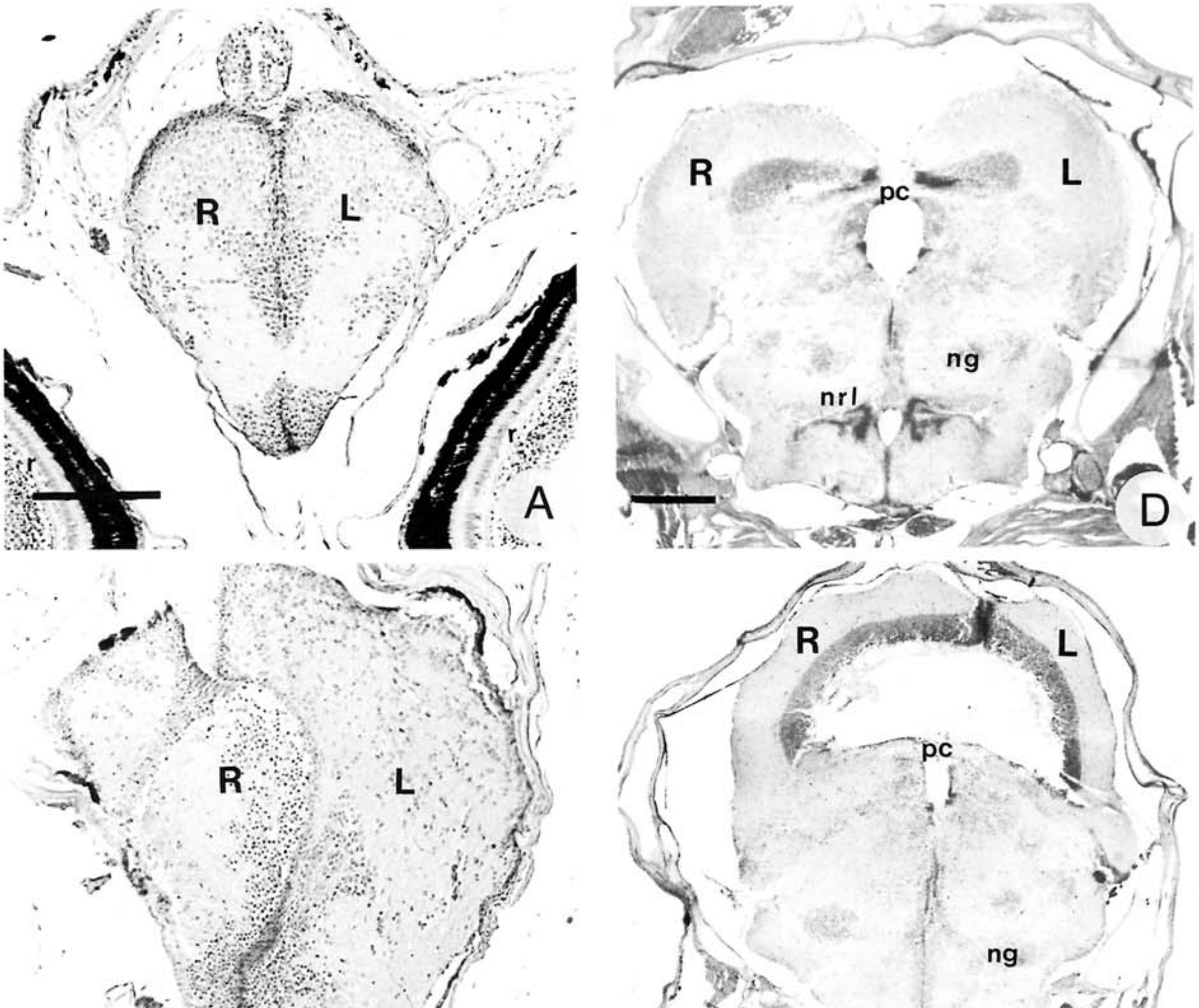

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Table 1. Quantitative analysis of the volume of the telencephalic hemispheres and the optic tectum during metamorphosis

W		Telencephalic hemispheres			Optic tectum		
		M.V.	S.D.	Dif. %	M.V.	S.D.	Dif. %
2	R	8,057,904	370.90	3.2	27,577,067	875.89	2.38
	L	7,799,766	459.90		26,918,442	792.29	
3	R	9,836,374	649.15	44.5	41,975,760	607.48	26.7
	L	17,726,391	1,338.88		30,767,319	797.25	
5	R	19,972,770	1,132.50	36.8	138,664,563	4,750.80	13.5
	L	27,297,300	948.50		119,955,566	4,260.50	
7	R	53,878,966	2,316.50	23.3	186,195,256	2,984.70	13.5
	L	70,239,166	2,822.30		160,782,933	1,969.40	
15	R	214,074,953	3,076.60	20.9	613,110,000	3,247.00	0.03
	L	270,719,453	3,688.40		612,899,500	3,329.03	

W = Weeks post-hatching; M.V. = mean volume (μm^3); Dif. % = percentage difference; R = right; L = left; S.D. = standard deviation.

morphic processes have been completed and that the definitive pattern of telencephalic development has been established.

Regarding the optic tectum, two weeks after hatching a slight volumetric difference between the two hemi-tecta was detected, the one on the right being the larger (table 1; fig.2, 3D). A week later this volumetric difference was even greater (fig.2, 3E), then reduced in percentage over the following weeks. At seven weeks after hatching, the percent difference was reduced by half, and it disappeared in specimens of 15 weeks (table 1, fig.2, 3F).

The asymmetries were observed at all levels in both the telencephalic hemispheres and the optic tectum.

Discussion

The results of this study should be considered a qualitative approximation because of the number of specimens analysed, although they are sufficiently significant if the standard deviations are considered (see table 1).

The results obtained suggest that the effects of the metamorphic processes on the brain begin approximately during the second week after hatching, which agrees with the observations of Chabanaud [1948] on the development of general asymmetry in this species. The most dramatic changes take place in the third week post-hatching and are reflected in maximum degrees of telencephalic and tectal asymmetry (table 1; fig.2).

Telencephalic asymmetry may originate from the semi-atrophy of the right half of the olfactory system with respect to the migration of the corresponding naris [Grassé, 1958]; this means that the number of ipsilateral olfactory inputs that reach the left olfactory bulb and, consequently, the bulbo-telencephalic projections, will be more abundant than those of the nadiral side. In addition, telencephalic asymmetry may also be affected by mechanical restrictions on the growth of the right telencephalic hemisphere due to pressure exerted by the cranial cartilage when the right side of the head is turned and twisted [Chabanaud, 1948; Grassé, 1958]. Similar arguments may, at least partially, explain the tectal asymmetry observed during the metamorphic processes, in this case, however the retino-tectal afferents are contralateral. Accordingly, the left hemi-tectum receives projections mainly from the right eye, which, during this period, is migrating across the animal's head and is notably smaller in size than the fixed eye, thus suggesting that its degree of functionality is lower. On the other hand, in the case of the optic tectum, the mechanical impediments to growth caused by osseous metamorphosis are practically non-existent, since major changes in the cephalic bones basically occur in the frontal zones of the head; moreover, the optic ventricle, located under the tectum, would mitigate the pressure exerted by the cranial cartilages on the tectal structure.

Five weeks after hatching the modifications in the cranial bones are complete, so the pressure on the brain decreases. Migration of the exteroceptive organs has also been completed. This set of factors may be reflected in an appreciable reduction in the degree of asymmetry in the telencephalon and in the optic tectum (table 1).

Although the external morphological changes have been completed, functional stabilization of the migrating organs and brain adaptation to the new somatic conformation are not immediate. Thus, after reaching the maximum degree of asymmetry between the 5th and 7th weeks post-hatching (table 1), both the telencephalon and optic tectum undergo a reverse process. In the case of the optic tectum, this process is complete (table 1, fig. 2), since in adult specimens there is no difference in functionality between the migrating eye and the fixed one [Lara et al., 1990], and nor is there any mechanical limitation which may govern differences in growth betwen the two hemi-tecta. On the other hand, functional and mechanical factors in the telencephalic area persist, determining its permanent asymmetry (table 1, fig. 1). Accordingly, the results obtained with specimens sacrified 15 weeks after hatching suggest that at this stage the brain adaptations to the new anatomic and functional structure have been completed.

Finally, we consider it to be significant that during the metamorphic process the volumetric asymmetry of the telencephalon, a structure of ipsilateral reception, is inverse to that found in the optic tectum, which receives contralateral exteroceptive inputs. The fact that the pattern of asymmetry depends on the type of exteroceptive inputs highlights the functional significance of metamorphic asymmetry.

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