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Embryonic origin of gustatory cranial sensory neurons

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Abstract

Cranial nerves VII, IX and X provide both gustatory (taste) and non-gustatory (touch, pain, temperature) innervation to the oral cavity of vertebrates. Gustatory neurons innervate taste buds and project centrally to the rostral nucleus of the solitary tract (NTS), whereas neurons providing general epithelial innervation to the oropharynx project to non-gustatory hindbrain regions, i.e., spinal trigeminal nucleus. In addition to this dichotomy in function, cranial ganglia VII, IX and X have dual embryonic origins, comprising sensory neurons derived from both cranial neural crest and epibranchial placodes. We used a fate mapping approach to test the hypothesis that epibranchial placodes give rise to gustatory neurons, whereas the neural crest generates non-gustatory cells. Placodal ectoderm or neural crest was grafted from Green Fluorescent Protein (GFP) expressing salamander embryos into unlabeled hosts, allowing us to discern the postembryonic central and peripheral projections of each embryonic neuronal population. Neurites that innervate taste buds are exclusively placodal in origin, and their central processes project to the NTS, consistent with a gustatory fate. In contrast, neural crest-derived neurons do not innervate taste buds; instead, neurites of these sensory neurons terminate as free nerve endings within the oral epithelium. Further, the majority of centrally directed fibers of neural crest neurons terminate outside the NTS, in regions that receive general epithelial afferents. Our data provide empirical evidence that embryonic origin dictates mature neuron function within cranial sensory ganglia: specifically, gustatory neurons derive from epibranchial placodes, whereas neural crest-derived neurons provide general epithelial innervation to the oral cavity.

Keywords: Cranial sensory ganglia; Gustatory neurons; Taste buds; Axolotl; Amphibian; GFP; Neural crest; Placodes; Gangliogenesis

Introduction

Innervation of the oral cavity is provided by branchiomeric nerves, V, VII, IX and X, whose cell bodies are housed in cranial ganglia adjacent to the hindbrain (Gross et al., 2003; Hatini et al., 1999; Landacre, 1910, 1912; Northcutt and Brändle, 1995). Three broad types of sensory neurons are found in these ganglia, i.e., somatosensory, general viscerosensory, and special viscerosensory, which together provide non-gustatory and gustatory innervation to the oral and pharyngeal regions. Non-gustatory neurons include both somatosensory fibers, which terminate as free nerve endings in the non-taste bud-bearing regions of the oropharyngeal epithelia and project centrally to the nucleus of the spinal tract of V (SpV; Halsell et al., 1993; Martin and Mason, 1977; Phelan and Falls, 1991), and general visceral fibers, which innervate sensory receptors in the cardiovascular and gastric epithelia and project to the caudal solitary nucleus (NTS; Cechetto, 1987; Roth and Wake, 1985; Zhang and Ashwell, 2001). Gustatory neurons, which are also termed special visceral (see Finger, 1993), innervate specialized peripheral receptor organs, the taste buds, embedded in the epithelial surfaces of the mouth and pharynx, and project to the rostral solitary nucleus within the hindbrain (Herrick, 1944; Martin and Mason, 1977; Opdam and Nieuwenhuys, 1976; Smith and Davis, 2000).

In addition to their diverse sensory functions, neurons of cranial sensory ganglia VII, IX and X are derived from two distinct embryonic cell populations: the cranial neural crest, and neurogenic epibranchial placodes (Adelmann, 1925; Coghill, 1916; D'Amico-Martel and Noden, 1983; His, 1868; Landacre, 1910, 1912; Narayanan and Narayanan, 1980; Northcutt and Brändle, 1995). Neural crest cells arise at the dorsal-most region of the developing neural fold, migrate ventrolaterally, and a

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subset differentiate into sensory neurons within the ganglia of the branchiomeric nerves (Hall, 1989; Hörstadius, 1950; Le Douarin and Dupin, 1993). Neural crest cells also contribute glial cells to the cranial ganglia (D'Amico-Martel and Noden, 1983). The epibranchial placodes appear slightly later in development, after the cranial neural crest has begun to migrate, as a series of thickenings in the ectoderm just dorsal to each of the branchial arches. These placodes generate neurons which then coalesce with the neural crest-derived cells to form ganglia VII, IX and X (Baker and Bronner-Fraser, 2001; D'Amico-Martel and Noden, 1983; Gross et al., 2003; Landacre, 1933).

One ambiguity of cranial nerve development is how the different sensory identities of the neuronal subtypes of ganglia VII, IX and X are established. One possibility is that the embryonic origin restricts neuronal developmental potential to a particular sensory subtype. Many have speculated that the epibranchial placodes contribute gustatory neurons to these ganglia, while the neural crest supplies general epithelial afferents (Ayer-Le Lievre and Le Douarin, 1982; D'Amico-Martel and Noden, 1983; Davies and Lindsay, 1985; Gross et al., 2003; Herrick, 1901; Landacre, 1910, 1912; Landacre, 1921; Narayanan and Narayanan, 1980; Northcutt and Brändle, 1995; Stone, 1922; Yntema, 1937, 1943; Yntema, 1944). Several descriptive studies support the idea that gustatory neurons arise from the epibranchial placodes. For example, there is a reliable correlation between the number of gustatory fibers exiting the ganglion and the size of the epibranchial placode neuronal contribution, although the proportion of placodal versus crest-derived neurons to individual ganglia varies among species (Ayer-Le Lievre and Le Douarin, 1982; D'Amico-Martel and Noden, 1983; Herrick, 1901; Landacre, 1910, 1912; Narayanan and Narayanan, 1980; Northcutt and Brändle, 1995). Further, gustatory neurons are only present in cranial ganglia that have a neuronal contribution from the epibranchial placodes, again leading many investigators to assume that gustatory neurons are descendent from epibranchial placodes (Coghill, 1916; Gross et al., 2003; Landacre, 1910, 1933; Yntema, 1943). However, the inability to specifically label the neurites of placodal versus neural crest neurons has precluded attempts to discern the embryonic origins of the three subtypes of sensory neurons within the ganglia (somatosensory, general visceral, and special visceral), and none of the previous work conclusively defines a role for embryonic origin in mature neuron fate.

The present study focuses on resolving the embryonic origin (epibranchial placode or neural crest) of the sensory neurons within cranial ganglia VII, IX and X, which innervate taste buds. If placodal neurons are exclusively gustatory, these neurons will innervate only taste buds in the periphery, and project centrally to the gustatory hindbrain nucleus, the rostral NTS. Conversely, if neural crest-derived neurons are non-gustatory, providing solely general epithelial innervation, they will terminate as free nerve endings in the oral epithelium and project axons to the appropriate hindbrain region, namely, the SpV. To test these hypotheses, we used embryos of *Ambystoma mexicanum*, the axolotl. Taste buds in this aquatic salamander are easily recognized at early postembryonic or larval stages (Barlow et al., 1996), and the development and neuroanatomy of cranial ganglia VII, IX and X, including central termination fields, have been described in great detail in this genus (Coghill, 1916; Herrick, 1901; Herrick, 1948; Landacre, 1910, 1921, 1931, 1933; Northcutt et al., 2000; Northcutt and Brändle, 1995; Northcutt et al., 1994; Roth et al., 1993; Roth and Wake, 1985; Stone, 1922). By grafting epibranchial placode or neural crest precursors labeled cytoplasmically with GFP into unlabeled host embryos, we mapped the ganglionic location, and peripheral and central projection pattern of each population of descendant sensory neurons. We found a complete segregation of cell fate depending on embryonic origin as assessed by neuroanatomical criteria. Our results demonstrate that, with respect to the oropharyngeal region, gustatory neurons are indeed exclusively placodal in origin, whereas those derived from the neural crest are entirely non-gustatory.

Materials and methods

Animal housing and care

Wild-type pigmented, albino, and transgenic-GFP (Sobkow et al., 2006) embryos of the axolotl, *A. mexicanum*, were obtained from the Ambystoma Genetic Stock Center at the University of Kentucky (Lexington, KY). Embryos and larvae from stages 2 to 41 (Bordzilovskaya et al., 1989) were maintained in 20% Holtfreter's Solution (HF) at 22 °C, unless otherwise noted. Animal procedures were approved by University of Colorado IACUC.

GFP microinjection

Prior to availability of GFP transgenic axolotl embryos (Sobkow et al., 2006), embryos were injected with GFP mRNA at the one- to two-cell stage. GFP mRNA was made in vitro using the SP6 promoter of the pCS2mt-GFP plasmid, gift of Dr. Mike Klymkowsky, University of Colorado Boulder (Klymkowsky, 1999), and the mMessage mMachine kit (Ambion, Texas, USA). For injections, pigmented embryos were immersed in 0.1% formalin in 20% HF as an antifungal treatment, rinsed twice in 20% HF, and manually removed from their egg jellies. Dejellied embryos were stabilized in trenches cut into Sylgard-lined (Dow Corning, Michigan, USA) dishes in 100% HF supplemented with penicillin and streptomycin (400 μg/ml each) and gentamycin (25 mg/ml). Two to 5 nl of GFP mRNA (225 pg/nl in dH_2O) were injected into each blastomere at the two-cell stage. Injected embryos were kept in 10% HF to prevent exogastrulation and returned to 100% HF once gastrulation was complete.

Transplant microsurgery

Microsurgical procedures were adapted from those described previously (Barlow and Northcutt, 1995; Gross et al., 2003). Briefly, neural crest grafts were performed at stages 17–18 (mid-neurula), prior to crest migration (Falck et al., 2002; Northcutt and Brändle, 1995), whereas grafts of ectoderm containing the presumptive epibranchial placodes were performed at stage 19 (late neurula, Gross et al., 2003). In the case of pigmented donor embryos labeled via GFP mRNA microinjection, albino host embryos of the same stage were used. When transgenic GFP donor embryos were utilized, wild-type pigmented embryos were used as hosts. In preparation for surgery, embryos were immersed in 100% HF and held in position in beds made in Permoplast® Clay (AMACO, Illinois, USA). The subregion of the anterior neural fold (NC: Barlow and Northcutt, 1995) or the presumptive epibranchial placode ectoderm (EP: Gross et al., 2003) was removed cleanly from GFP-labeled donor embryos with flame-etched tungsten microneedles and transplanted isochronically and homotopically into host embryos whose own neural fold ($n=16$) or presumptive epibranchial placode-containing ectoderm ($n=33$) had been removed (Figs. 1B–F). All grafts were unilateral, with care taken to maintain the correct orientation of the transplanted tissue. Grafts were held in place with mild pressure by a clay bridge