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Amphibians provide new insights into taste-bud development

R. Glenn Northcutt and Linda A. Barlow

Until recently, the predominant model of taste-bud development was one of neural induction: ingrowing sensory fibers were thought to induce taste-bud differentiation late in embryonic development. Recent experimental studies, however, show that the development of taste buds is independent of their innervation. In amphibian embryos, the ability to generate taste buds is an intrinsic feature of the oropharyngeal epithelium long before the region becomes innervated. These studies indicate that patterning of the oropharyngeal epithelium occurs during gastrulation, and suggest that taste buds or their progenitors play the dominant role in the development of their own innervation.

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MUCH OF THE PNS of vertebrates arises from neurogenic placodes and the neural crest, embryonic tissues (Fig. 1) that are hallmarks of vertebrate development and underlie vertebrate origins[1–3]. The role of the neural crest is well documented in the genesis of the sensory ganglia of cranial and spinal nerves, and in the genesis and patterning of such diverse structures as the neurocranium, pharyngeal skeleton, teeth and other epidermal derivatives, including feathers and hair[4–6].

The role of neurogenic placodes, localized patches of tall columnar cells, in the development of the PNS is not as widely appreciated. These placodes form within the head ectoderm of all vertebrate embryos (Fig. 1). The most rostral of these placodes, the olfactory placode, invaginates to form the receptors and nerves of the olfactory complex (olfactory and vomeronasal organs and nerves), as well as the closely associated ganglion cells of the nervus terminalis[7,8]. More caudally situated profundal or trigeminal placodes, or both, contribute neurons to the compound sensory ganglion of the trigeminal nerve[3,9], whose fibers innervate much of the skin of the head. The remaining neurogenic placodes can be divided into a dorsolateral series and a ventrolateral (epibranchial) series, adjacent to the developing hindbrain and pharyngeal pouches, respectively (Fig. 1). The dorsolateral series consists of an octaval (otic) placode that invaginates to form the sensory maculae of the inner ear and the sensory ganglion of the eighth nerve[10], and, in fish and many amphibians, an additional six placodes that give rise to the electroreceptive and mechanoreceptive organs of the lateral-line system and the cranial nerves that innervate these receptors[11–16].

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Epibranchial placodes were initially implicated in the development of the gustatory system, primarily on the basis that they were extremely well developed in embryonic catfishes[17], which develop extensive fields of taste buds that cover their entire body surface. This correlation was subsequently reinforced by the experimental observation that the epibranchial placodes contribute neurons to the sensory ganglia of the facial, glossopharyngeal and vagal nerves[9]; these are the only nerves that innervate taste buds in vertebrates. In spite of this correlation, there is still no experimental evidence of whether the neurons that innervate taste buds arise from the neural crest or epibranchial placodes, or both.

Unfortunately, there are few experimental studies on the development of the gustatory system of vertebrates: manipulation of avian and mammalian embryos is quite difficult at later embryonic stages, and it is precisely during this late phase that taste buds develop. Culture of embryonic tissues of amniotes is also limited to short periods of time, which has further precluded the study of taste-bud development[18,19]. However, amphibian embryos offer an alternative developmental model that avoids many of these difficulties. These embryos are resilient to surgical manipulation at any stage of embryogenesis, and embryonic tissues develop normally in culture for up to several weeks. Embryos of the Mexican axolotl, *Ambystoma mexicanum*, are particularly useful because the embryonic cells of the wild type contain cytoplasmic melanin granules that provide a robust, endogenous marker with which to track the fate of the cells when they are transplanted into albino hosts. A number of recent experimental studies using embryonic axolotls[20–22] have provided new insights into the embryonic origin of taste buds

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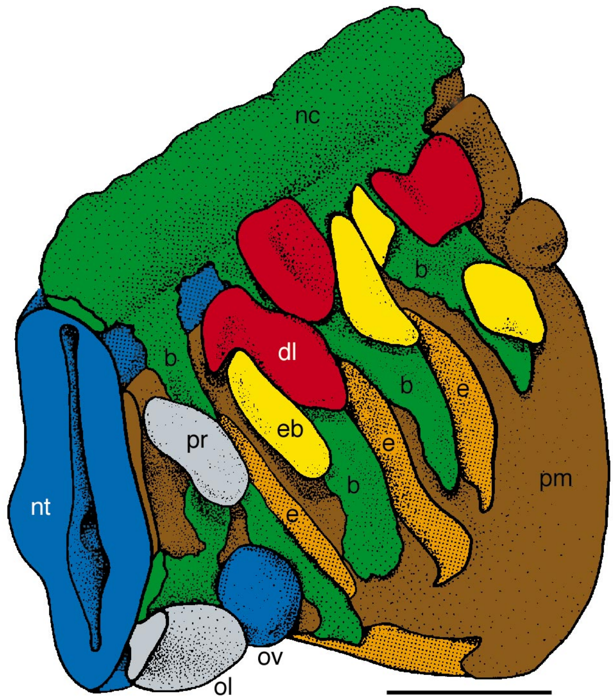

Fig. 1. ***Distribution of neurogenic placodes and neural crest in the developing head of an axolotl.*** *In a dorsolateral reconstruction of the head (rostral is to the left) at an early stage of development, the neural crest (nc) caps the dorsal surface of the neural tube (nt), but bands of crest cells (b) are beginning to stream ventrally over the surface of the paraxial mesoderm (pm). The rostral paraxial mesoderm will form the extrinsic eye muscles, whereas the more caudal paraxial mesoderm will form the branchiomeric muscles, which are separated by evaginated pharyngeal pouches (e) of endoderm. At this stage, the paraxial mesoderm has not yet completed its migration, and pharyngeal endoderm (orange) is still visible ventrally. The bands of neural crest will eventually form the proximal portions of the sensory ganglia of the cranial nerves that innervate the branchiomeric muscles, as well as the pharyngeal skeleton and overlying dermis of the skin. At this developmental stage, two rostral neurogenic placodes can be identified near the optic vesicle (ov): the olfactory placode (ol), which will give rise to the peripheral olfactory system; and the profundal placode (pr), which will give rise to the profundal (deep opthalmic) and/or trigeminal nerves. More c audally, a dorsolateral series of placodes (dl) will give rise to the receptors and cranial nerves of the inner-ear and lateral-line system, and a ventrolateral or epibranchial series of placodes (eb) will give rise to distal portions of the sensory ganglia of the facial, glossopharyngeal and vagal nerves. Scale bar, 500 µm.* Modified from Ref. 3.

and the tissues responsible for their induction. These insights reveal the inadequacies of earlier models of taste-bud development, and enable an alternative model to be proposed.

Embryonic origin of taste buds

Epibranchial placodes, like the dorsolateral placodes, might give rise to receptor organs as well as the nerves that innervate them[1,2]. If this is the case, then placodal cells must migrate into the oral epithelium where they differentiate into taste buds. Subsequently, the taste buds become innervated by the ingrowing fibers of the sensory neurons of the facial, glossopharyngeal and vagal nerves, which also arise from the epibranchial placodes. However, homotopic transplantation of patches of skin containing epibranchial placodes from pigmented axolotl embryos into albino hosts at the same developmental stage (Figs 2A and 3A) revealed that the epibranchial placodes only produce the sensory neurons of the nerves that innervate taste buds (Fig. 3B), not the taste buds themselves[20].

The essential role of the neural crest in the development of teeth and pharyngeal elements indicates that crest cells migrate into the oropharyngeal region before the differentiation of taste buds[4,6] and it has therefore been suggested that taste buds might arise from a population of crest cells[23]. Homotopic transplantation of a portion of the cephalic neural fold known to contribute to the branchial arches[6] from pigmented axolotl embryos into albino hosts at the same developmental stage (Fig. 2B) resulted in pigmented cells in all known neural crest-derived tissues, such as the cartilages of the branchial arches (Fig. 3C), cranial nerve ganglia, odontoblasts, melanocytes and mesenchymal cells of the future dermis, but pigmented cells were not found in taste buds[20].

Because taste buds do not arise from the neural crest or epibranchial placodes, it is likely that these sensory organs arise locally from the oropharyngeal epithelium. To test this hypothesis, a fluorescent lipophilic dye, DiI, was microinjected into the dorsal lip of axolotl embryos at the onset of gastrulation (Fig. 2C). This region is known to give rise to the cephalic endoderm

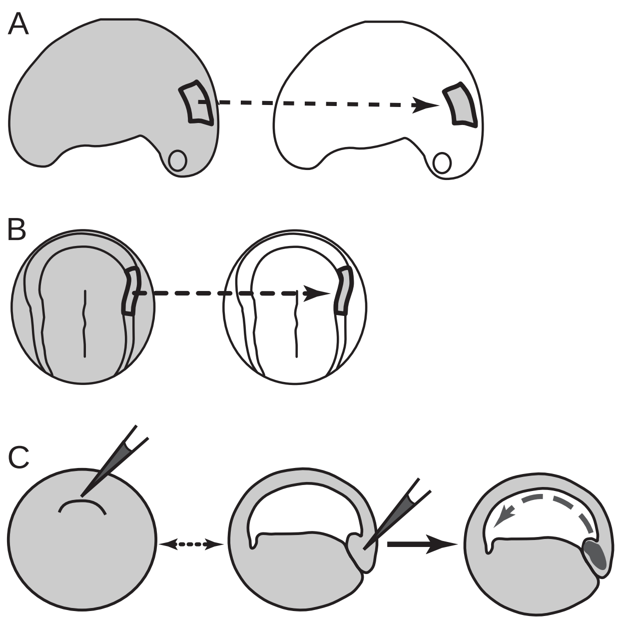

Fig. 2. ***Experimental manipulations used to determine the embryonic origin of taste buds.*** *Each cell of a wild-type axolotl embryo is provisioned with maternally supplied melanin granules, which serve as a robust marker of cell fate when wild-type cells are placed in an albino host. The unilateral transplantation of pigmented epibranchial placodal ectoderm into albino host embryos* **(A)** *is used to demonstrate that the epibranchial placodes contribute neurons to the cranial nerve ganglia that innervate taste buds but do not give rise to taste buds. The unilateral transplantation of a pigmented neural fold into an albino host embryo* **(B)** *is used to demonstrate that neural crest cells do not give rise to taste buds. Microinjection of the lipophilic dye, DiI, into presumptive pharyngeal endoderm at the onset of gastrulation* **(C)** *reveals that taste buds arise directly from pharyngeal endoderm. In* A*, both early tailbud embryos are shown in lateral view, with anterior to the right. In* B*, the neural plate-stage embryos are shown in dorsal view, with the anterior at the top. In C, the left and middle embryos, the animal pole is at the top: the middle embryo represents an embryo at the same early gastrula stage shown in sagittal view. In the right embryo, the broken arrow outlines the path that the presumptive pharyngeal endoderm will involute during gastrulation.*