RALPH ANKEN AND FRANCK BOURRAT

BRAIN ATLAS OF THE MEDAKAFISH

Oryzias latipes

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EDITIONS

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INTRODUCTION

The medaka as a model

Teleostean fishes form by far the most abundant (in term of number of species) and the most diversified group of all vertebrates. They comprise some 21,000 species and represent 96% of all extant fishes, or nearly half of all extant vertebrates (Nelson, 1984).
Yet their importance in biological studies has been comparatively modest, at least until recently. To limit ourselves to the field of neurobiology, much of the current knowledge has been gained using either more « complex » models (i.e. mammals) -this is especially true in neuroanatomy, neuroendocrinology, and physiology of systems-, or, on the contrary, « simpler » models, such as insects, molluscs or worms.
This latter approach has turned out to be particularly fruitful in developmental neurobiology and in neurophysiology of single neurons, or of simple neuronal circuits (e.g. Broadie et al., 1993; Chalfie and Au, 1989; Chalfie et al., 1985; Goodman et al., 1984; Goodman and Shatz, 1993; Stent et al., 1992).

Even developmental neurobiologists who focused on vertebrates have often favored the amphibian model (e.g. the classical studies of Sperry, 1963; or, more recently, the continuing success of Xenopus as a laboratory animal: Chien and Harris, 1994; Holt and Harris, 1993), or the chicken (e.g. Landmesser, 1984; Le Douarin, 1993). This is due to the fact that these animals combine external development and large embryos, and are therefore amenable to experimental embryology techniques (grafting, microsurgery, etc..).

This situation is quickly changing, however, and teleostean fishes are on the verge of gaining a prominent position in the field of vertebrate developmental biology, including developmental neurobiology. This is largely due to the evolution of this field itself, which has, in the last decade, tremendously benefitted from the tools of molecular genetics.

In this context, some teleostean species may be considered as almost ideal models: their attractive features include a small size, high fecundity, external development, transparency of the eggs and embryos and short generation time, at least as concerns vertebrates (2-3 months from egg to egg).

A species fullfilling all these criteria is the zebrafish, *Danio rerio*, a Cypriniforme (Cyprinoidei) from India. It owes its success largely to the fact that it is amenable to large-scale genetic studies (amongst small teleosts, its fecundity is especially high); indeed, two genome-wide saturation screens for developmental mutants, using chemical mutagenesis, have been recently performed in the zebrafish (see Development Suppl., 1996, for a first systematic account of the results obtained by the group of Nüsslein-Volhart in Tübingen, and that of Driever in Boston). In addition, the early steps of the development of this small fish (fate map at the blastula and gastrula stages, etc...) have been thoroughly analyzed, thanks mostly to the efforts of G. Streisinger, C. Kimmel and collaborators, who initially made this model popular (Kimmel, 1989; Kimmel and Warga, 1988).

Another teleost which fullfills the criteria summarized above is the medaka, *Oryzias latipes*, a Atherinomorpha (Cyprinodontoidei) from Japan and Eastern Asia. This fish had long been an important laboratory animal in Japan, and a great wealth of knowledge had been gathered on its biology and some aspects of its development (see, for example, Iwamatsu, 1993, and references herein). It should be mentioned that in this group of teleosts (Atherinomorpha) is also present the swordtail fish (*Xiphophorus helleri*, a close relative of the medaka), which has been for decades a model in carcinogenesis (Malitschek et al., 1995) and sex determination studies (see, for example, Kallman, 1984).

The prolonged interest of several Japanese groups for the medaka has led to the production of numerous well characterized strains, particularly several inbred strains (Hyodo-Taguchi and Sakaizumi, 1993). It has also allowed the development of genetic methods such as the production of haploids, tetraploids, etc..., a feature that it shares with the zebrafish.
Compared to this otherwise similar model (in terms of general biology of the species), the medaka develops slower (10 days from fertilization to hatching, as compared to 48 h for the zebrafish), and is considerably more robust. Indeed, the zebrafish is a purely tropical fish, whereas the medaka can stand low temperatures for prolonged periods of time (in Japan, they are known to survive in winter in frozen ponds). This feature, besides its obvious practical interest for the maintenance of laboratory animals, can also be used to control the developmental pace, which can be slowed almost at will, a characteristic often useful in experimental embryology. Another indication of the robustness of the medaka can be seen in the fact that it recently became the first vertebrate to successfully reproduce in weightless conditions aboard a space shuttle (Ijiri 1995).
To these advantageous features can be added the fact that it has a significantly smaller genome than the zebrafish, and that it is the first vertebrate in which an active transposable element has been found (Koga et al., 1996).

Aim of the present atlas

The primary purpose of the present work is to provide the medaka scientific community with a tool for neuroanatomical studies, and especially to allow the precise localization of gene expression patterns. Indeed, among the rapidly growing number of genes cloned and sequenced, in teleost fishes as well as in other vertebrates, a majority show an expression in the central nervous system (CNS), either during the development, or in the adult, or both. This situation, of course, is to be expected, since more genes are expressed in the brain than in any other tissue, in every species examined (Chaudhari and Hahn, 1983; Snider and Morrison-Bogorad, 1992).

There is an abundant literature dealing with various aspects of fish neuroanatomy. Nevertheless, these studies were often performed in a comparative perspective, which lead to the analysis of very specialized or primitive species (see also the later section dealing with nomenclature).

In contrast, very few studies have covered the entire CNS of a given fish species. To our knowledge, only four such atlases have ever been published: that of Burr (1928) on the short sunfish *Orthagoriscus mola*, a work that has become somewhat obsolete; that of Maler et al. (1991) on the electric fish *Apteronotus leptorhynchus*, a work that cannot be readily used because electric fishes have a number of specialized encephalic structures, the homologs of which are difficult to recognize in other teleosts; and finally the recent atlases of Anken and Rahmann (1994) on the swordtail fish *Xiphophorus helleri*, and of Wulliman et al. (1996) on the zebrafish *Danio rerio*.

Despite the above-mentioned similarities in some of their biological characteristics, the medaka and the zebrafish are not at all closely related (see Fig. 1). The phylogenetic distance between them is indeed large enough often to render the precise identification of individual brain nuclei arduous and uncertain at best, if one looks at the CNS of one species using the atlas of the other.

On phylogenetic grounds, the work of Anken and Rahmann (1994) can be used at least as a rough guide for the medaka CNS. This study, however, comprises a relatively small number of topologically arranged transverse sections, and completely lacks the respective sagittal and horizontal sections. Moreover, the « miniaturization » of the medaka reflects itself in the structure of its brain, which cytoarchitectonically appears somewhat alike that of a larval swordtail fish (which is only marginally covered in the *Xiphophorus* atlas). For these reasons, it is impractical to use this latter book in the course of a study on medaka CNS.

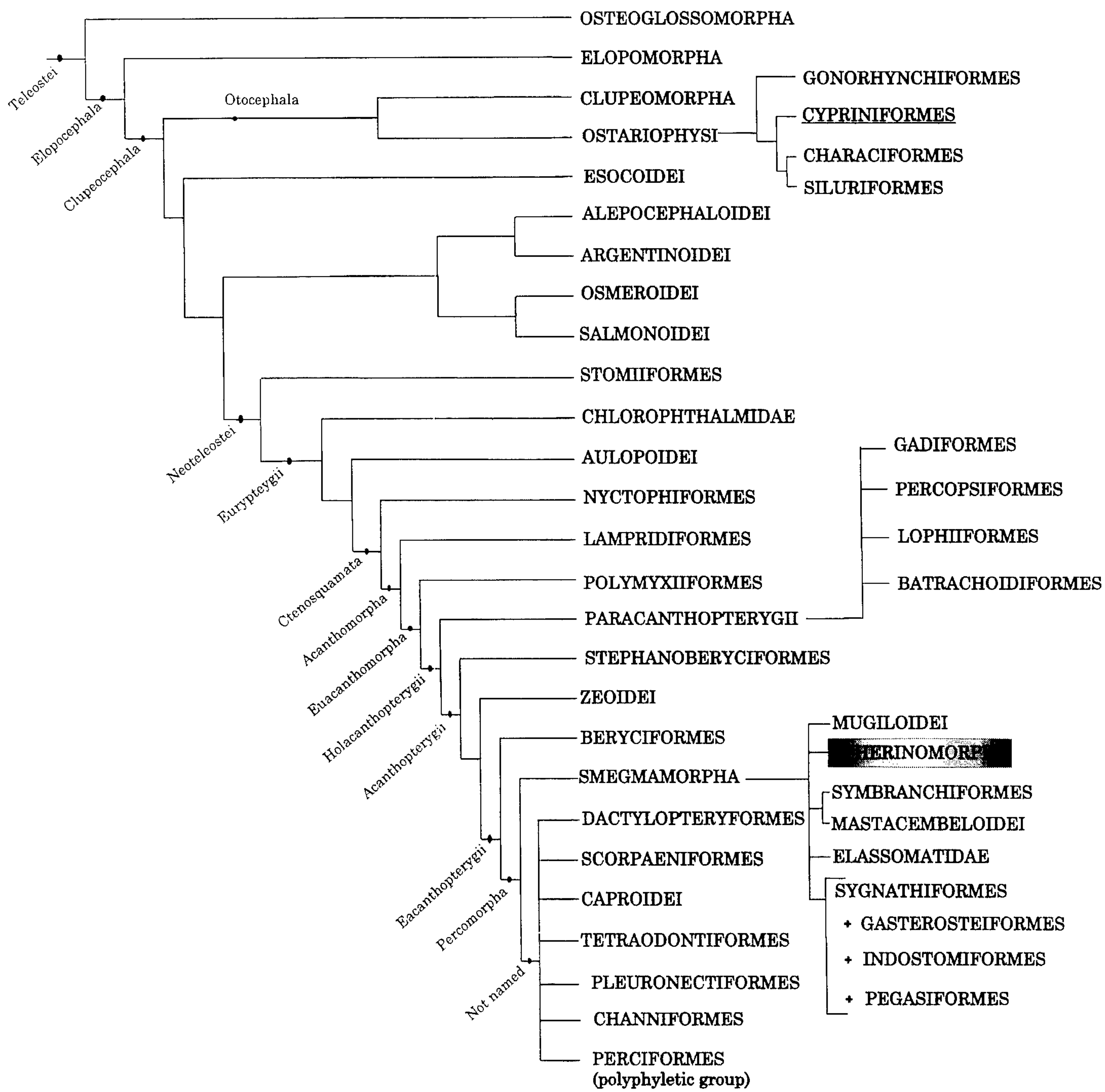

Figure 1:
A modern view of the phylogeny of teleostean fishes.

Adapted from Stiassny, Parenti and Johnson (1996) "Interrelationships of Fishes", Academic Press
Adaptation: courtesy of G. Lecointre.
The medaka belongs to the Atherinomorpha (shaded box).
The zebrafish belongs to the Cypriniformes (underlined).

Although the primary purpose of the present atlas is to provide a tool for medaka neuroanatomists, it will equally allow comparisons between the organization of the CNS in various teleosts. Neuroanatomists should now be able to put in register the features of close relatives (swordtail fish versus medaka, goldfish versus zebrafish, see Rupp et al., 1996), and distant relatives (zebrafish versus medaka or swordtail fish). Such comparative approaches should be especially fruitful in the field of developmental neuroanatomy, or developmental genetics, since a considerable number of genes are, or will soon be, cloned both in *Danio rerio* and in *Oryzias latipes*.

MATERIALS AND METHODS

Medaka strain used and histological procedures

We used young adult medakas (about two months of age; body length: 2-3 cm) of the orange-red strain (kindly provided by Pr. A. Shima, Tokyo University). The fishes were raised in 20 l tanks (density: about 30 fishes/tank) at 25°C, with a 12h day/12 h night cycle. They were fed twice a day with commercial dry fish food, and once a day with live brine shrimp (*Artemia salina*) larvae.

The fishes were killed by immersion in chilled water, and fixed in Bouin's fixative for at least one week at 4°C. The brains were not dissected out, in order to: 1) allow a precise and « natural » orientation for the various planes of sections, and 2) facilitate the identification of cranial nerves.

Whole fishes (n=20) were wax-embedded, and serially cut at 8 μm in either the transverse, horizontal or sagittal plane. The sections were stained with cresyl violet-thionine.

In addition, we prepared sections of older fishes (about 1 year of age; body size > 5 cm), following the same procedures, to evaluate the degree of maturity of the CNS of the young adults used for this atlas. We found that, although the brains of the old fishes are larger -they continue to grow all over the animal's life, a well-documented phenomenon in teleosts, see for example Zupanc and Horschke (1995) for a recent report, there are no qualitative differences with those used for the present work, which, therefore, can be considered as fully mature.

Preparation of the brain atlas, and guidelines for its use

Some external features of the medaka brains are given first (Fig. 2). Then the respective levels of sections in the transverse, horizontal and sagittal planes are presented (Fig. 3).

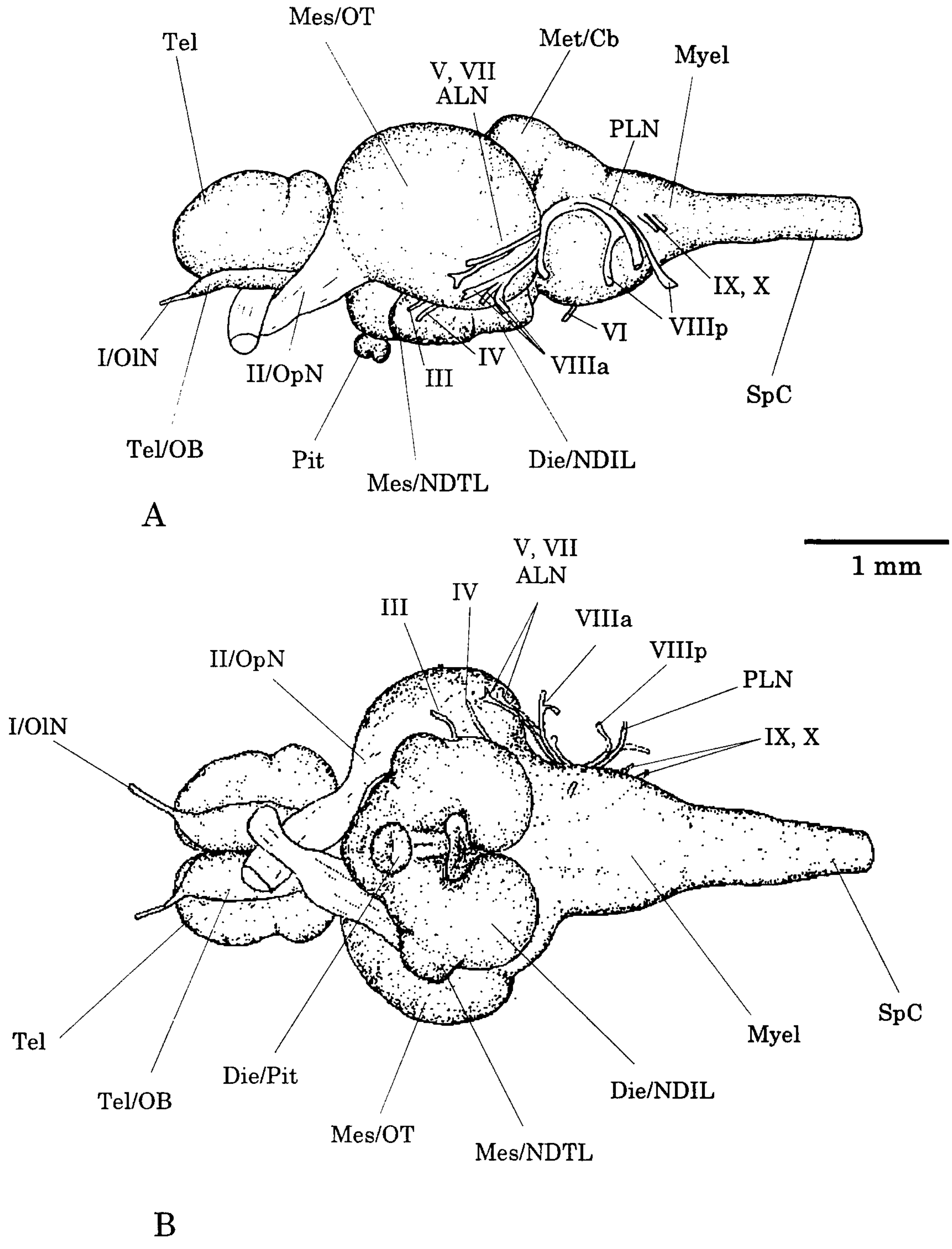

Figure 2:
Semi-schematic lateral (A) and ventral (B) views of adult medaka brains.

Abbreviations: ALN: anterior lateral line nerve; Cb: corpus cerebelli; Die: diencephale; Mes: mesencephale; Met: metencephale; Myel: myelencephale; NDIL: nucleus diffusus of lobus inferioris (of hypothalamus); NDTL: nucleus diffusus of torus lateralis; OB: olfactory bulb; OlN: olfactory nerve; OpN: optic nerve; OT: optic tectum; Pit: pituitary; PLN: posterior lateral line nerve; SpC: spinal cord; Tel: telencephale.

I: olfactory nerve; II: optic nerve; III: oculomotor nerve; IV: trochlear nerve; V: trigeminal nerve; VI: abducens nerve; VII: facial nerve; VIII: octavus nerve (a: anterior ramus; p: posterior ramus); IX: glossopharyngeal nerve; X: vagus nerve.