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Short Communication

Retinoic acid repatterns axolotl lateral line receptors

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ABSTRACT The effects of all-trans retinoic acid on the development of the lateral line placodes of axolotls was studied. Late gastrula and early neurula were exposed to 10^{-7} to 10^{-5} M retinoic acid for one hour and then reared until they would normally be feeding larvae. As in other vertebrates, the extent of the developmental abnormalities is concentration dependent. Those embryos exposed to the highest concentration of retinoic acid failed to form much of the forebrain and midbrain, including the olfactory, optic and otic organs, which were reduced or absent. Although all lateral line placodes continued to generate fully formed receptors and cranial nerves, the production of neuromasts and the organization of these receptors into lines were markedly reduced. Equally important, all of the placodes at the highest concentration of retinoic acid failed to generate ampullary organs, thereby indicating a strong posteriorizing effect of retinoic acid on these placodes.

KEY WORDS: *electroreceptor, lateral line, neuromast, retinoic acid, salamander*

Vertebrate heads are characterized by a number of segmentally arranged features along their anteroposterior axis: pharyngeal arches and pouches, branchiomeres, cranial nerves, neuromeres, etc. Many of these features are derived from neural crest and neurogenic placodes (Northcutt and Gans, 1983) whose patterning during development is critical for the formation of a complex head. The lateral line system is a set of features that arises from a serially repeated series of dorsolateral placodes (Fig. 1) and whose fates differ along the anteroposterior axis of the head (Stone, 1922; Smith *et al.*, 1990; Northcutt *et al.*, 1994). For example, in most aquatic salamanders, the first four lateral line placodes (Fig. 1, ad, av, mid, and st) give rise to both electroreceptive ampullary organs and mechanoreceptive neuromasts (Fig. 2), whereas the posterior lateral line placode (Fig. 1, p) only gives rise to the trunk neuromasts (Northcutt *et al.*, 1995). Individual lateral line placodes also differ from each other by forming unique patterns of neuromast lines and by giving rise to multiple lateral line nerves that enter the medulla sequentially in the anteroposterior axis (Northcutt, 1992; Northcutt and Brändle, 1995).

Patterning of the anteroposterior axis can be disrupted during development (Dursten *et al.*, 1989; Sive *et al.*, 1990; Holder and Hill, 1991) by the application of exogenous all-trans retinoic acid (RA). When administered during neurulation, RA has a "posteriorizing effect" in that it suppresses the differentiation of anterior features and/or stimulates the differentiation of posterior features. For example, when RA is administered to *Xenopus* embryos during gastrulation, the tadpoles lack olfactory organs and eyes, as well as much of the forebrain and midbrain, but they have an enlarged hindbrain (Dursten *et al.*, 1989). Similar results have been reported in chicks, where administration of RA repatterns the first branchial arch (Plant *et al.*, 2000) and transforms the maxillary prominence into a second frontonasal mass (Lee *et al.*, 2001).

Little information is available on the effects of RA on the development of the lateral line system. Holder and Hill (1991) reported the reduction of the ganglion of the anterior lateral line nerve and enlargement of the ganglion of the posterior lateral line nerve in zebrafish, but they did not comment on other features of the lateral line system. For this reason, axolotl embryos were exposed to various concentrations of RA during early neurulation in order to assess the effects of RA on the development of their lateral line system. Although the time frame for the specification and determination of the lateral line placodes in axolotls has not been established, it is likely the embryos were exposed to RA prior to determination of the placodes (Schlosser and Northcutt, 2001). Axolotls were chosen because the development and organization of the lateral line system is best understood in this species.

Larvae exposed to 10^{-7}M RA were superficially indistinguishable from control stage 43 larvae (Fig 3A). The same six major lateral lines that occur on the head of normal stage 43 larvae (Fig.

Abbreviations used in this paper: RA, retinoic acid.

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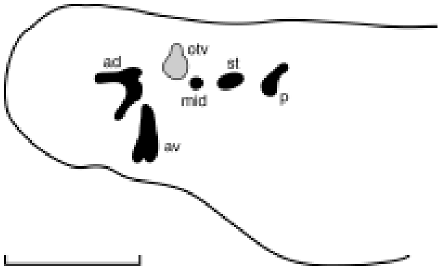


Fig. 1. Camera lucida drawing of a flat mount of the head ectoderm of a normal stage-35 axolotl embryo. *Relative position and size of the dorsolateral placodes. ad, anterodorsal; av, anteroventral; mid, middle; otv, otic vesicle; p, posterior; st, supratemporal placodes. Bar, 1 mm.*

4) could be recognized in those larvae exposed to 10^{-7}M RA (Fig. 4). The supraorbital and infraorbital lines, which are derived from the anterodorsal placode, were, however, shifted posteriorly in these larvae, so that the otic gap, a patch of ectoderm normally free of lateral line receptors, was reduced. These larvae also showed slight reduction in the number of ampullary organs (Fig. 4).

Larvae exposed to 10^{-6} M RA appeared distinctly abnormal. The eyes and head were smaller, the number of primary gill filaments was reduced, and these filaments had fewer secondary filaments. The body was shorter, and some abdominal edema was apparent. These larvae lacked an otic gap, and the number of neuromasts in the supraorbital, infraorbital, and preoperculomandibular lines was reduced (Fig. 4). The middle and supratemporal lines, which are derived from the same named lateral line placodes, were shifted ventrally, and individual lines were no longer recognizable. The number of ampullary and pit organs was also significantly reduced (Fig. 4).

Larvae exposed to 10^{-5} M RA were extremely microcephalic in appearance (Fig. 3B), with eyes and otic organs that were tiny or nonexistent. The gills and their primary filaments were severely reduced, and the trunk was considerably shorter than that of the controls. These larvae also exhibited severe abdominal edema. The number of neuromasts was severely reduced, and the organization of the remaining neuromasts was so disrupted that it was impossible to discern which lines were represented by these receptors (Fig. 4). However, in spite of the disruption to lateral line receptor fields, all neuromasts were fully differentiated and of normal size. Finally, ampullary and pit organs were totally absent.

Larvae exposed to 10^{-5} M RA displayed, in addition, greatly distorted cranial nerves (Fig. 5). These larvae lacked olfactory, optic, and octaval nerves, and the trigeminal nerve, when present, was reduced to scattered ganglionic cells displaced far rostrally. The remaining cranial nerves were seen to enter the medulla via a single ganglionic mass, which could be divided into an extremely large dorsal component, composed of lateral line neurons, and a ventral component, composed of the sensory neurons of the branchiomeric nerve.

In developing axolotls, RA clearly respecifies the positional values of the lateral line placodes and other cephalic structures. Those larvae exposed to high concentrations of RA experienced severe reductions in the rostral head, similar to those reported in zebrafish (Holder and Hill, 1991) and *Xenopus* (Durston *et al.*, 1989). Unlike what happens to the inner ear (Holder and Hill, 1991), however, the lateral line is fully differentiated when exposed to high concentrations of RA. In spite of the severe reductions in head structures, none of the rostral lateral line placodes were lost, as even those embryos exposed to 10^{-5} M RA still developed superficial ophthalamic and buccal rami whose ganglionic cells are derived from the most rostral placode, the anterodorsal placode. All of the placodes, however, generated far fewer neuromasts, and the organization of these neuromasts into specific lines was badly disrupted. Perhaps most interesting is the fact that none of the lateral line placodes generated ampullary and pit organs, as would

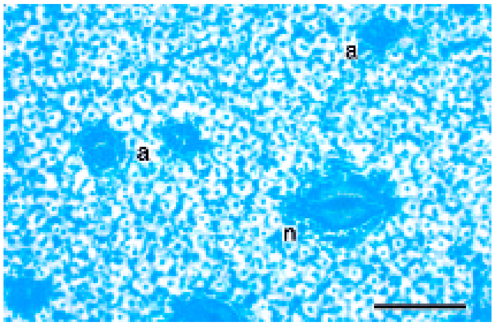


Fig. 2. Photomicrograph of ampullary organs (a) and a neuromast (n). *Flat mount of the head ectoderm of a normal axolotl larva. Bar, 200 μm.*

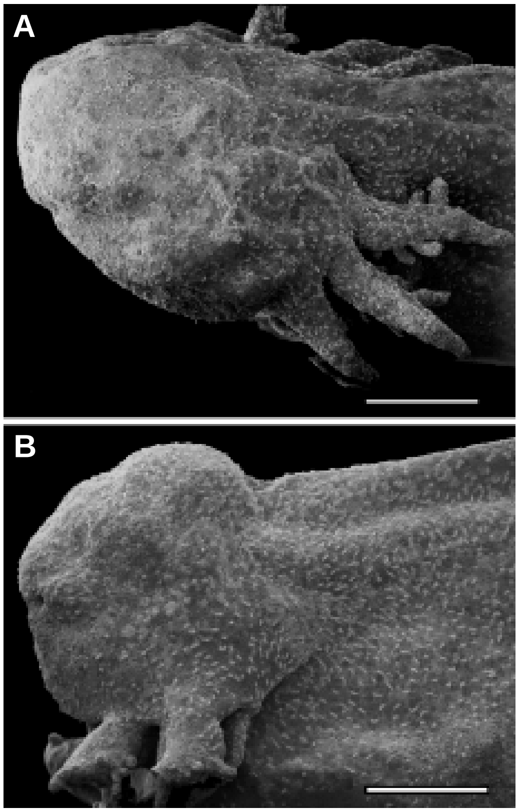
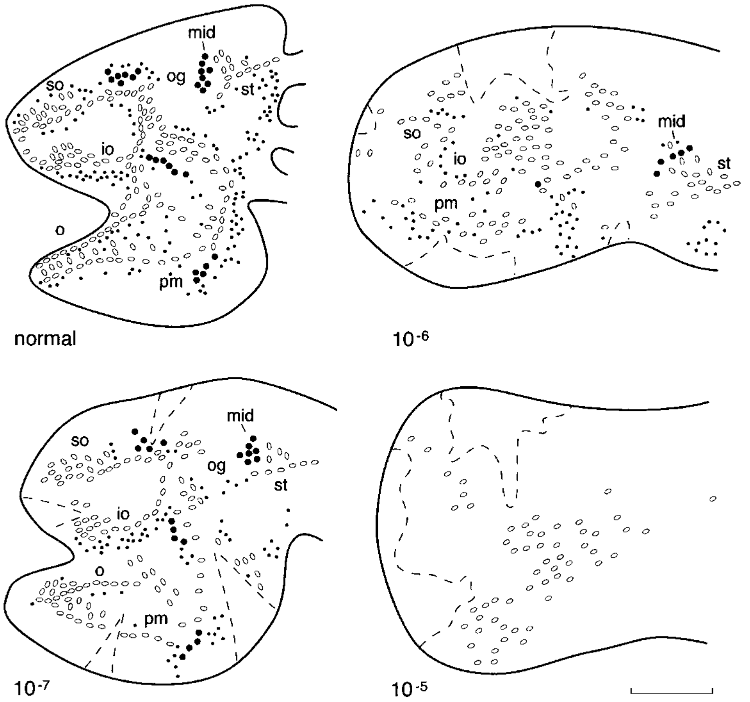


Fig. 3. Scanning electron micrograph of the lateral surface of the head of a stage-43 axolotl larva. (A) *Control and* **(B)** *larva exposed to 10^{-5} M retinoic acid. Bar, 500 μm.*



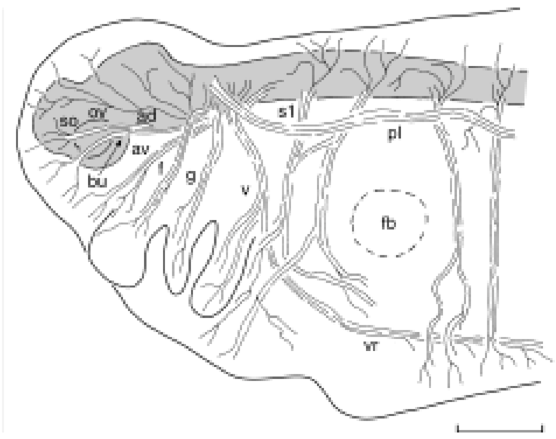


Fig. 4 (left). Camera lucida drawings of flat mounts of the left head ectoderm of stage-43 axolotl larvae. *Distribution of ampullary organs (small dots), neuromasts (open ovals) and a special class of neuromasts termed pit organs (large dots) after exposure to* 10^{-7}*,* 10^{-6} *and* 10^{-5} *M retinoic acid for one hour during neurulation. Abbreviations: io, infraorbital; mid, middle; o, oral; og, otic gap; pm, preoperculomandibular; so, supraorbital; st, supratemporal lateral lines. Bar, 1 mm.*

Fig. 5 (right). Camera lucida drawing of immunohistochemically visualized cranial nerves of a stage-43 axolotl larva. *The larva was exposed to* 10^{-5} *M retinoic acid for 1 hour. Abbreviations: ad, anterodorsal lateral line nerve; av, anteroventral lateral line nerve; bu, buccal ramus; f, facial nerve; fb, forelimb bud; g, glossopharyngeal nerve; ov, optic vesicle; pl, posterior lateral line nerve; so, superficial ophthalmic ramus; s1, first spinal nerve; v, vagal nerve; vr, vagal visceral ramus. Bar, 400* μ*m.*

normally occur. Since ampullary organ primordia normally form prior to complete differentiation of neuromasts and neuromasts were fully differentiated in embryos exposed to the highest RA concentrations, the fact that ampullary organs did not form cannot be due to a truncation of lateral line developmental programs. The lack of ampullary organs reflects the posteriorizing effect of RA; even the most rostral placodes mimicked the most posterior placodes in receptor development. This is particularly evident in the greatly increased size of what appears to be the sensory ganglion of the posterior lateral line nerve, which appears to be due to some sort of fusion of all lateral line ganglia. This apparent fusion of lateral line ganglia is similar to what has been seen in zebrafish (Holder and Hill, 1991). It remains to be determined, however, whether these effects are due to the direct action of RA on the placodes, or whether they occur indirectly due to changes in neural crest and/or the medulla.

Experimental Procedures

Embryonic axolotls, *Ambystoma mexicanum,* incubated at 23-26ºC from fertilized eggs (wild type) by the Indiana Axolotl Colony, Indiana University, were staged following the normal developmental tables of Bordzilovskaya *et al.* (1989). Embryos from late gastrula-early neurula stages (stages 12-14) were exposed to either 10^{-5}, 10^{-6}, or 10^{-7} M all-trans retinoic acid (Sigma Chemical Co.), dissolved in Holtfreter's solution containing 1% dimethyl sulfoxide (DMSO), for one hour in the dark, then rinsed five times in fresh Holtfreter's solution and 1% DMSO. Retinoic acid solutions were prepared immediately prior to each experiment. Subsequent rearing occurred in Holtfreter's solution until stage 43, when feeding commences. At this stage, the embryos were euthanized in 0.01% tricaine methanesulfonate (Sigma) and preserved for analysis. Additional embryos from stages 12-14 were exposed to Holtfreter's solution and 1% DMSO for one hour and reared as controls. Conclusions were based on a minimum of 24 experimental cases (i.e. 8-10 individuals, each, from three different egg clutches).

All embryos were fixed in either 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) or Dent's fixative (20 ml DMSO and 80 ml absolute methanol). The embryos fixed in paraformaldehyde were used for scanning electron microscopy, ectodermal skin mounts, or histology (glycol methacrylate-embedded transverse serial sections). The Dent's fixed embryos were used to visualize the cranial nerves immunohistochemically, using an antibody against acetylated α-tubulin (Sigma). These procedures are outlined in detail in Northcutt *et al.* (1994).

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