

## DEVELOPMENT OF THE ELECTROSENSORY LATERAL LINE LOBE IN THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*, WITH REFERENCE TO THE ONSET OF SWIMMING AND FEEDING BEHAVIORS

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We describe the development of the medullary electrosensory lateral line lobe (ELL) of *Ictalurus punctatus*, the channel catfish, with reference to the onsets of three behaviors — undulatory swimming, schooling, and exogenous feeding. *Ictalurus punctatus* embryos and larvae were staged according to a modification of Armstrong and Child (1962), where hatching occurs as early as stage 40. Using nissl-stained histological sections we report that: 1) lateralis innervation is established by stage 32; 2) the lateralis area is distinguished as a subpial, laterally-positioned cellular region by stages 33-34; 3) lateralis neurons differentiate by stages 42-43; 4) the cerebellar crest forms by stages 45-46; 5) the ELL is distinguished from the (mechanosensory recipient) medial nucleus by stages 48-49; and 6) a morphologically mature ELL forms by stage 53. Swimming and schooling occur prior to the formation of a mature ELL. The onset of feeding correlates with morphological maturity of the ELL.

**Keywords:** neuronal substrates; octavolateralis systems; medulla; Siluriformes

### INTRODUCTION

During development, the timing of any particular behavioral onset requires an assembled neuronal circuitry (e.g. Blaxter, 1986; Browman, 1989). Thus, behavioral studies augment anatomical studies in determining the establishment of functioning neuronal substrates, especially under circumstances where physiological experimentation is difficult (Coghill, 1929; Hagedorn *et al.*, 1988). For example, in the red sea bream (*Pagrus major*; Sparidae), Toyoda and Uematsu (1994) demonstrate that the tectum grows most rapidly as visual feeding becomes established, gustatory nuclei differentiate as larvae assume benthivory and that growth in the cerebellum corresponds to an improved motor performance.

Catfishes (suborder Siluroidei) are remarkable sensory animals. While perhaps best known for their epidermal taste buds — catfishes have been referred to as 'swimming tongues' (Atema, 1969, 1971; Caprio *et al.*, 1993) — they have also evolved electrosensory capabilities (Parker and van Heusen, 1977; Finger, 1986).

Catfish electrosensation has been implicated in feeding on actively swimming prey, orientation within an environment, and social interactions (Finger, 1986). Regarding feeding, Peters and Bretschneider (1972), Roth (1972), Peters and Meek (1973), and Peters and van Wijland (1974) have shown that potential prey species produce low-frequency alternating current or direct current electric fields that are within the range of detectability of ictalurid catfishes (Finger, 1983, 1986, 1988; Kalmijn, 1988). Kalmijn (1974, 1988) demonstrated that catfishes can use the electric sense alone to locate live prey and to initiate feeding activity. Roth (1972) has shown electrophysiologically that ampullary electroreceptors respond to potentials generated by live fishes at a distance of 5–10 cm.

Roth (1968), Kalmijn (1974), Peters and van Wijland (1974, 1993) and Kalmijn *et al.* (1976 a, b) have also shown that catfishes use electric cues for orientation. McCleave *et al.* (1971) and Peters and Bretschneider (1972) demonstrated that freshwater habitats consist of detectable direct current fields that are stable enough to be used as landmarks. The source of such electrical variations may be chemical dissimilarities, bottom composition, springs, tributaries, vegetation beds and other differences in the biotic and abiotic environment. Freshwater catfishes can learn to discriminate between hiding places on the basis of the polarity of nonuniform and uniform electric fields (summarized in Kalmijn, 1988).

The question arises: how does the development of the electrosensory system compare with the onsets of behavioral functions thought to be mediated by electrosensory cues? To address this question we examined the onsets of three behaviors — undulatory swimming, schooling and exogenous feeding — with reference to the morphological development of the first order medullary electroreceptive nucleus, the electrosensory lateral line lobe (ELL).

## MATERIALS AND METHODS

*Ictalurus punctatus* eggs were purchased from Osage Catfishes, Osage Beach, Missouri during the early summers of 1994 and 1995 and were grown at the Iowa Lakeside Laboratory, Milford, Iowa. Eggs were incubated in a flow-through, lake water system. Following hatching, larvae were raised in aerated lake water in which 50% of the water was replaced daily. For food, post-larvae were offered a dense assortment of zooplankton, predominantly *Daphnia* sp., collected from nearby West Lake Okoboji.

Embryos and larvae of *Ictalurus punctatus* were staged based on a scheme similar to that proposed by Armstrong and Child (1962). In *I. punctatus*, hatching occurs between stages 40 and 43. In 1995, larvae in any particular stage were larger than in 1994.

To examine the ELL, 37 animals representing a developmental series between stages 30 and 53 (total lengths [TL] between 6.0 and 10.0 mm), as well as two juveniles (40 and 42 mm TL) and one adult (170 mm TL) were first anesthetized in 3-aminobenzoic acid ester (methanesulfonate salt, Sigma, St. Louis, U.S.A.) and immersion-fixed in 4% paraformaldehyde dissolved in phosphate buffered saline (PBS, pH 7.2). The craniums of older larvae were opened to facilitate penetration of the fixative into the brain. Juveniles and adults were transcardially perfused according to Lannoo *et al.* (1989). Animals were stored in fixative at room temperature and transported to our laboratory in Indiana. Prior to being processed for histological analysis, the stomachs of animals were examined to determine whether they had begun to feed.

The procedures used for histological analysis were routine (Kiernan, 1990), and are briefly summarized here. Following dehydration by immersion in a graded series of alcohols, whole animals (larvae) or brains (juveniles and adults) were embedded in paraffin and sectioned at either 6  $\mu\text{m}$  (1994) or 10  $\mu\text{m}$  (1995) on a rotary microtome. Ribbons of sections were mounted in order onto microscope slides, deparaffinized, rehydrated, stained with 0.1% cresyl violet acetate, dehydrated and coverslipped using Permount<sup>®</sup> (Fisher, Fair Lawn, NJ, U.S.A.) as the mounting medium. Sections were examined using a Jenalumar compound microscope or a Nikon SMZ-U dissecting microscope. Photographs were taken using either Kodak T-max<sup>®</sup> 100 or Tech Pan<sup>®</sup> film. For the descriptions presented here, we have primarily relied on the animals collected in 1995 and sectioned at 10  $\mu\text{m}$  in the transverse plane. Animals collected and sectioned in 1994, and animals sectioned parasagittally, were used to supplement these basic descriptions.

## RESULTS AND DISCUSSION

### Behavioral Onsets

Swimming and schooling. *Ictalurus punctatus* embryos begin moving the caudal portion of their tail at stage 28, but undulatory swimming does not begin until about stage 43. Larvae are more likely to swim when part of an aggregation ('schooling'; in our tanks this typically occurred in corners) or in a current. Describing aggregations, Ryder (1883:227) noted: 'When first hatched [stage

40–43], on the sixth to eighth day [following egg deposition], the young [*I. punctatus*] exhibited a tendency to bank up or school together like young salmon. They also, like young salmon, tended to face or swim against the currents in the aquarium, a habit common, in fact, to most young fishes recently hatched. At stage 48 larvae begin to exhibit negative geotropism, or 'swimming up'.

Feeding. Describing the onset of feeding, Ryder (1883:228) wrote: 'By the 13th day the yolk [yolk] had been absorbed [about stage 48] and the young fish were ready to feed. On the 15th day after oviposition it was found that they would feed.' Indeed, we observed first feeding in our fish at stage 52 or 53.

### Development of the ELL

The presence of both the lateral portion (electrosensory component) of the anterior ganglion (Finger, 1986) and the ganglion of the posterior lateral line nerve (electro- and mechanosensory components) was observed at stages 30–31. However, we could not demonstrate a connection between these ganglia and the brain at this age and thus could not confirm the presence of the central projections of these neurons.

The central projections of the lateral anterior and posterior ganglia were unequivocally demonstrated in our material by stage 32 (Figure 1a,b). These projections became increasingly robust with subsequent growth. At this point in development, the medulla appears as a nearly solid mass of neurons and neuroblasts except in the most ventral regions, which were asomatic (see Figure 1c) and consist of fascicles of decussating (arcuate) fibers, visible under differential interference contrast microscopy.

At stage 33–34, the lateralis innervation was well established. The medullary lateralis region could be distinguished as a dense cellular region located at the extreme lateral edge of the fourth ventricle (Figure 1c).

At stages 36–37, and 39–40, the lateralis region and its innervation were more robust than at previous stages, but remained qualitatively similar to their earlier appearance and therefore are not shown here. At stage 39–40, the lateralis area extended along the neural axis from the level of the caudal cerebellum through the level of the otic capsule.

By stage 42–43, cells in the lateralis area had begun to differentiate (Figure 1d). In this region, clusters of large neurons were positioned ventral and medial to smaller, undifferentiated cells.

At stages 45–46, the cells of the lateralis area continued to differentiate. The cerebellar crest was beginning to form (Figure 2a). This structure was similar to the molecular layer in other regions of the cerebellum and was positioned

subpially, above the cellular layers of the lateralis region and below what appears to be a lateralis growth zone (Figure 2a; see also Lannoo and Lannoo, 1994, 1995).

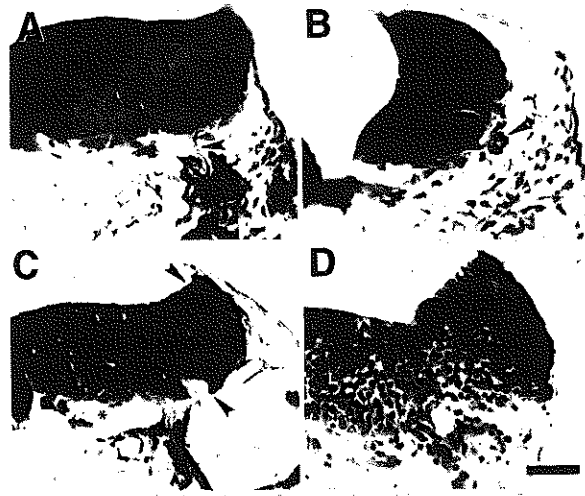


FIGURE 1 Development of the lateralis region in *Ictalurus punctatus* embryos. The connections between the anterior ganglion (A) and the ganglion of the posterior lateral line nerve (B) are demonstrated at stage 32 (arrowheads). C) The lateralis region (delimited by the arrowheads) is distinguished as a dense cellular region located at the extreme lateral edge of the fourth ventricle at stage 33–34. In this section note the large dorsal region of neuronal cell bodies and the smaller ventral somatic region (asterisk). D) Cells in the lateralis area begin to differentiate at stage 42–43. Scale bar: 25  $\mu\text{m}$ .

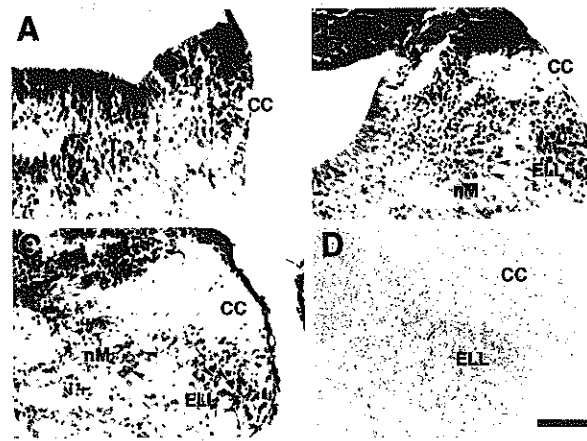


FIGURE 2 Development of the lateralis region in *Ictalurus punctatus* demonstrating the development of the cerebellar crest (CC) and the differentiation of the mechanoreceptive medial nucleus (nM) from the electrosensory lateral line lobe (ELL). A stages 45–46; B) stages 48–49; C) stage 53; and D) adult. B, C) note the large, differentiating crest cells (arrowheads). Scale bar: A, B, C, = 50  $\mu\text{m}$ ; D = 200  $\mu\text{m}$ .

During stages 48–49, distinct populations of crest cells, one located subpially and one more medially, could be distinguished (Figure 2b). These populations appeared to represent the morphological differentiation of the ELL from the mechanosensory medial nucleus. Within the ELL, the lamination of undifferentiated small cells overlying more differentiated large cells was present rostrally. In contrast, caudally within the ELL, cells of various sizes and stages of differentiation were intermingled. At these stages the cerebellar crest was well formed and recognizable, larger rostrally than caudally. At stage 50, the cerebellar crest differentiated into two recognizable regions: one over the ELL with a concave ventral surface, and one over the medial nucleus with a convex ventral surface.

By stage 53, the lateralis region had begun to form a distinct lobe (Figure 2c), visible on the surface of the brain. Within the ELL, cellular regions had begun to be recognizable, with large crest cells clustered dorsally. Subsequent growth was in a caudolateral direction. In adults, the ELL develops into a large lobe which, unlike in larvae, was capped only by the cerebellar crest (Figure 2d; Finger, 1986).

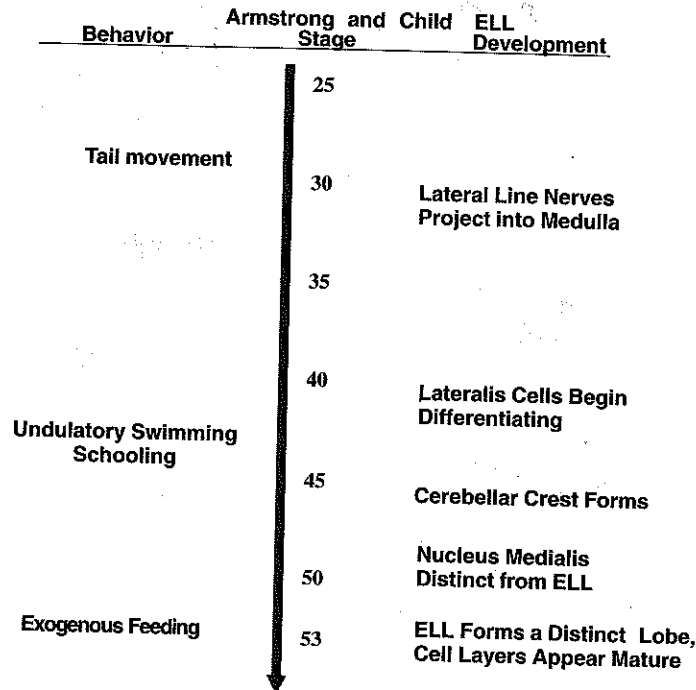


FIGURE 3 Stages in the development of *Ictalurus punctatus* illustrating the timing of the onset of swimming, schooling, and feeding behaviors relative to the formation of structures within the ELL.

### Timing of Development of ELL Circuitry and Implications for Behavioral Onsets

All of the components of the *Ictalurus punctatus* ELL were present and in a morphologically mature position by stage 53 (see also Lannoo and Lannoo, 1994, 1995). Assuming that these criteria indicate a functioning electrosensory system, this system does not mature in time to support undulatory swimming and schooling behaviors, but does mature in time to support exogenous feeding (Figure 3). This conclusion — that swimming begins before, and feeding begins after, the appearance of a morphologically mature ELL in *Ictalurus punctatus* — represents a correlation, and therefore must be interpreted with caution. To state definitively that the onset of feeding in this species depends upon a functioning electrosensory system requires the following demonstrations: 1) that functional maturity in the ELL is tied to morphological maturity; 2) that the higher-order connections necessary to integrate electrosensation with other neuronal systems are present and 3) that electroreception is required for feeding in larvae after yolk absorption.

### Acknowledgments

We dedicate this paper to the memory of Walter Heiligenberg. In 1987, Walter invited M.J.L. into his laboratory for a month to work with Heinrich Vischer on the development of ELL circuitry in gymnotoid electric fishes. It is difficult to consider aspects of ELL development without remembering the excitement of working in Walter's lab, and the afternoon trip we took out to the U.S. Navy's Top Gun base to collect zooplankton to feed the electric fish larvae.

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