The Role of Innervation in Induction and Differentiation of Taste Organs: Introduction and Background

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ABSTRACT: To establish lingual receptive fields that are the basic unit of taste function, ganglion cells must extend neurites to peripheral and central targets and form connections. This symposium concerns developmental interactions between the geniculate, trigeminal and petrosal ganglia and peripheral taste organs, the gustatory papillae and resident taste buds. Investigators present data from organ and tissue culture, from mice with targeted gene deletions and from grafting experiments, in pursuit of principles that direct early innervation of the taste system. The lingual ganglia and the taste papillae initially develop independently, but then become reciprocally dependent as ganglia derive neurotrophin support from gustatory papillae and the papillae require sensory innervation for growth and morphogenesis. The issue of subsequent taste bud induction is discussed with results from amphibian and mammalian models, yielding conclusions that are not yet totally convergent. However, an essential role for sensory innervation in mammalian taste bud differentiation and acquisition of appropriate quantitative relations between ganglion cells and target organs is clearly demonstrated. A working outline is presented for periods of ganglion cell/target organ independence and interdependence during early innervation of the peripheral taste system.

A functioning gustatory system is essential to survival. It is not surprising, then, that behavioral responses to taste stimuli are demonstrable in the human newborn on the day of birth and indeed, neural substrates for the sense of taste are functional in the mammalian fetus.[1,2] The early development of neural pathways that transmit sensory input from lingual taste organs to the central nervous system must derive initially from the cranial ganglia devoted to gustation: the geniculate ganglion innervating anterior tongue taste buds; the petrosal ganglion innervating posterior tongue taste buds and gustatory papillae; and, in addition, the trigeminal ganglion providing sensory innervation to the gustatory papillae on the anterior tongue but not to the taste buds *per se.*

To establish taste pathways during development, neurites extend from the ganglion cells not only to gustatory organs within the tongue, but also to the brainstem in the central nervous system. In this way, receptive fields are established that are the basic unit of sensory function. Therefore, development of the gustatory ganglia is central to establishment of taste sensation. This symposium on *The Role of Innervation in Induction and Differentiation of Taste Organs* focuses on current issues related to developmental interactions and the formation of connections between the taste ganglia and their peripheral target organs, the gustatory papillae and resident taste buds (FIG. 1). In this introduction, I outline some of the key questions related to early innervation of the peripheral taste system and indicate how symposium speakers address aspects of

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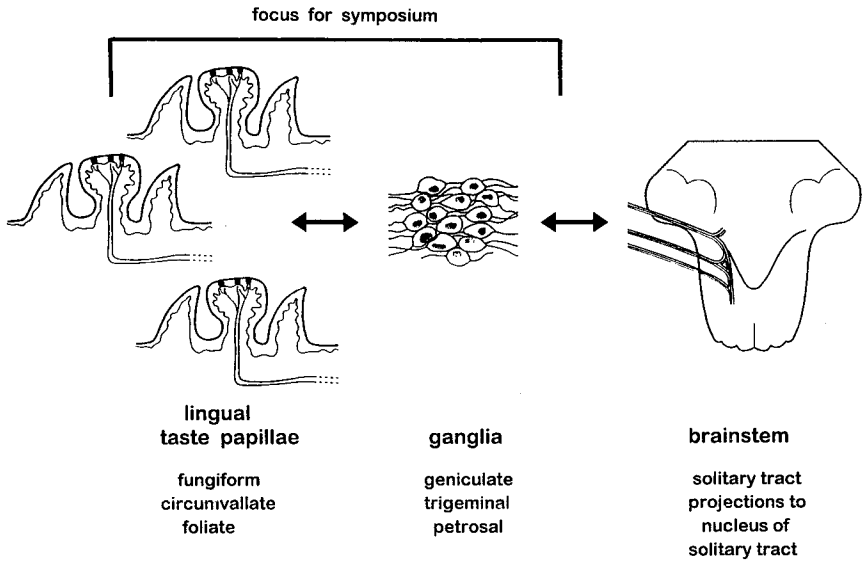

FIGURE 1. To establish lingual taste pathways, neurites must extend from sensory ganglia to innervate peripheral targets in the tongue and central targets in the brainstem. This symposium focuses on initial development in the peripheral taste system, and interactions between the ganglia that innervate the tongue and the lingual taste organs, the gustatory papillae and their resident taste buds.

these questions. The papers that follow this introduction represent a subset of the presentations from the symposium; papers from L. F. Reichardt and A. M. Davies are not included.

A major issue in understanding ganglion cell/taste organ interactions relates to the nature of environmental factors, whether local or derived from the target organs, that support ganglion cell development and differentiation. Although the biology of taste ganglia has not been extensively explored, neurotrophins are among the factors that have recognized roles in development of various sensory neuron populations.[3-5] Indeed, recent evidence indicates that neurotrophins also play a key role in the survival and differentiation of cells in the geniculate and petrosal ganglia, the major taste ganglia, and the trigeminal ganglion.[4,6] Furthermore, new data presented at this meeting demonstrate a direct and selective neurotrophin effect on neurite outgrowth from the embryonic geniculate ganglion (FIG. 2).[7]

At the symposium, Louis Reichardt and Alun Davies provided a broad neuroscience perspective on the role of neurotrophins in supporting survival of sensory ganglion cells, before, during and after target contact. These presentations made clear the specificity of neurotrophin effects on various sensory ganglia and the importance of developmental stage in these effects, because neuron populations can switch dependence from one neurotrophin to another. Related to the timing issue are questions about the source of neurotrophin support for developing ganglia. In general, the sensory ganglia develop independently of target-derived support, relying on local factors, and then become target dependent with the arrival of axons in the target field.[8] Although the Reichardt and Davies presentations indicated a depth of understanding that is emerging

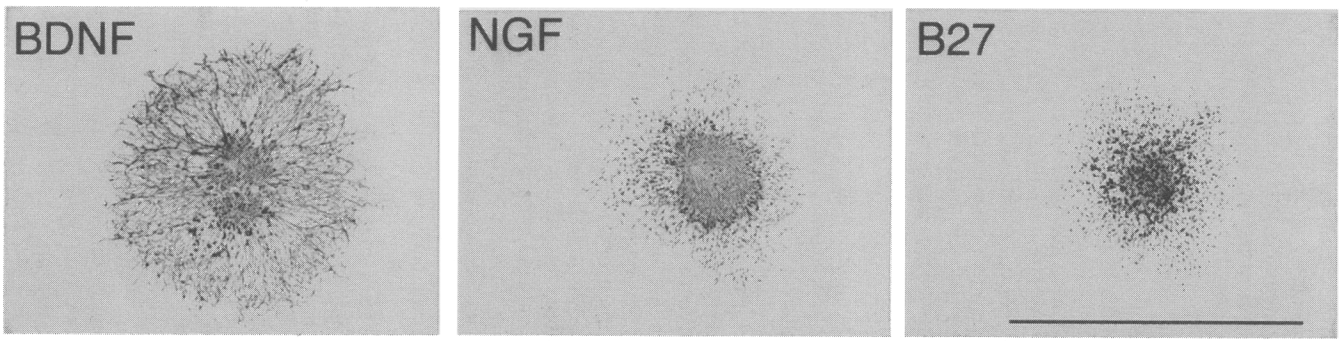

FIGURE 2. Explants of geniculate ganglia from embryonic rat, dissected at gestational day 16 (E16) and maintained in culture for 24 hours with exogenous brain-derived neurotropin factor (BDNF), 10 ng/ml; nerve growth factor (NGF), 50 ng/ml; or standard medium alone, B27. Cultures were immunoreacted with an antibody to protein gene product (PGP) 9.5. BDNF supports extensive neurite outgrowth, whereas few neurites extend with NGF and almost none with B27. *Scale bar* = 2 mm.

about specific events and signaling pathways in ganglion cell dependence on neurotrophins, the data were based primarily on dorsal root and trigeminal ganglia. Knowledge about gustatory ganglia is virtually nonexistent in comparison.

The geniculate and petrosal gustatory ganglia begin to develop between gestational days 11 and 12 (E11 and E12) in embryonic rat,[9] whereas the tongue does not begin to form until E13.[10] Clearly, then, target-derived neurotrophins could not support the taste ganglia in the earliest stages of development, but could participate in the differentiation of ganglion cells at later stages.

Bernd Fritzsch extends the symposium discussion about neurotrophin support for ganglion development by comparing the nature of the cross-talk between neurons and peripheral targets in establishing sensory systems that originate from placodally derived ganglia: the auditory, vestibular, lateral line, electroreceptive and taste systems. Based on analysis of mice with targeted mutations in neurotrophin genes or in the Trk family of receptor kinase genes, his paper suggests possible parallels between auditory and vestibular systems, and the taste system, in aspects of ganglion cell dependence on brain-derived neurotrophin factor (BDNF) and neurotrophin-3 (NT3). In addition, Fritzsch points out that while knowledge is emerging about the nature of signals from the peripheral targets back to ganglion cells, essentially nothing is known about molecular signals from the ganglia to target organs.

For subsequent papers in the symposium there is a shift in emphasis from the ganglia that innervate sensory organs to development of the peripheral target organs themselves, specifically the gustatory papillae. To determine any potential role for target organs in interactions with growing neurites, it is first necessary to understand the origins of the peripheral targets. Formation of the gustatory papillae is initially characterized by a local thickening of the tongue epithelium to form a placode, with condensation of mesenchymal cells directly underneath (FIG. 3). Subsequently there is a series of invaginations and evaginations, as epithelium and mesenchyme interact to form the taste papilla with an epithelial covering over a mesenchymal core.

A major question in the chemosensory field centers on mechanisms that direct induction and early development of the taste papillae (FIG. 4). Papilla induction is a key element in the events that contribute to taste circuit formation, because on the mammalian tongue, taste buds reside only in taste papillae. However, lack of basic knowledge presents important obstacles to attempts to study mechanisms of papilla induction. For example, there is no detailed description of the series of epithelial and mesenchymal changes that occur, at a cellular or molecular level, in early induction; nor is it known whether epithelium or mesenchyme has the initial induction role.

Associated with the question of induction is the formation of fungiform papillae in a patterned array on the anterior tongue. It has been noted since early scanning electron microscopic studies of the embryonic rat tongue that the taste papillae do not form randomly on the tongue, but in a distinctive pattern that is quite similar across embryos[1,11] (FIG. 5). The patterned distribution of papillae suggests possible similarities between taste organs and other epithelial specializations, including teeth and vibrissae, in the nature and regulation of embryonic tissue interactions during development.

Recently, a role for sensory innervation has been experimentally *excluded* in directing induction of both single papillae and multiple papillae in the typical anterior tongue pattern.[10] Organ cultures of embryonic rat tongue dissected and maintained *in vitro* without intact sensory innervation demonstrate fungiform papilla formation in large numbers and linear arrays on anterior tongue (FIG. 6). At the same time, experiments to describe expression patterns for various genes that direct formation of epithelial organs provide evidence that molecular products of these patterning genes might be important in directing papilla formation.[12,13] For example, in mouse fungiform papilla at early developmental stages there is localized expression of mRNA for *sonic hedgehog*

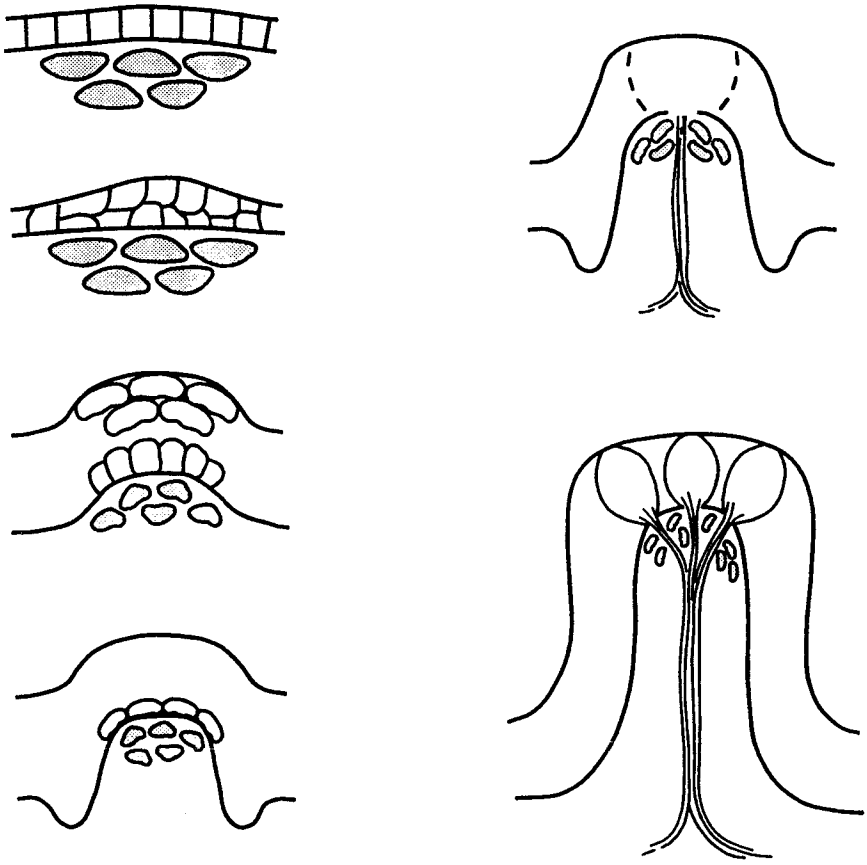

FIGURE 3. Diagram of the formation and morphogenesis of the fungiform papilla, from induction through taste bud acquisition. Induction is characterized by a series of interactions between lingual epithelium and underlying mesenchyme cells, to form a placode. Subsequent tissue evaginations and invaginations reflect exchanges between epithelium and mesenchyme to form the taste papilla, comprised of an epithelial covering over a core of connective tissue. In the densely innervated, advanced papilla, taste buds develop in the apical epithelium.

and members of the gene family for bone morphogenetic proteins *(e.g., BMP-4)*.[12] At these same early stages, *distal-less-3* mRNA is selectively expressed in mouse fungiform papilla.[13] Data presented at this meeting expand on these demonstrations.[14]

Beyond induction, continued gustatory papilla development includes extensive growth and morphogenesis (FIG. 7). The extracellular matrix molecules, laminin and tenascin, participate in cell and tissue interactions that establish papilla form and permit the extensive innervation of the central papilla core.[15] Whereas nerves are not necessary for papilla induction, in these later stages of papilla development, innervation is an essential element for appropriate morphogenesis. This is apparent in mice with targeted gene deletions for the neurotrophin, BDNF. In BDNF null mutant mice, there are substantial reductions in size and number of neurons in geniculate, petrosal and trigem-

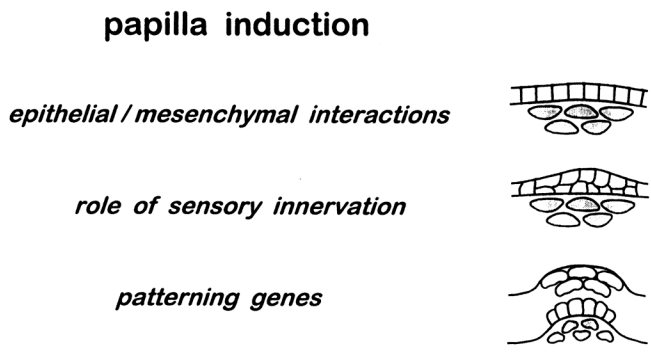

FIGURE 4. Diagram of taste papilla induction. To regulate initial epithelial and mesenchymal exchanges that form the papilla placode and early papilla, at least two mechanisms can be considered: via sensory innervation, and/or gene products active at sites of embryonic organ formation.

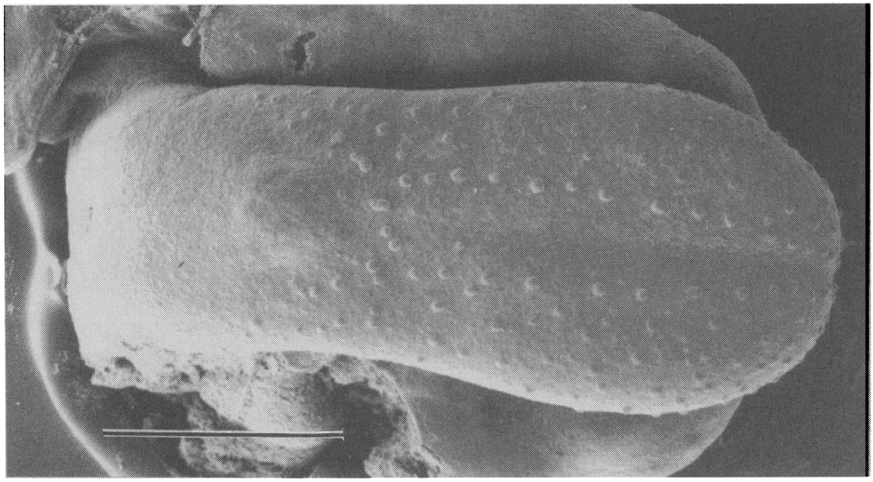

FIGURE 5. Scanning electron micrograph of embryonic rat tongue at 16E. The patterned distribution of fungiform papillae in rows on the anterior tongue is apparent. *Scale bar* = 1 mm.

inal ganglia.[4,6] With this reduction in the source of sensory innervation for gustatory papillae is an associated reduction in size of circumvallate and fungiform papillae, and an aberrant form of circumvallate papilla.[16–18]

Therefore, although papillae initially form independently of direct inductive influences from sensory innervation, subsequent development and maintenance is neurally dependent. In parallel, although the sensory ganglia develop in advance of any contact with a target organ (or indeed before the tongue is even formed) and therefore independently of target support, subsequent target influence on the ganglion presumably occurs. Thus, a reciprocal set of interactions would be established: ganglion cells de-

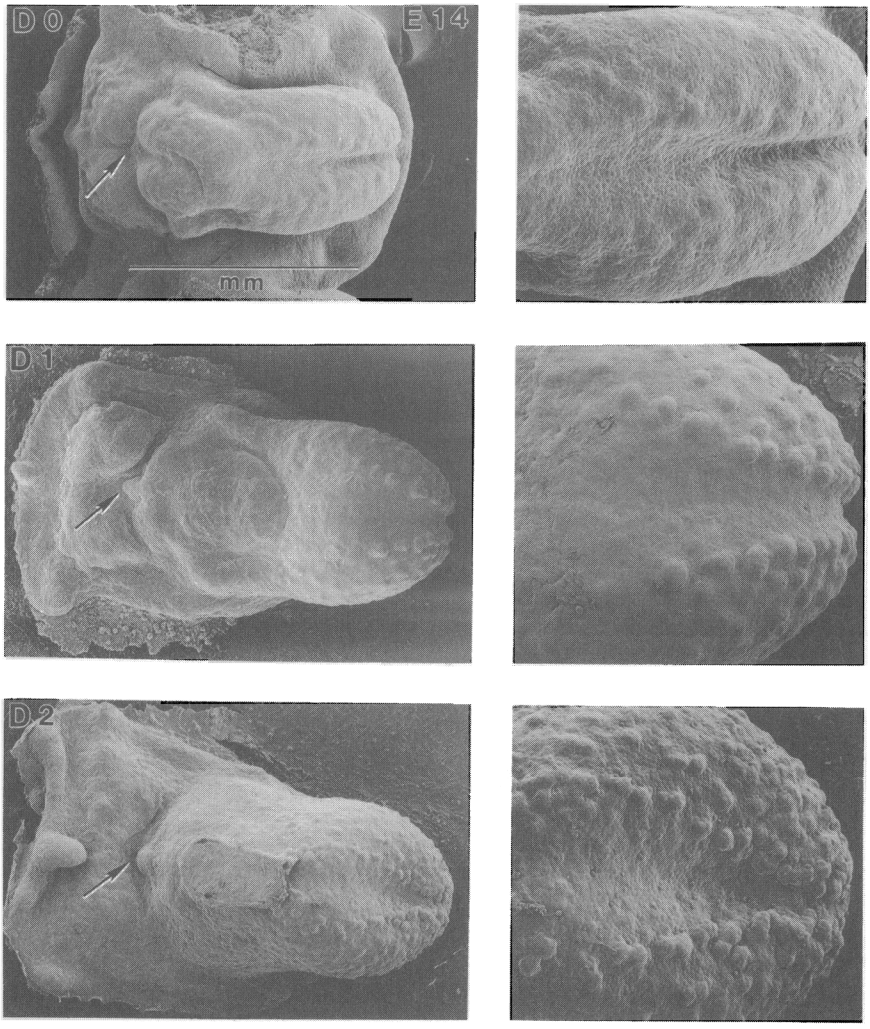

FIGURE 6. Scanning electron micrographs of embryonic rat tongues dissected at 14E and maintained in organ culture. Tongues are illustrated at low *(left column)* and high *(right column)* power. **DO:** Embryonic day tongue taken at 14E when cultures are established. The caudal border of the anterior tongue *(arrow)* is noted. Numerous small eminences that represent incipient fungiform papillae are apparent on the anterior tongue. **D1:** After one day in culture the tongue has increased in length and fungiform papillae are well formed and prominent in rows on the anterior of the cultured tongue. **D2:** The fungiform papillae are even more distinctive on the anterior tongue after two days in culture. Because the tongues are cultured in the absence of intact sensory ganglia, these experiments exclude a role for sensory innervation in papilla induction. Complete data are in Mbiene, MacCallum & Mistretta.[10]

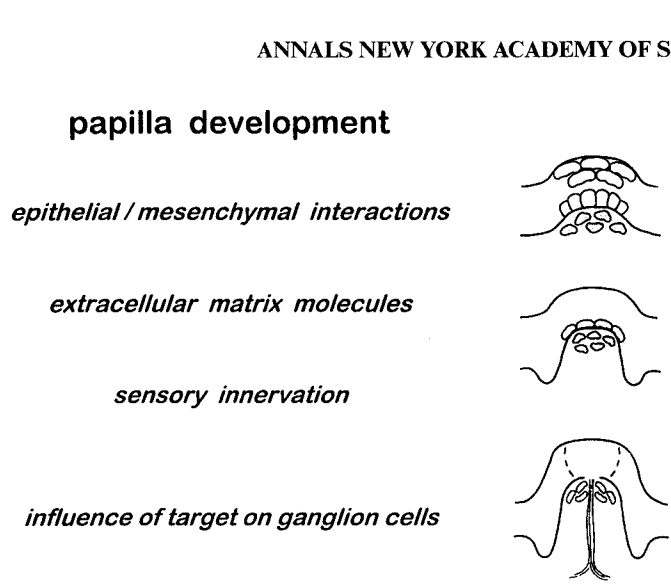

FIGURE 7. During papilla development, epithelial/mesenchymal interactions continue to participate in forming the papilla. At these stages, extracellular matrix molecules and sensory innervation have demonstrated roles in papilla morphogenesis and growth. The papilla may begin to reciprocally sustain ganglion cells, via neurotrophins.

velop without target influence, but become dependent on taste papillae for neurotrophin support once the papillae are innervated; papillae develop without ganglion cell innervation but become dependent on innervation as the neurites grow into the target field. This is discussed further in a summary outline (see below, FIG. 10).

If at stages of target organ innervation, ganglion cells derive support from neurotrophins supplied by the papillae, then neurotrophins should be demonstrable *in vivo* at appropriate stages of papilla development. In this symposium, Christopher Nosrat presents distributions of mRNA expression for various neurotrophins in embryonic rat tongue and papillae, and in turn, describes effects of neurotrophin deletion on tongue innervation and papilla development in neurotrophin knockout mice. Nosrat proposes that gustatory system development utilizes BDNF as an active neurotrophin, whereas the tongue somatosensory system utilizes NT3. Bruce Oakley presents additional, converging data in his paper on gustatory papilla development in mice with mutated genes for BDNF, NT3, and NT4.

Once the gustatory papillae develop to an advanced morphology, the papillae not only are extensively innervated but also contain an appropriate number of taste buds (FIG. 8). Developmental issues shift from a focus on papilla induction and development to an emphasis on taste bud induction and development. In rat there is a pause of several days between the initial appearance of taste papillae at E14 and the first appearance of taste buds at E20, the day before birth.[1, 11, 19] During the intervening period, bundles of neurites grow into the connective tissue core of the embryonic taste papillae.[20] The extent to which there is a comparable delay before taste bud formation in mammals with lengthy gestations—sheep, rhesus monkey, and human—is not clear from the literature.[21]

The field of taste has long accepted the view that taste bud cells are induced to differentiate from epithelial cells in gustatory papillae once gustatory nerves in the taste papilla core have penetrated the epithelial basement membrane.[1, 19] However, in a recent

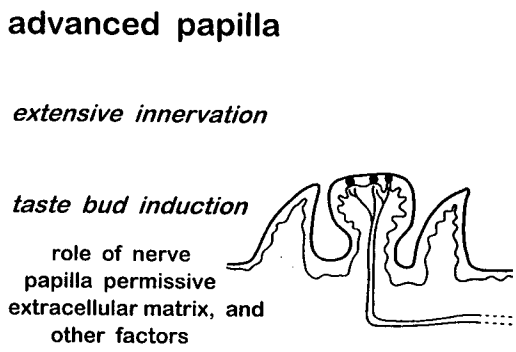

FIGURE 8. The advanced papilla is extensively innervated, with taste buds (number varies according to species) in the apical epithelium. In regulating taste bud induction, potential roles for papilla innervation, the papilla itself and other factors must be considered.

series of experiments that replicate and extend those of the early embryologist Stone,[22, 23] investigators have concluded that taste buds do not require innervation for induction or 'complete differentiation'.[24] In this symposium, Linda Barlow presents results from these recent studies and proposes a model for taste bud development and innervation.

Barlow's conclusions that taste buds do not require innervation for initial induction stand in contrast to data from neurotrophin knockout mice, in which taste bud number is substantially reduced in association with reduced numbers of innervating neurons from sensory ganglia (symposium papers by Nosrat and Oakley). However, taste *papilla* size, number and shape are altered in the knockout mice, so taste bud loss may be secondary to the papilla derangement. And in fact Fritzsch's symposium paper concludes from study of trkB knockout mice that whereas taste ganglia *do not develop* in these animals, taste buds *do form* on the tongue in fungiform papillae.[25]

Therefore, investigators do not draw totally convergent conclusions from mouse knockout studies, and investigators using mouse mutants do not all draw similar conclusions to those of Barlow and colleagues. It is important to note that the latter investigators use an amphibian species, the axolotl salamander, animals with unique developmental and regenerative processes.[26] Furthermore, the axolotl taste buds do not reside in papillae but in a mucosa, and might relate more directly to mammalian taste buds in nonlingual locations such as the soft palate and epiglottis.[1, 27]

A final consideration in discussing interactions between growing nerves from ganglion cells and their peripheral target organs is the issue of acquisition of the appropriate number of papillae and/or taste buds in the receptive field of a single ganglion cell (FIG. 9). Robin Krimm presents data in this symposium making several key points: one taste bud in rat is innervated by several geniculate ganglion cells on average; the number of ganglion cells innervating a taste bud correlates with taste bud size in young adults; and, ganglion cell/taste bud relations are established progressively during development through a period of at least 40 days postnatal. Therefore, it is clear that the acquisition of correct numbers of taste buds in the receptive field of a single taste ganglion cell results from lengthy, direct interactions between neurons and targets.

Based on results discussed in the symposium and this introductory paper, a summary of current knowledge on ganglion cell/peripheral target organ interactions during early innervation of taste organs is presented (FIG. 10). Ganglia and taste papillae are independent of each other for initial development. The gustatory ganglia must de-

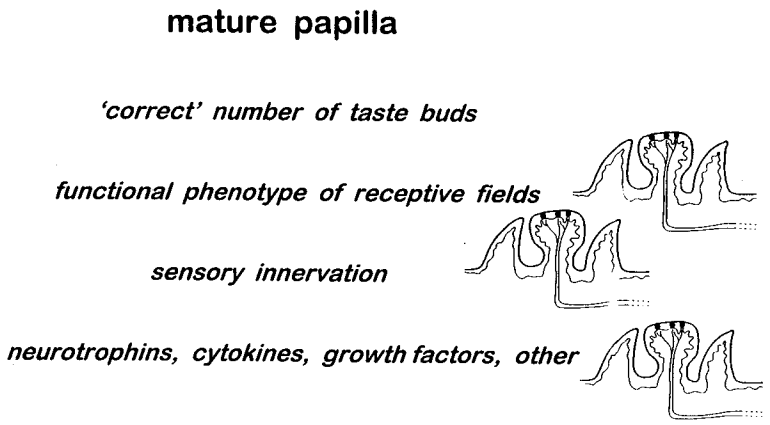

FIGURE 9. The mature taste papilla has a species-appropriate number of taste buds and ganglion cell innervation, so that papillae are organized in functional receptive fields.

rive initial support from local neurotrophins and/or other factors, as suggested for other neuron populations,[28,29] but this is not yet established. Recent literature suggests that products of patterning genes may direct the series of inductive exchanges between tongue epithelium and mesenchyme that result in formation of gustatory papillae.

Once ganglion cells extend neurites into the immature gustatory papillae, interdependence ensues, with ganglion cells deriving neurotrophin support from innervated papillae, and papillae dependent on nerves for maintenance and continued morphogenesis. With continued papilla development, taste buds form in specific locations of the papilla epithelium. Whether sensory innervation has an inductive role in mammalian taste bud formation is not known; however, once formed, taste buds are neurally dependent for survival and acquisition of appropriate size and numbers. Therefore, it is clear that beyond induction there is a demonstrated neural dependence for taste bud differentiation. Because larger taste buds are innervated by more ganglion cells than are smaller buds, there also is apparent continued support of ganglia from the peripheral target organs.

Although there is an emphasis in this symposium on the role of neurotrophins in supporting ganglion cell differentiation, certainly other molecules must contribute to gustatory ganglion maintenance. Furthermore, the mechanisms that guide growing neurites to appropriate target organs have not been extensively discussed in the symposium, but direction to and contact with correct targets are essential for receptive field development. Thus, the symposium is not all-inclusive in considering important issues in early taste development. However, the symposium presents central directions that currently engage investigators who study early innervation of taste organs and provides a working summary for the current state of knowledge and the numerous remaining questions in taste organ induction and differentiation.

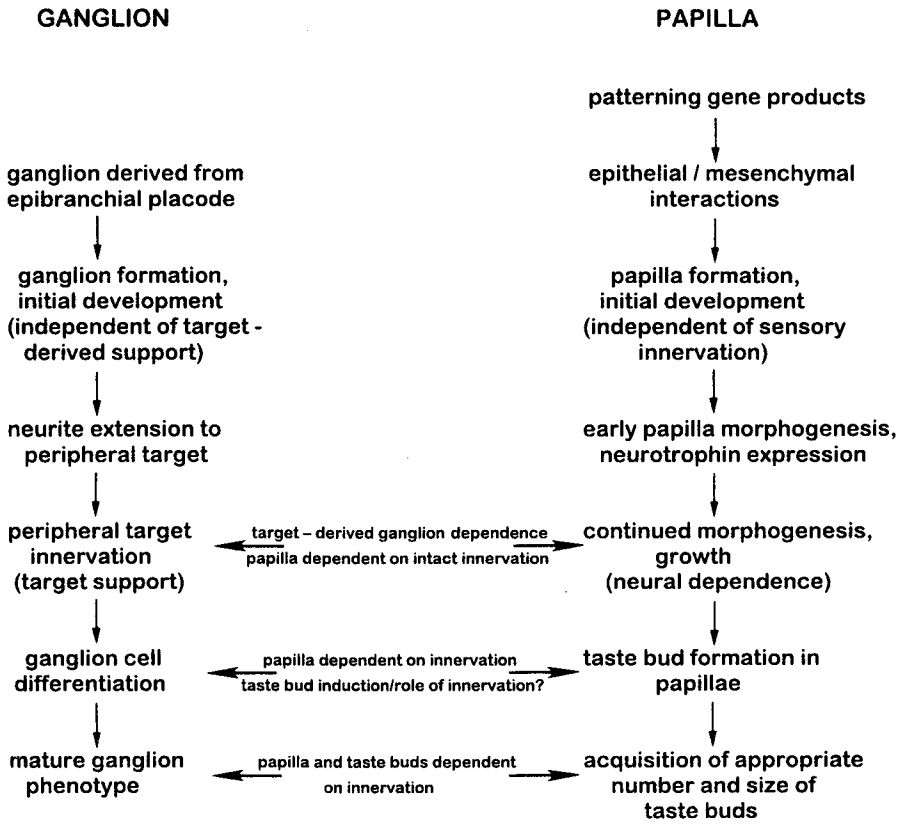

FIGURE 10. Diagram of a working outline for independent and interdependent stages of ganglion and papilla development, during early innervation of the peripheral taste system. Both ganglion and taste papilla develop independently, but interact when neurites extend to innervate papillae, and papillae produce neurotrophins. Subsequent dependence of peripheral taste organs on innervation has been demonstrated; and neurotrophin-dependence of ganglia is also established, presumably derived from taste papillae. See text for full discussion.

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