Brain Research, 586 (1992) 176–180

BRES 25294

Zebrin II distinguishes the ampullary organ receptive map from the tuberous organ receptive maps during development in the teleost electrosensory lateral line lobe

Michael J. Lannoo [a], Leonard Maler [b] and Richard Hawkes [c]

[a] *The Muncie Center for Medical Education, Indiana University School of Medicine and Department of Physiology and Health Sciences, Ball State University, Muncie, IN 47306 (USA),* [b] *Department of Anatomy, Faculty of Medicine, The University of Ottawa, Ottawa, Ont K1H 8M5 (Canada) and* [c] *Department of Anatomy and the Neuroscience Research Group, Faculty of Medicine, The University of Calgary, Calgary, Alta., T2N 4N1 (Canada)*

(Accepted 5 May 1992)

Key words: Electrosensation; Mechanosensation; Pyramidal cell; Map formation; Ontogeny; Phylogeny

In weakly electric gymnotiform teleosts, monoclonal antibody anti-zebrin II recognizes developing pyramidal cells in the ampullary organ-receptive medial segment of the medullary electrosensory lateral line lobe (ELL) and in the mechanoreceptive nucleus medialis. Developing pyramidal cells in the remaining three tuberous organ-receptive lateral ELL segments are unreactive. These results suggest that certain biochemical features of the ELL ampullary organ-receptive medial segment are more similar to the nucleus medialis than to the tuberous organ-receptive ELL segments, and support the hypothesis that the ampullary system evolved from mechanosensory precursors.

From sensory input to motor output the electrosensory system of gymnotiform teleosts is one of the best understood vertebrate sensory systems[9]. This system contains a high degree of order and is experimentally accessible, making it a good candidate model for understanding the general rules underlying organization in the developing nervous system[12,14,22,24]. Furthermore, an evolutionary history has been proposed; the electrosensory system is believed to have evolved from the mechanosensory system[3,6,20], which is present in all teleosts. Here, using immunocytochemical techniques, we report a transient developmental feature, shared by the mechanosensory system and a portion of the electrosensory system, that supports this evolutionary scenario.

The lateral line sensory system of gymnotiforms consists of three basic receptor organ types: neuromast organ mechanoreceptors, ampullary organ electroreceptors and tuberous organ electroreceptors. Each receptor type projects, by way of bipolar afferent neurons, to a separate lateral line nucleus or subnucleus within the brainstem medulla. Mechanoreceptive afferents project to the nucleus medialis (nM). Both types of electroreceptors project to the electrosensory lateral line lobe (ELL). In particular, ampullary organ afferents project to the medial segment of the ELL (MS-ELL), and tuberous organ afferents trifurcate to send collaterals to each of the three lateral segments of the ELL (LSs-ELL). Electroreceptor inputs to all four segments of the ELL form somatotopic maps; each map is mirror-image oriented with respect to its neighbor(s). Furthermore, the ELL is laminated; primary afferents, descending ELL inputs, ELL efferents, and major cell types segregate. Because of this organization we have been studying aspects of ELL formation as a model for understanding the development of neuronal ordering. One goal has been to use immunocytochemical methods to find monoclonal antibodies (mabs) that label cell types specific to individual maps. Here we show that mab anti-zebrin II recognizes the pyramidal cells in the MS-ELL during development, and therefore distinguishes this segment from the LSs-ELL. Fur-

Correspondence: M.J. Lannoo, The Muncie Center for Medical Education, Indiana University School of Medicine and Department of Physiology and Health Sciences, Ball State University, Muncie, IN 47306, USA. Fax: (1) (317) 285 1050.

thermore, nM pyramidal cells also express zebrin II during this time. This result indicates a transient biochemical feature shared by the nM and MS-ELL that is not shared by the MS-ELL and the LSs-ELL, and suggests a phylogenetic link between the mechanosensory system and the ampullary electrosensory system.

Mab anti-zebrin II was generated in Balb/c mice immunized against a crude homogenate of the hindbrain of the teleost, *Apteronotus leptorhynchus*[2,13,14]. Hybridoma supernatant was used undiluted, plus 0.1% Triton X-100. These high concentrations of supernatant gave good labelling of dendrites and axons without generating background reactivity.

Eigenmannia (presumably *E. lineata*) were bred in the laboratory by reproducing the key environmental conditions of their tropical rainy season[7,10,11,23]. Larvae were fed zooplankton and maintained at 28°C. Developmental time is defined by days post-spawning[23,24]. Larvae used in the study were aged 4, 6, 8, 10, 12, 14, 17, 16, 29, and 34 days post-spawning, and were the same animals used in a previous study[13]. Four larvae were examined at each age except day 4, where three animals were used, and day 34, where two animals were used. Animals were anesthetized in MS 222 (3-aminobenzoic acid ethyl ester, Sigma, St. Louis), fixed by immersion, cryoprotected[14,25], and stored at −20°C, as described previously[13,14]. Immunocytochemistry was performed on 30- or 40-μm sections of brainstem cut transversely.

Previous studies have shown that mab anti-zebrin II recognizes a single 36 kDa polypeptide present in cerebellar Purkinje cells in a number of species widely separated phylogenetically, including other fishes, birds and mammals[1,2,5,8,14]. Within the gymnotiform teleosts, zebrin II immunoreactivity is present during development in all Purkinje cells within the corpus cerebellum, the valvula cerebellum, and the anterior eminentia granularis[2,13]; terminology and identifications follow previous studies[4,18]. Zebrin II expression in the gymnotiform cerebellum is shown schematically in Fig. 1a. Purkinje cells within the medial and posterior eminentia granularis do not label. Extracerebellar axons from the corpus cerebellum, and, apparently, the anterior eminentia granularis, also label. Labelled Purkinje cell axons are observed projecting through the tractus cerebello–acousticolateralis along the lateral margin of the

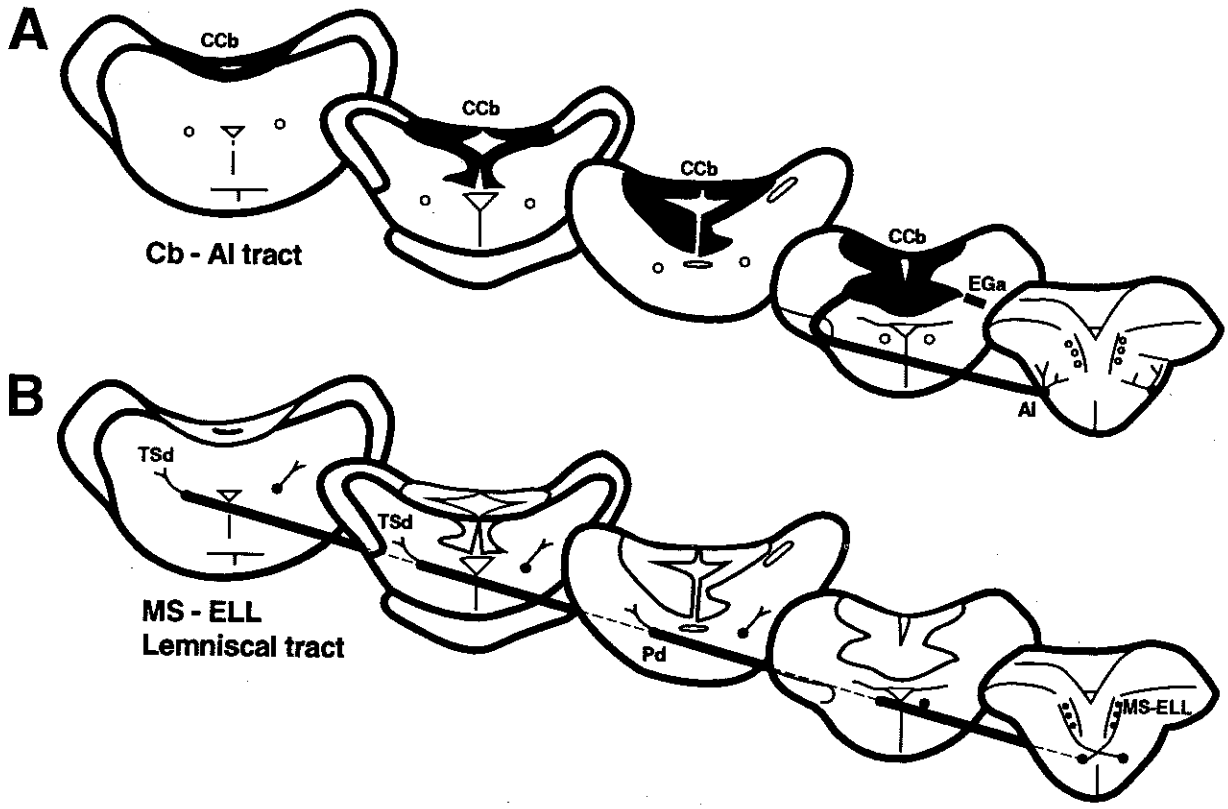

Fig. 1. Schematic drawing illustrating the differences between the two brainstem systems expressing zebrin II during development in the teleost, *Eigenmannia*. A: the uncrossed tractus cerebello-acousticolateralis (tCb-Al) projecting from the cerebellum to the hindbrain, specifically to the dorso-octavolateralis nucleus (DO) and the nucleus medialis (nM) described previously[2,13,14]. B: the crossed ascending lemniscal system from the MS-ELL to the isthmal preeminentialis dorsalis (Pd) and the midbrain dorsal torus semicircularis (TSd) reported here. For simplicity, zebrin II$^+$ nM cells are not indicated.

brain, and terminating in the nM and the dorsal octavolateralis nucleus (Figs. 1a and Fig. 2). In adults this labelling pattern is the same, with the important exception that some medial cells within the valvula cerebellum lose their immunoreactivity[13]. Other teleost adults, for example the zebrafish (*Brachydanio rerio*; Cypriniformes), the black bullhead (*Amieurus melas*; Siluriformes) and the mormyrid *Gnathonemus petersii*, conform to the gymnotiform pattern[2,14,19].

Here we report for the first time that zebrin II expression occurs in a non-Purkinje cell type, namely the pyramidal cells of the developing MS-ELL and the nM. Between day 14 and 17 post-spawning in *Eigenmannia*, pyramidal cells within the MS-ELL begin to express zebrin II (Fig. 2c,d). As with Purkinje cells, zebrin II labelling in pyramidal cells is present within the cell soma, except the nucleus, and throughout the dendritic and axonal processes. Zebrin II expression in

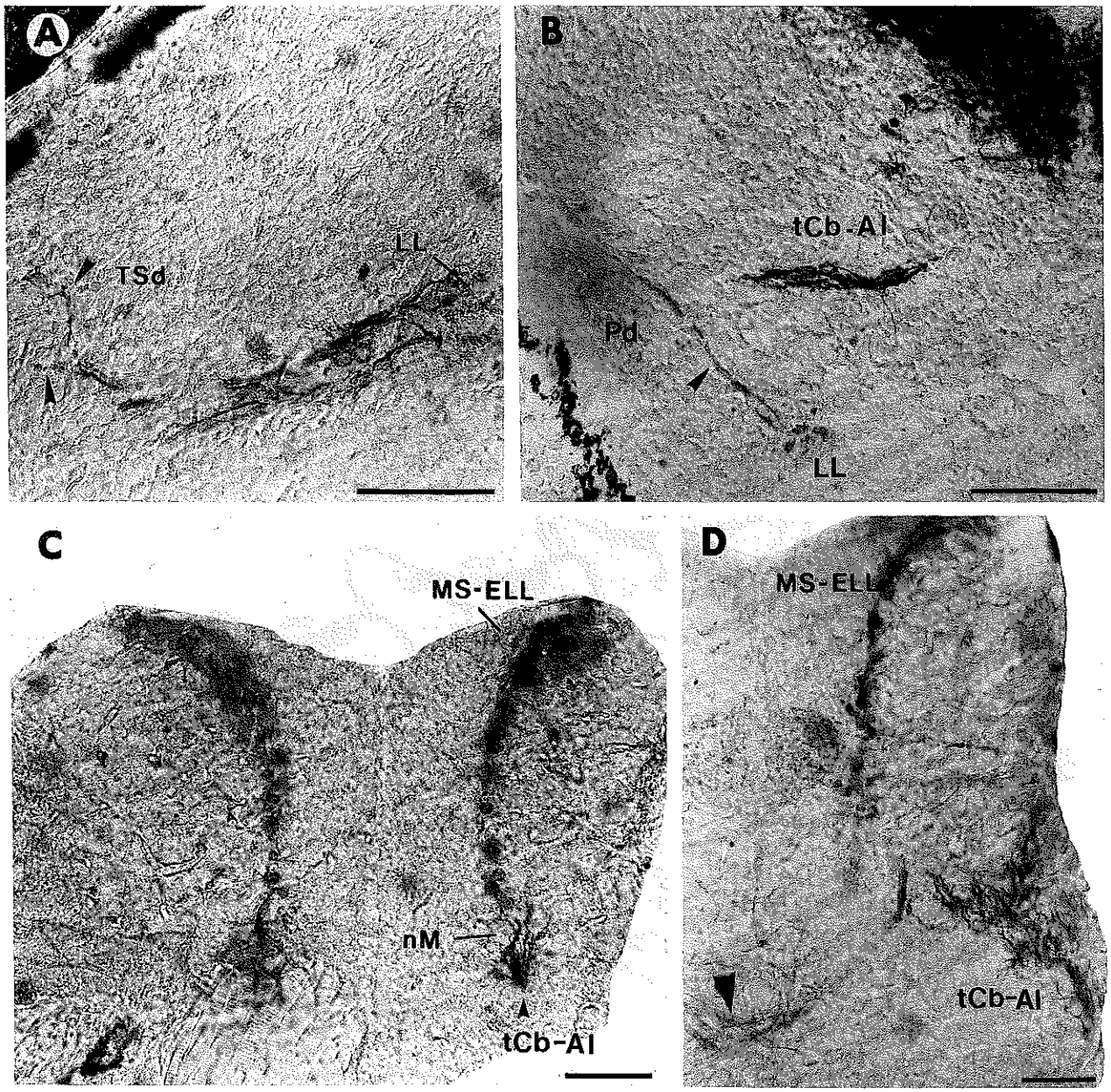

Fig. 2. Details of zebrin II expression in the MS-ELL. In each micrograph, except A, dorsal is up; in A dorsal is to the upper left. A: axons (arrowheads) coursing from the lateral lemniscus (LL) into the dorsal torus semicircularis (TSd) B: axons (arrowhead) coursing from the lateral lemniscus (LL) into the nucleus preeminentialis dorsalis (Pd). Note the descending tractus cerebello-acousticolateralis (tCb-Al) axons running dorsomedially. C: pyramidal cell bodies in the MS-ELL; note the vertical orientation of the cell lamina, and the tCb-Al fibers that course to the nucleus medialis (nM). D: the decussation (arrowhead) of MS-ELL pyramidal cell axons as they course towards the contralateral lateral lemniscus (not shown). Bars: A,B = 100 μm; C,D = 50 μm.

pyramidal cells is transitory – adult pyramidal cells are zebrin II$^-$. In larval pyramidal cells zebrin II expression corresponds to the period of neurite outgrowth, as axons emerge, decussate, and course through the contralateral lateral lemniscus (Fig. 1b). Pyramidal cell axon collateral branches are visible as they course to the isthmal nucleus preeminentialis dorsalis (Fig. 2b) and the mesencephalic torus semicircularis dorsalis (Fig. 2a).

Pyramidal cells in the nM (previously described as crest cells[16]) also express zebrin II. The timing of zebrin II expression in the nM corresponds to its expression in the MS-ELL (Fig. 3). Pyramidal cells in the nM are identified by their position in relation to the developing ELL (Fig. 3a[12]) and by the fact that they receive Purkinje cell innervation, which is also zebrin II$^+$ (Fig. 3b[13,14]). The labelling of nM cells is weaker than in the MS-ELL but specific; in contrast, cells in other brainstem nuclei are unlabelled.

Adult MS-ELL and nM pyramidal cells are zebrin II$^-$, meaning that zebrin II must be suppressed at some point during development. We could not determine the timing of this zebrin II supression except to note that it occurs between day 34 (the age of our oldest animals) and adulthood.

During development, mab anti-zebrin II is able to distinguish MS-ELL pyramidal cells from the pyramidal cells in the three lateral ELL segments. Therefore, zebrin II expression provides a biochemical means of distinguishing between the targets of the two principal types of electroreceptor inputs, i.e. ampullary organ receptive and tuberous organ receptive maps. Ampullary and tuberous segments within the ELL receive separate primary afferent inputs, suggesting that a molecule such as zebrin II might be used by ingrowing afferents to find their correct target map. Zebrin II, however, cannot itself be the candidate molecule because this polypeptide is expressed after the primary afferents have become established. Primary afferents begin entering the ELL at around day 5 post-spawning[12] (or at about 9 mm total length[22]), while zebrin II is initially expressed in the nM between day 14 and day 17. In mammalian Purkinje cells zebrin II expression also occurs after cerebellar afferents have become established[15,21].

The ELL appears to have evolved from the nM or a similar structure in non-electrosensory teleosts by a two step process: (1) the establishment of the ampullary organ receptive segment characteristic of siluriforms (catfishes); and (2) the establishment of the three tuberous organ receptive segments characteristic of gymnotiforms[3,6]. The pattern of zebrin II immunoreactivity reported here supports this interpretation. In particular, because zebrin II is present in only one of the two electrosensory subsystems and the mechanosensory system, its expression in the brainstem cannot be directly related to either mechanoreception or electroreception. Instead, the presence of zebrin II suggests a phylogenetic link between the mechanoreceptive system and the ampullary electroreceptive system, and supports the hypothesis that electroreceptive components arose from mechanoreceptive precursors.

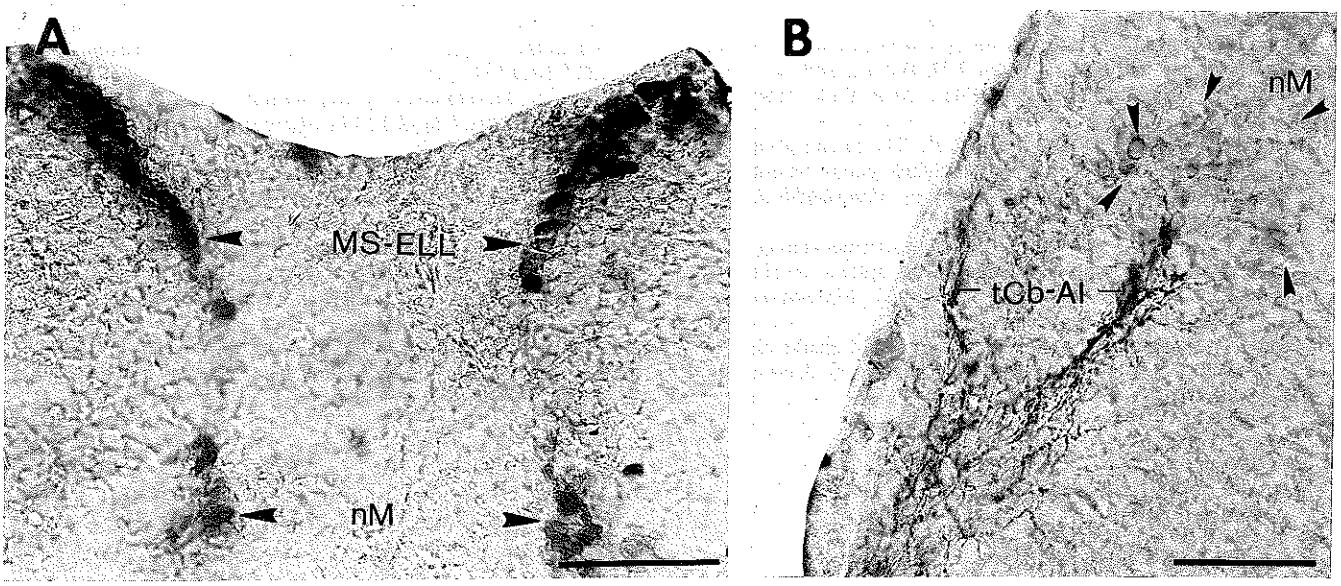

Fig. 3. Zebrin II immunoreactive pyramidal cells in the nucleus medialis (nM). A: nM pyramidal cells located ventral to, and clearly separate from, the MS-ELL. B: a cluster of nM cells (indicated by arrowheads) at the termination of the medial branch of the tractus cerebello-acousticolateralis (tCb-Al). In both micrographs notice that nM labelling is less intense than labelling in the MS-ELL. Bars = 50 μm.

Traditionally, cytoarchitectural similarity has been the basis for uniting the ampullary organ receptive MS-ELL with the tuberous organ receptive ELL maps. However, in addition to the zebrin II expression reported here two features of the MS-ELL suggest a more distant relationship between the MS-ELL and the LSs-ELL. First, while pyramidal cell axons from the LSs-ELL course anteriorly out of the ELL then decussate prior to entering the lateral lemniscus, MS-ELL pyramidal cell axons first decussate to enter the lateral lemniscus and then course anteriorly[17]. Secondly, the MS-ELL develops from a germinal zone that is distinct from the tuberous germinal zone(s)[12,22]. Unlike the tuberous maps, which develop in a horizontal orientation, the MS-ELL first develops in a vertical orientation then shifts to a horizontal orientation with growth. Together, these three distinct features of the MS-ELL, zebrin II expression, efferent trajectory, and developmental pattern, argue for an ampullary MS-ELL map much more distinct from the tuberous LSs-ELL maps than the histological evidence alone would indicate.

Drs. Walter Heiligenberg and Heinrich Vischer kindly provided the developmental series of *Eigenmannia* larvae. Susan J. Lannoo assisted in preparing the figures. Danette Pratt created Fig. 1. This project was supported by the Medical Research Council (Canada), Indiana University School of Medicine seed funds, and NIH Grant NS30702 to M.J.L.

1 Brochu, G., Maler, L. and Hawkes, R., Zebrin II: a novel antigenic marker of cerebellar compartmentation in fish and rat, *Soc. Neurosci. Abstr.*, 14 (1988) 495.
2 Brochu, G., Maler, L. and Hawkes, R., Zebrin II: a polypeptide antigen expressed selectively by Purkinje cells that reveals compartments in rat and fish cerebellum, *J. Comp. Neurol.*, 291 (1990) 538–552.
3 Bullock, T.H., Northcutt, R.G. and Bodznick, D.A., Evolution of electroreception, *Trends Neurosci.*, 5 (1982) 50–53.
4 Carr, C.E. and Maler, L., Electroreception in gymnotiform fish: central anatomy and physiology. In T.H. Bullock and W. Heiligenberg (Eds.), *Electroreception*, Wiley, New York, 1986, pp. 319–373.
5 Doré, L., Jacobson, C.D. and Hawkes, R., The organization and postnatal development of zebrin II antigenic compartmentation in the cerebellar vermis of the grey opossum, *Monodelphis domestica, J. Comp. Neurol.*, 291 (1990) 431–449.
6 Finger, T., Bell, C.C. and Carr, C.E., Comparisons among electroreceptive teleosts: why are electrosensory systems so similar? In T.H. Bullock and W. Heiligenberg (Eds.), *Electroreception*, Wiley, New York, 1986, pp. 465–481.
7 Hagedorn, M. and Heiligenberg, W., Court and spark: electric signals in the courtship and mating of gymnotiform electric fish, *Animal Behav.*, 33 (1985) 254–265.
8 Hawkes, R. and Gravel, C., The modular cerebellum, *Prog. Neurobiol.*, 36 (1991) 309–327.
9 Heiligenberg, W.F., *Neural Nets in Electric Fish*, MIT Press, Cambridge, 1991.
10 Kirschbaum, F., Environmental factors control the periodical reproduction of tropical electric fish, *Experientia*, 31 (1975) 1159–1160.
11 Kirschbaum, F., Reproduction of the weakly electric fish *Eigenmannia virescens* (Rhampichthyidae, Teleostei) in captivity. I. Control of gonadal recrudescence and regression by environmental factors, *Behav. Ecol. Sociobiol.*, 4 (1979) 331–355.
12 Lannoo, M.J., Vischer, H. and Maler, L., The development of the electrosensory nervous system of *Eigenmannia* (Gymnotiformes). II. The electrosensory lateral line lobe, midbrain, and cerebellum, *J. Comp. Neurol.*, 294 (1990) 37–58.
13 Lannoo, M.J., Brochu, G., Maler, L. and Hawkes, R., Zebrin II immunoreactivity in the rat and in the weakly electric teleost *Eigenmannia* (Gymnotiformes) reveals three modes of Purkinje cell development, *J. Comp. Neurol.*, 309 (1991) 1–19.
14 Lannoo, M.J., Ross, L., Maler, L. and Hawkes, R., Development of the cerebellum and its extracerebellar Purkinje cell projection in teleost fishes as determined by zebrin II immunocytochemistry, *Prog. Neurobiol.*, 37 (1991) 329–363.
15 Leclerc, N., Gravel, C. and Hawkes, R., Development of parasagittal zonation in the rat cerebellar cortex: mab Q113 antigenic bands are created postnatally by the supression of antigenic supression in a subset of Purkinje cells, *J. Comp. Neurol.*, 273 (1988) 399–420.
16 Maler, L., Finger, T. and Karten, H., Differential projections of ordinary lateral line receptors and electroreceptors in the gymnotid fish, *Apteronotus (Sternarchus) albifrons, J. Comp. Neurol.*, 158 (1974) 363–382.
17 Maler, L., Sas, E., Carr, C.E. and Matsubara, J., Efferent projections of the posterior lateral line lobe in gymnotiform fish, *J. Comp. Neurol.*, 211 (1982) 154–164.
18 Maler, L., Sas, E., Johnson, S. and Ellis, W., An atlas of the brain of the electric fish *Apteronotus leptorhynchus, J. Chem Neuroanat.*, 4 (1990) 1–38.
19 Meek, J., Hufmans, T.G.M., Maler, L. and Hawkes, R., The distribution of zebrin II in the gigantocerebellum of the mormyrid fish (*Gnathonemus petersii*), compared with other teleosts, *J. Comp. Neurol.*, 316 (1992) 17–31.
20 Singh, S. and New, J., Central topography of electro- and mechanosensory lateral line afferents in the channel catfish, *Ictalurus punctatus, Soc. Neurosci. Abstr.*, 17 (1991) 319.
21 Sotelo, C., Bourrat, F. and Triller, A., Postnatal development of the inferior olivary complex in the rat. II. Topographic organization of the immature olivocerebellar projection, *J. Comp. Neurol.*, 222 (1984) 177–199.
22 Viete, S., Development of the multiple maps of the electrosensory lateral line lobe (ELL) in *Eigenmannia, Soc. Neurosci. Abst.*, 17 (1991) 1405.
23 Vischer, H., The development of lateral line receptors in *Eigenmannia* (Teleostei, Gymnotiformes). I. The mechanoreceptive lateral line system, *Brain, Behav. Evol.*, 33 (1989) 205–222.
24 Vischer, H., Lannoo, M.J. and Heiligenberg, W., The development of the electrosensory nervous system in *Eigenmannia* (Gymnotiformes). I. The peripheral nervous system, *J. Comp. Neurol.*, 290 (1989) 16–40.
25 Watson, R.E., Wiegand, S.J., Clough, R.W. and Hoffman, G.E., Use of cryoprotectant to maintain long-term peptide immunoreactivity and tissue morphology, *Peptides*, 7 (1986) 155–159.