Development 103 Supplement, 185–193 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

Tissue interactions and cell differentiation: neurone–sensory cell interaction during otic development

THOMAS R. VAN DE WATER

Laboratory of Developmental Otobiology, Departments of Otolaryngology and Neuroscience, Albert Einstein College of Medicine, 1410 Pelham Parkway South, Bronx, NY 10461, USA

Summary

Statoacoustic ganglion neurones (SAG) are produced by the same group of cells (otic placode) that produce all of the receptor cells that populate the sensory areas of the inner ear. The observation that ingrowth of SAG neurites to presumptive sensory areas of the inner ear preceded cytodifferentiation of those receptor cells suggested a causal relationship. Results from *in vivo*, *in ovo* and *in vitro* studies do not support a causal relationship. These studies support the hypothesis that the programme for labyrinthine sensory cell differentiation is intrinsic and does not require the extrinsic stimulus of neuronal interaction to trigger its expression. In contrast, developing statoacoustic ganglion neurones appear to require a trophic influence that is supplied by either their peripheral or central target tissues for their survival and maturation *in vitro*. A mechanism for the ingrowth of SAG dendrites to their appropriate target sites within the inner ear proposes that attractant fields produced by areas of differentiating sensory cells act to guide the nerve growth cones of ingrowing SAG neurites to the appropriate tissues. Preliminary results from a heterochronic series of SAG implants to common age otocysts suggest that these SAG neurones are capable of responding to the attractant fields which are produced by presumptive labyrinthine sensory epithelium over an extended period of otic development. Both *in ovo* and *in vitro* studies suggest that spatiotemporal patterns of extracellular matrix molecules may be important components of the attractant fields which are produced by the sensory areas of the developing inner ear and may ultimately result in the specificity of their neuronal connections.

Key words: sensory cell, neurone, trophic support, attractant field, extracellular matrix, inner ear, otic placode, N-CAM, L-CAM, Ng-CAM.

Introduction

Neurones of the statoacoustic ganglion (SAG) develop a high degree of specificity within the mature inner ear, but exhibit plasticity during development (Whitehead & Morest, 1985; Pujol, 1986). It is unlikely that neuronal networks are wholly genetically determined since the genome could not possibly specify the myriad of connections that have to be made within an intact organism. To begin to understand the mechanism by which this specificity is achieved (e.g. tonotopic organization of the organ of Corti), an understanding at the cellular level of neurone–sensory cell interactions is a prerequisite.

This article reviews recent findings that add to our knowledge of otic development and the interplay between SAG neurones and sensory cells that compose both the vestibular and auditory sensory receptors.

Origins of the statoacoustic ganglion

There is direct experimental evidence (Campenhout, 1935; Yntema, 1937) that the neurones that compose the amphibian SAG are wholly of placodal origin. There is no direct evidence showing the origins of these neurones in mammals and there are supporters for both placode (Halley, 1955; Batten, 1958) and neural crest (Bartelmez, 1922; Deol, 1967) contributions to the neuronal population of this ganglion. A comprehensive study of the origins of avian cephalic

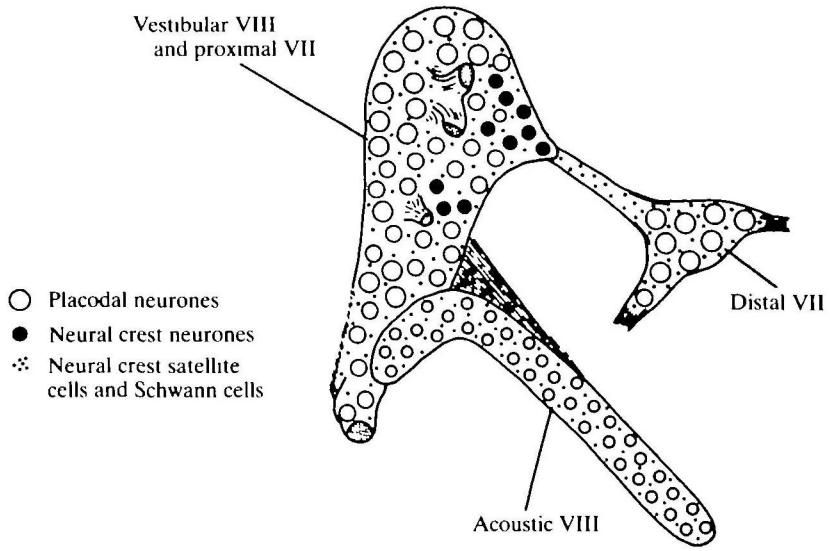


Fig. 1. Lateral view of right VII–VIII ganglionic complex of a 12-day-old chick embryo indicating the origins of neurones, satellite cells and Schwann sheath cells.

ganglia (D'Amico-Martel & Noden, 1983) using chick–quail interspecies transplants has shown that all of the acoustic and most, if not all, vestibular neurones of the SAG are derived from the otic placode (Fig. 1). A later analysis of chimaeric chick–quail labyrinths and statoacoustic ganglia that were not wholly derived from the placodal transplants define the site of origin of the SAG neurones (Noden, 1984). Both vestibular and auditory neurones originate from a common area of otic epithelium which goes on to form the medial wall of the utricule (Fig. 2). Therefore, most neurones of the SAG originate from a site quite different from their eventual peripheral targets (e.g. organ of Corti). This is significant when postulating a mechanism by which these SAG neurones send their dendritic processes to the appropriate sites within the developing inner ear. These facts preclude the possibility that migrating neuroblasts establish tracts that later serve a substrate guidance role (Letourneau, 1979) for the nerve growth cones of these neurones as they innervate their appropriate inner ear sensory receptors.

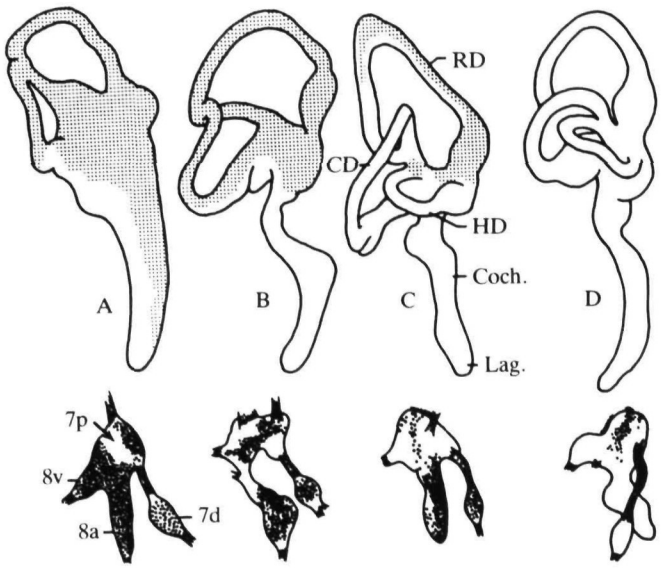


Fig. 2. Statoacoustic ganglion. A–D illustrate the different patterns of labelling in reconstructed membranous labyrinths following grafting of small pieces of quail dorsolateral surface ectoderm (A–C) or the neural crest (D) at the midmyelencephalic level. Stippling indicates quail epithelium. Below are reconstructions of ganglia associated with the seventh and eighth cranial nerves in each of these four hosts. Dots indicate quail neurones. These reconstructions are used to map the origins of each part of the membranous labyrinth and of both the vestibular and acoustic neurones. RD, HD and CD, rostral, horizontal and caudal semicircular ducts; Coch., cochlear duct; Lag., lagena; 7p, 7d, proximal (root) and distal (geniculate) ganglia, respectively; 8a, 8v, acoustic and vestibular ganglia.

Neurone–sensory cell interactions (also see Davies, this volume)

Nerves can exert trophic effects on the sensory receptors that they innervate (e.g. taste buds; Guth, 1969). Ingrowth of SAG dendrites to their presumptive peripheral target sites precede overt cytodifferentiation of these target sensory cells in several species (chick, Knowlton, 1967; rat, Weibel, 1957; mouse, Sher, 1971; lamprey, Thornhill, 1972). These observations coupled with thymidine labelling of the developing mouse labyrinth (Ruben, 1967), which shows that the oldest hair cells of Corti's organ are last to differentiate, suggested a causal relationship between ingrowth of SAG dendrites and cytodifferentiation of otic sensory epithelium. Observations of nerve fibre presence associated with otic sensory

epithelium cytodifferentiation in cultured reaggregates of chick otocysts (Orr, 1968) strengthened the 'fait accompli' acceptance of the theory that ingrowth of SAG dendrites induces cytodifferentiation of otic sensory epithelium.

An *in vitro* study (Van De Water, 1976) utilizing mouse otocysts explanted either 'with' or 'without' SAG neurones directly addressed the question of whether or not these neurones and their neuritic processes exert a trophic effect on the differentiation of both vestibular and auditory sensory epithelia. Analysis of a total of 180 inner ear explants of three gestational age groups representing three distinct stages of otocyst–SAG interaction did not show any differences (light microscopy) in cytodifferentiation of either auditory or vestibular sensory epithelia (Fig. 3A,B). A similar series of 'with' or 'without' SAG otic explants (Dragone & Van De Water, 1988) excised from 13-day (gestational) mouse embryos have confirmed these findings (Van De Water, 1976) using immunocytochemical detection of neurone-specific enolase as a marker for both auditory (Fig. 4A,B) and vestibular hair cell differentiation (Dechesne, Sans & Keller, 1985; Dechesne & Pujol, 1986).

The hypothesis that inner ear sensory cells do not require an extrinsic trophic stimulus as could be supplied by SAG neurones to cytodifferentiate is also supported by ultrastructural findings from the developing macula utriculus of the mouse (Van De Water,

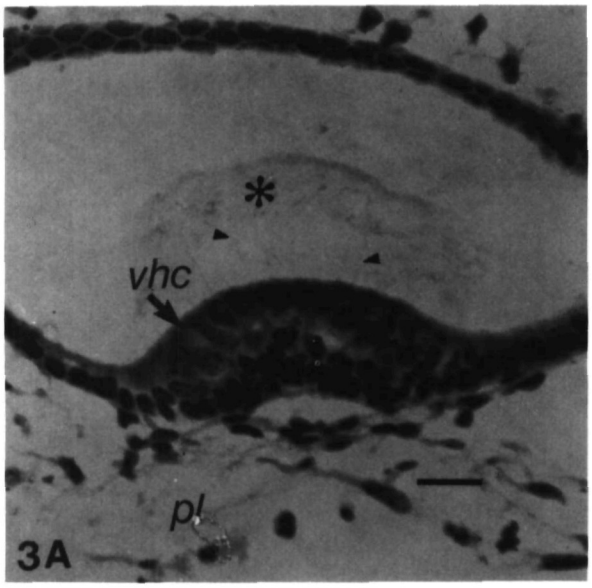
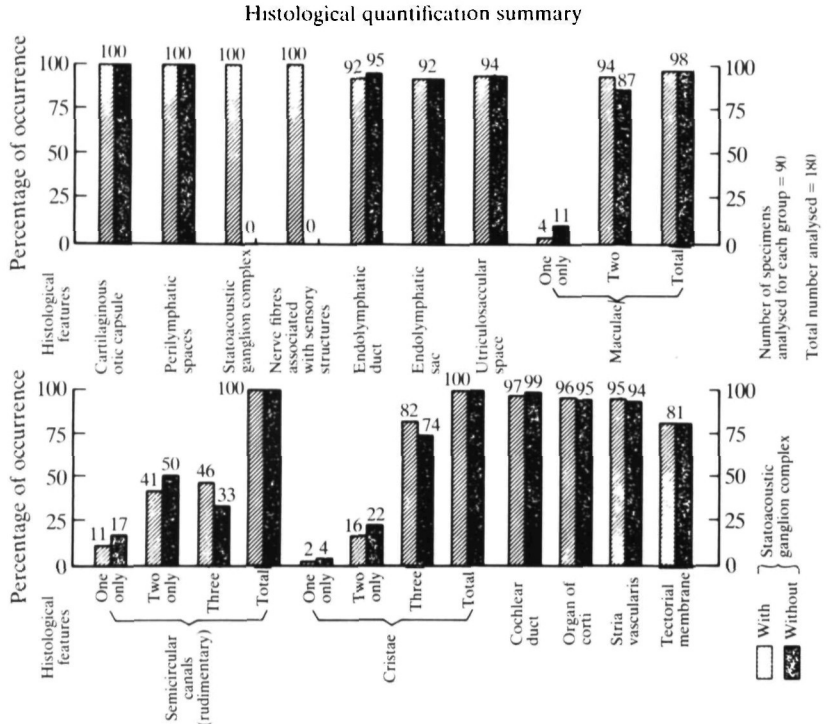


Fig. 3. (A) 13-gestation day 'without' SAG inner ear explant, after 8 days *in vitro*. A normal crista ampullaris with hair cells (*vhc*) that have bundles of sensory hairs (arrowheads) projecting from their apical surfaces and a well-developed cupula (*) in the endolymphatic lumen. No neuronal elements are present either at the base of the hair cells or in the perilymphatic spaces (*pl*). Bar, 20 μm. (B) Combined results of histological quantification of 11-, 12- and 13-day-old 'with' and 'without' statoacoustic ganglion, SAG, otic explants that were cultured until the equivalent of gestation day 21.



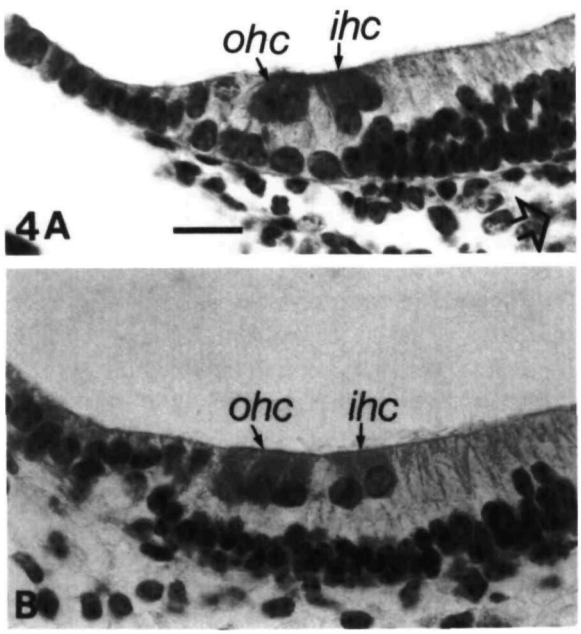


Fig. 4. Gestation day-13 inner ear explants, after 7 days *in vitro*. Stained (PAP method) for the presence of bound neurone-specific enolase, NSE, antibody. (A) An organ of Corti that developed in a 'with' SAG explant. Both inner (*ihc*) and outer (*ohc*) hair cells stain positive for the presence of NSE. A bundle of nerve fibres is also stained in the perilymphatic space (open arrow) below Corti's organ. (B) An organ of Corti that developed in a 'without' SAG explant. Both inner and outer hair cells stain positive for the presence of NSE. No nerve fibres are present. Bar, 20 μm.

Wersall, Anniko & Nordeman, 1978) and labelling studies of the embryonic rat vestibular epithelia (Sans & Chat, 1982). These findings are strengthened by the observations in the developing chick inner ear that differentiation of all labyrinthine hair cells is normal and their presynaptic specializations are maintained even though their nerve terminals are absent as a result of *in ovo* administered β-bungarotoxin (Hirokawa, 1977). Embryonic chick auditory receptors that are rendered aneural and grafted to the chorioallantoic membrane also develop normally in every respect (Fig. 5) when compared to chick cochlea grafts that were transplanted and developed with an intact SAG (Corwin & Cotanche, 1985).

The lateral line is capable of regenerating its sensory receptors in the absence of lateral line sensory nerves (Speidel, 1947; Jorgensen & Flock, 1976), however, there is a limited but detectable trophic influence of these nerves on the number of sensory receptors that populate the regenerated tails which may take several months post-regeneration to express its effects (Speidel, 1948; Borden & Corwin, 1985). In

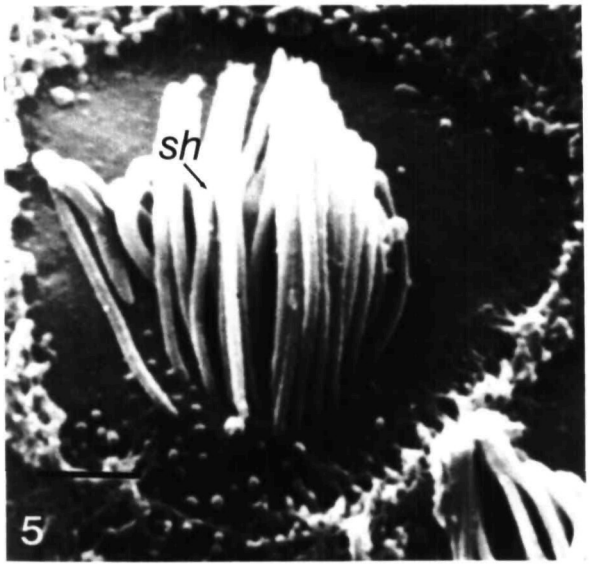


Fig. 5. The apical surface of a chick auditory hair cell that developed in an embryonic day (ED 5) chick cochlea that was surgically denervated and grafted to a chorioallontic membrane where it developed until the equivalent of ED 17. The sensory hairs (*sh*) are normal in every respect for this stage (i.e. ED 17) of auditory hair cell development. Bar, 1 μm.

the developing inner ear, analysis of *in vitro* developed sensory receptors did not indicate an effect on either sensory cell size or number (Van De Water, 1976). Definitive proof is provided by the SEM analysis of grafted neural and aneural embryonic chick cochlea; quantification of the overall sensory cell pattern which includes cell locations, cilia size and number per individual sensory cell (Fig. 5) as well as sensory hair bundle orientation shows no differences (Corwin & Cotanche, 1985). These observations show that the inner ear, unlike the lateral line, does not exhibit any demonstrable trophic response to the presence of SAG neuronal elements and that the programme for sensory cell differentiation is intrinsic.

Sensory cell–neurone interactions (also see Davies, this volume)

Dependence of neurones on their target tissues during development is the basis of the theory that competition between dendrites or axons for connections determines survival during the developmental period of normal cell death (Landmesser & Pilar, 1978). Some natural cell death occurs in the developing chick SAG shortly after the establishment of synaptic contacts with both peripheral and central targets (Ard & Morest, 1984). *In vitro* experiments that manipulated ganglion–target tissue interactions in otic explants of both chick (Ard, Morest & Hauger,

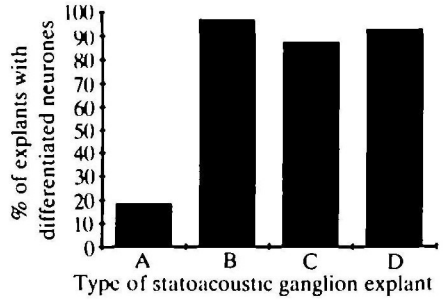
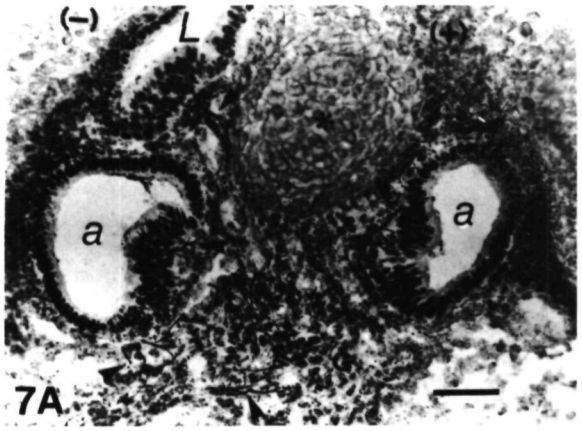


Fig. 6. A histogram depicting the survival rate of the four types of SAG explants after 14 days *in vitro*. Type A, isolated ganglion (18·5 %); type B, ganglion and sensory epithelium (97·2 %); type C, ganglion and rhombencephalic tissue (87·5 %); type D, ganglion and sensory epithelium and rhombencephalic tissue (93·1 %).

1985) and mouse embryos (Zhou & Van De Water, 1987*a*) show that both peripheral (sensory epithelium) and central (brainstem) target tissues provide trophic support of SAG neurones (Fig. 6). Recent *in vitro* studies (Dragone & Van De Water, 1988) examining this question with the aid of antibodies raised against neurone-specific enolase show that peripheral target tissue (i.e. 100 % of explants with NSE + neurones; average 70 neurones/explant) provides better trophic support of neuronal differentiation than central target tissue (i.e. 33 % of explants with NSE + neurones; average 2 neurones/explant). These findings are consistent with a trophic role for neurone–target tissue interactions that support neuronal survival and maturation in both avian and mammalian statoacoustic ganglia. Both peripheral and central targets are implicated with the peripheral target providing more active trophic support of these neurones *in vitro*.

Regenerating lateral line receptors deprived of their natural sensory nerves attract nearby spinal nerves (Speidel, 1948). Van De Water (1976) proposed that presumptive sensory epithelium of the inner ear prior to overt cytodifferentiation undergoes chemodifferentiation and establishes attractant fields that act to direct ingrowing SAG neurites towards areas of differentiating sensory epithelia. The first experimental results to support this attractant field hypothesis was provided by cocultures of two otic explants that shared a single SAG (Van De Water & Ruben, 1983, 1984; Anniko & Van De Water, 1986). These otocyst pairs were oriented so that the ganglion of the 'with' SAG explant was adjacent to the site of SAG removal of the closely opposed 'without' SAG otocyst. Analysis of these cocultured otocysts show that SAG nerve fibres grew into the otic sensory epithelia within both of these inner ear explants (Fig. 7A,B). This phenomenon occurred in both 11- and 12·5-day otocyst cocultures, but was not observed



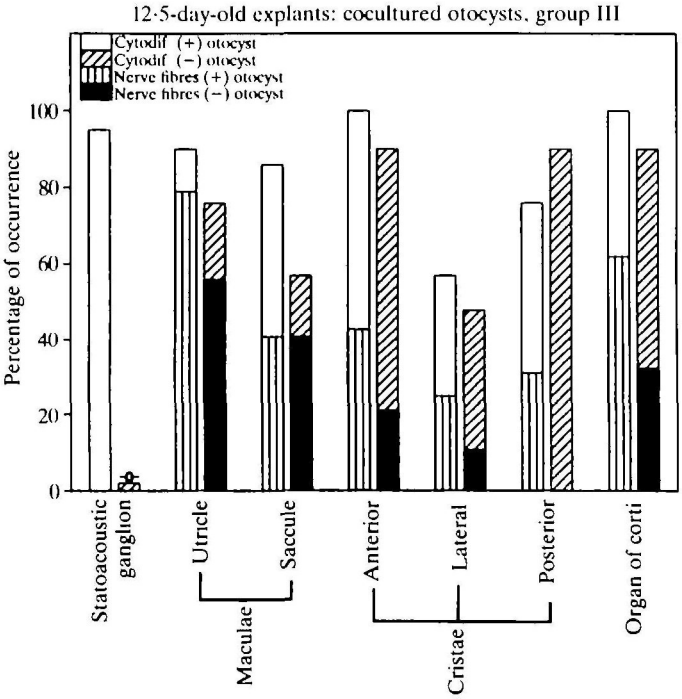


Fig. 7. (A) Cocultured 12·5-day-old otic explants which shared a single statoacoustic ganglion, after 8 days *in vitro*. An asterisk indicates a common cartilaginous capsule wall. Nerve fibres (arrowheads) are seen in association with anterior cristae (*a*) of both the 'with' SAG (+) and 'without' SAG (−) otic explants and with lateral crista (*L*) in (−SAG) explant. Bar, 80 μm. (B) A histogram of histological quantification data of 12·5-day-old cocultured 'with' and 'without' SAG inner ear explants.

in cocultures of inner ears derived from 14-day mouse embryos. Cultures of 12-day otocyst that had their SAGs excised and replaced by 10·5-day trigeminal ganglia provide evidence that these otic epithelium attractant fields are not specific for SAG neurones (Van De Water & Ruben, 1984). Trigeminal neurites were regularly seen in association with both vestibular and auditory sensory cells of these heterotypic

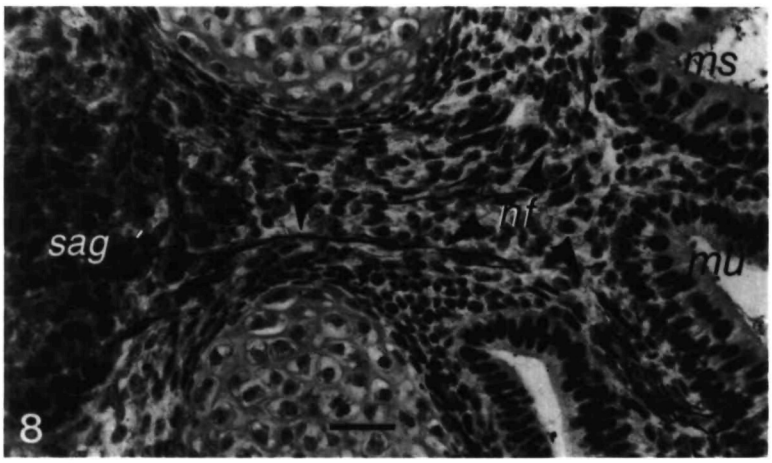


Fig. 8. Heterochronic SAG implant. Gestation day-12 otocyst that had its SAG extirpated and replaced with a gestation day-14 SAG, after 8 days *in vitro*. Neurones (*sag*) of the day-14 ganglion extend neurites (*nf*) to both the maculae of the utricle (*mu*) and saccule (*ms*) in this section. Nerve fibres were associated with all of the vestibular and auditory sensory receptors that developed in this explant. (Bodian nerve fibre stain). Bar, 40 μm.

ganglia implant cultures. Other studies where multiple targets are interacted with a ganglion explant have also demonstrated that target tissue need not be the natural target of a ganglion to stimulate directed outgrowth of neurites from that ganglion (Ebendal & Jacobson, 1977; Pollack, Muhlach & Liebig, 1981). This observation that otic sensory epithelium can attract neurites from an implanted heterotypic ganglion suggests that the attractant fields produced by this epithelium are of a general nature and actual specificity might rely more on spatial and temporal factors as a mechanism for the attraction of specific SAG neurites to their appropriate target sites.

Study of an elasmobranch inner ear which continues to grow and add new sensory cells throughout its life shows that the auditory neurones of this labyrinth expand their terminal arbours continually and preferentially orient toward the site of postembryonic hair cell production in the macula neglecta (Corwin, 1985). This finding also supports the hypothesis of attractant fields as a mechanism for guiding the growth cones of these continually growing neurones to the areas of newly produced hair cells within this auditory receptor.

To define better the temporal aspects of the SAG ability to respond to attractant fields, a staged series of SAG were implanted into a common age (i.e. 12-day) otic explant. This stage otocyst is in an active stage of SAG neuritic ingrowth to the as yet undifferentiated otic epithelium (Sher, 1971) and at a stage where neurites from both homo- and heterotypic ganglia implants are attracted to areas of differentiating sensory cells (Van De Water & Ruben, 1984). A homochronic control (i.e. 12-day) and heterochronic series of SAG (i.e. excised from 13-, 14-, and 15-day embryos) were implanted into 12-day otic explants and then allowed to develop for 8 days *in vitro*. Analysis of the resultant specimens show that all of these SAG implants sent fibres to areas of both vestibular (Fig. 8) auditory sensory receptors. These preliminary results suggest that SAG neurones retain their ability to extend neurites to differentiating otic sensory cells for an extended period during development of the inner ear.

Extracellular matrix

Fibronectin (Rogers *et al.* 1983), laminin (Rogers *et al.* 1983; Hammarback, Palm, Furcht & Letourneau, 1985) and collagen (Roufa, Johnston & Bunge, 1983) have all been shown to promote neurite outgrowth. A cell adhesion molecule, N-CAM, has been shown to increase in the innervated sensory areas of the chick inner ear while L-CAM increased in the nonsensory areas (Richardson, Crossin, Chuong & Edelmann, 1987). This study also noted changes in the expression of both neurone–glial cell adhesion molecules, Ng-CAM and cytotactin during SAG nerve fibre invasion of the otic sensory epithelia. These authors suggest that the spatiotemporal expression of extracellular matrix molecules are significant factors in establishing patterns of innervation and specificity of neuronal connections in the chick inner ear. A preliminary study of SAG explants cultured on the surface of HEMA hydrogels which contained matrix molecules (Zhou & Van De Water, 1987*b*) shows that the mouse SAG responds to the two extracellular matrix molecules tested (i.e. collagen type I and fibronectin) by extending neurites.

Conclusions

Fig. 9 summarizes the origins of the SAG and the interplay between neurones and sensory cells of the inner ear that occur during the development of this complex sensory receptor (Van De Water, 1986). The neurones that compose the SAG are primarily of placodal origin with a minimal contribution of neural crest cells. All of the SAG supporting cells are derived from the cephalic neural crest. The hypothesis that cytodifferentiation of inner ear sensory hair

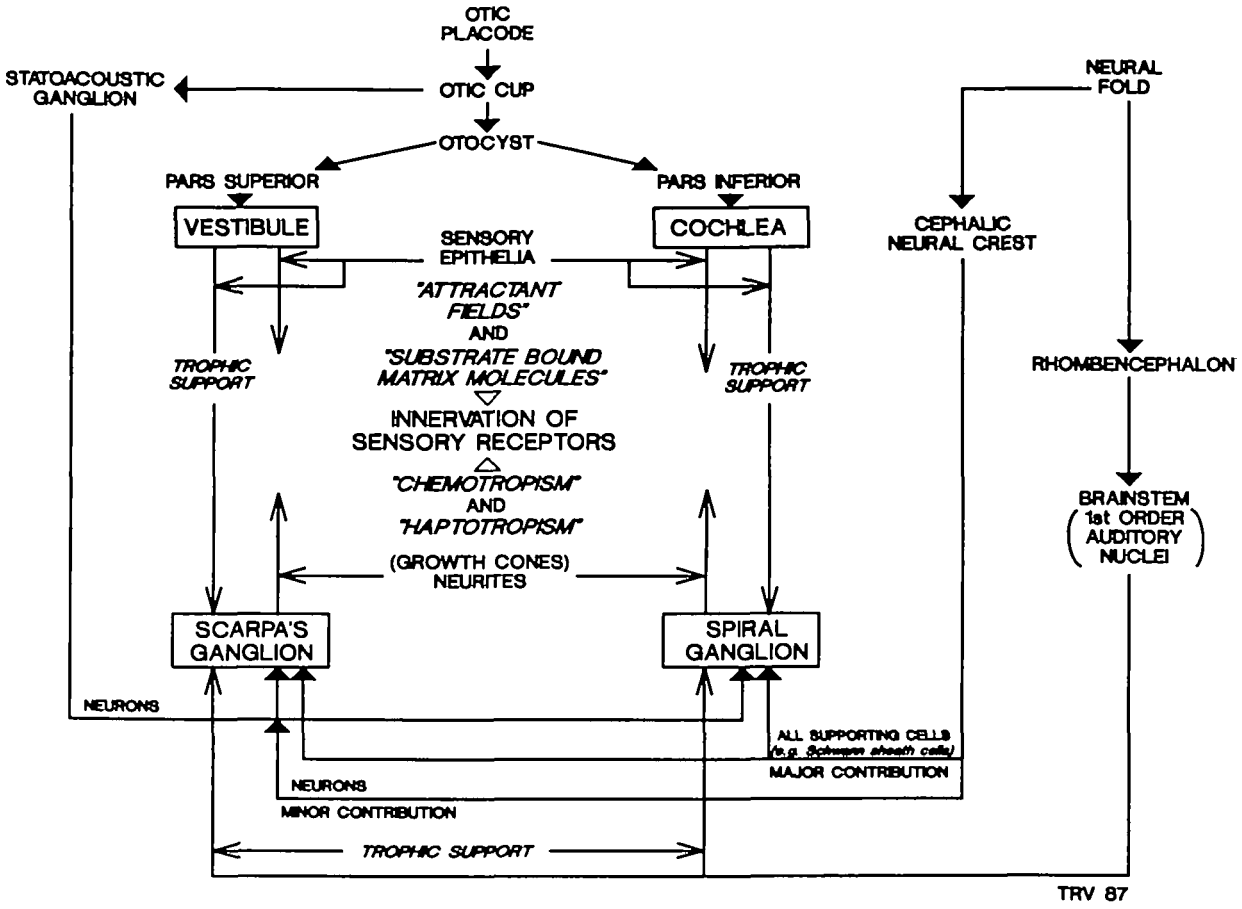


Fig. 9. A flow chart depicting the major developmental interactions that influence and control the differentiation of inner ear sensory structures and the establishment of their innervation. Otic components in boxes represent final inner ear structures. Lines with solid arrowheads trace the sequence of changes that occur during the development of inner ear structures, while lines with open arrowheads indicate influences that operate at each stage of otic development. Influences that as yet lack definitive proof but are strongly suggested by indirect evidence are indicated by open arrowheads without lines.

cells (i.e. both vestibular and auditory) is intrinsic to these cells and does not depend upon the extrinsic stimulus that could be provided by the neuronal elements of the SAG to trigger its expression is supported by both *in vivo*, *in vitro* and *in ovo* studies. In contrast, the neurones that compose the SAG are dependent upon the trophic support provided by both peripheral and/or central target tissues for survival and maturation. Recent *in vitro* and *in vivo* studies support the hypothesis that differentiating inner ear sensory epithelia produce attractant fields that guide the ingrowth of SAG neurites. The neurones of the SAG appear to be able to respond to these attractant fields for an extended period during labyrinthine development. The exact mechanism by which these attractant fields are produced (e.g. biochemical extracellular matrix: Varon, Manthorpe & Williams, 1984; electrical: Patel & Poo, 1982; substrate guidance: Letourneau, 1979; Carney & Silver, 1983) by the differentiating sensory cells of the inner ear is currently not known. The attractant fields appear to have broad effects and are not specific for SAG neurites, therefore, temporal–spatial information and the mechanism by which they are generated will be vital to understanding how such a process can establish specificity of neuronal connections within the inner ear. The spatiotemporal patterns of extracellular matrix molecules (ECM) are suggested to be a significant factor in the establishment of the patterns of innervation within the inner ear. These ECM results are provocative but await more definitive proof of their actual role in the establishment of patterns of innervation within the developing inner ear.

Many of the studies cited in this paper that were performed by the author were supported by grants from NIH (NS 08365), March of Dimes-Birth Defects Foundation (I-372) and The Deafness Research Foundation. Thanks for the typing of this paper are given to Mina Pozzuto. Vera Galinovic-Schwartz is thanked for all of her excellent nerve fibre stains and other histological preparations. The author also thanks Drs D'Amico-Martel and

Noden for the use of Fig. 1, Dr Noden for Fig. 2 and Drs Corwin and Cotanche for Fig. 5 of this paper.

References

ANNIKO, M. & VAN DE WATER, T. R. (1986). Synaptogenesis in cocultured inner ear explants which share a single statoacoustic ganglion. *Acta Otolaryngol.* (Stockh) **102**, 415–422.

ARD, M. D., MOREST, D. K. & HAUGER, S. H. (1985). Trophic interactions between the cochlea vestibular ganglion of the chick embryo and its synaptic targets in culture. *Neurosci.* **16**, 151–170.

ARD, M. D. & MOREST, D. K. (1984). Cell death during development of the cochlear and vestibular ganglia of the chick. *Int. J. devl. Neurosci.* **2**, 535–547.

BARTELMEZ, G. W. (1922). The origin of the otic and optic primordia in man. *J. comp Neurol.* **34**, 201–232.

BATTEN, E. H. (1958). The origin of the acoustic ganglion in the sheep. *J. Embryol. exp. Morph.* **6**, 597–615.

BORDEN, P. C. & CORWIN, J. T. (1985). Hair cells in the lateral line are trophically dependent upon innervation for both maintenance and regeneration. *Soc. Neurosci. Abstr.* **11**, 270.

CAMPENHOUT, E. (1935). Experimental researches on the origin of the acoustic ganglion in amphibian embryos. *J. exp. Zool.* **72**, 175–193.

CARNEY, P. R. & SILVER, J. (1983). Studies on cell migration and axon guidance in the developing distal auditory system of the mouse. *J. comp. Neurol.* **215**, 359–369.

CORWIN, J. T. (1985). Auditory neurons expand their terminal arbors throughout life and orient toward the site of postembryonic hair cell production in the macula neglecta in elasmobranchs. *J. comp. Neurol.* **239**, 445–452.

CORWIN, J. T. & COTANCHE, D. A. (1985). Development of hair cells: location-specific patterns of cilia number, cilia length, and bundle orientiation differentiate after denervation and transplantation ot embryonic cochleae. *Soc. Neurosci. Abstr.* **11**, 245.

D'AMICO-MARTEL, A. & NODEN, D. M. (1983). Contributions of placodal and neural crest cells to avian cranial peripheral ganglia. *Am. J. Anat.* **166**, 445–468.

DECHESNE, C. J. & PUJOL, R. (1986). Neuron-specific enolase immunoreactivity in the developing mouse cochlea. *Hearing Res.* **21**, 87–90.

DECHESNE, C. J., SANS, A. & KELLER, A. (1985). Onset and development of neuron-specific enolase immunoreactivity in the peripheral vestibular system of the mouse. *Neurosci. Lett.* **61**, 299–304.

DEOL, M. S. (1967). The neural crest and the acoustic ganglion. *J. Embryol. exp. Morph.* **17**, 533–541.

DRAGONE, L. L. & VAN DE WATER, T. R. (1988). Neuron specific enolase expression in cultures of normal and aneural otic explants, with isolated statoacoustic ganglia and ganglia with target tissue. *Assoc. Res. Otolaryngol. Abotr.* **11**, 152.

EBENDAL, T. & JACOBSON, C. O. (1977). Tissue explants affecting extension and orientation of axons in cultured chick embryo ganglia. *Expl Cell Res.* **105**, 379–387.

GUTH, L. (1969). 'Trophic' effects of vertebrate neurons. *Neurosci. Res. Prog. Bull.* **7**, 1–73.

HALLEY, G. (1955). The placodal relations of the neural crest in the domestic cat. *J. Anat.* **89**, 133–152.

HAMMARBACK, J. A., PALM, S. L., FURCHT, L. T. & LETOURNEAU, P. C. (1985). Guidance of neurite outgrowth by pathways of substratum-absorbed laminin. *J. Neurosci. Res.* **13**, 213–220.

HIROKAWA, N. (1977). Disappearance of afferent and efferent nerve terminals in the inner ear of the chick embryo after chronic treatment with β-Bungarotoxin. *J. Cell Biol.* **73**, 27–46.

JORGENSEN, J. M. & FLOCK, A. (1976). Non-innervated sense organs of the lateral line: development in the regenerating tail of the salamander *Ambystoma mexicanum*. *J. Neurocytol.* **5**, 33–41.

KNOWLTON, V. Y. (1967). Correlation of the development of membranes and bony labyrinths, acoustic ganglia, nerves and brain centers in the chick embryo. *J. Morph.* **121**, 179–208.

LANDMESSER, L. & PILAR, G. (1978). Interactions between neurons and their targets during in vivo synaptogenesis. *Fedn. Proc. Am. Socs. exp. Biol.* **37**, 2016–2022.

LETOURNEAU, P. C. (1979). Cell-substratum adhesion of neurite growth cones, and its role in neurite elongation. *Expl Cell Res.* **124**, 127–138.

NODEN, D. M. (1984). The use of chimeras in analyses of craniofacial development. In *Chimeras in Developmental Biology* (ed. N. M. LeDouarin & A. McLaren), pp. 241–280. London: Academic Press.

ORR, M. F. (1968). Histogenesis of sensory epithelium in reaggregates of dissociated embryonic chick otocysts. *Devl Biol.* **17**, 39–44.

PATEL, N. & POO, M. M. (1982). Orientation of neurite growth by extracellular electric fields. *J. Neurosci.* **2**, 483–496.

POLLACK, E. D., MUHLACH, W. L. & LIEBIG, V. (1981). Neurotrophic influence of mesenchymal limb target tissue on spinal cord neurite growth "in vitro". *J. comp. Neurol.* **200**, 393–405.

PUJOL, R. (1986). Synaptic plasticity in the developing cochlea. In *The Biology of Change in Otolaryngology* (ed. R. J. Ruben, T. R. Van De Water & E. W. Rubel), pp. 47–54. Amsterdam: Elsevier Science Publishers BV (Biomedical Division).

RICHARDSON, G. P., CROSSIN, K. L., CHUONG, C. M. & EDELMANN, G. (1987). Expression of cell adhesion molecules during embryonic induction. III. Development of the otic placode. *Devl Biol.* **119**, 217–230.

ROGERS, S. L., LETOURNEAU, P. C., PALM, S. L., MCCARTHY, J. & FURCHT, L. T. (1983). Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. *Devl Biol.* **98**, 212–220.

ROUFA, D. G., JOHNSTON, M. I. & BUNGE, M. B. (1983). Influence of ganglion, non-neuronal cells and substratum on neurite outgrowth in culture. *Devl Biol.*

99, 225–239.

RUBEN, R. J. (1967). Development of the inner ear of the mouse: A radioautographic study of terminal mitoses. *Acta Otolaryngol. (Stockh) Suppl.* **220**, 1–44.

SANS, A. & CHAT, M. (1982). Analysis of temporal and spatial patterns of rat vestibular hair cell differentiation by tritiated thymidine radioautography. *J. comp. Neurol.* **206**, 1–8.

SHER, A. (1971). Embryonic and postnatal development of the inner ear of the mouse. *Acta Otolaryngol. (Stockh) Suppl.* **285**, 1–77.

SPEIDEL, C. C. (1947). Correlated studies of sense organs and nerves of the lateral line in living frog tadpole. I. Regeneration of denervated organs. *J. comp. Neurol.* **87**, 29–55.

SPEIDEL, C. C. (1948). Correlated studies of sense organs and nerves of the lateral line in living frog tadpoles. II. The trophic influence ot specific nerve supply as revealed by prolonged observations of denervated and reinnervated organs. *Am. J. Anat.* **82**, 277–320.

THORNHILL, R. A. (1972). Development of the labyrinth of the lamprey. *Proc. R. Soc. Lond.* B. **181**, 175–198.

VAN DE WATER, T. R. (1976). Effects of removal of the statoacoustic ganglion complex upon the growing otocyst. *Ann. Otol. Rhinol. Laryngol.* **85** *Suppl.* **33**, 1–32.

VAN DE WATER, T. R. (1986). Determinants of neuron-sensory receptor cell interaction during development of the inner ear. *Hearing Res.* **22**, 265–277.

VAN DE WATER, T. R. & RUBEN, R. J. (1983). A possible embryonic mechanism for the establishment of innervation of inner ear sensory structures. *Acta Otolaryngol.* (Stockh) **95**, 470–479.

VAN DE WATER, T. R. & RUBEN, R. J. (1984). Neurotrophic interactions during in vitro development of the inner ear. *Ann. Otol. Rhinol. Laryngol.* **93**, 558–564.

VAN DE WATER, T. R., WERSALL, J., ANNIKO, M. & NORDEMAN, H. (1978). Development of the sensory receptor cells in the utricular macula. *ORL*, 297–304.

VARON, S., MANTHORPE, M. & WILLIAMS, L. R. (1984). Neuronotrophic and neurite-promoting factors and their clinical potentials. *Devl Neurosci.* **6**, 73–100.

WEIBEL, E. R. (1957). Zur Kenntnis der Differenzierungsvorgange in Epithel des Ductus Cocholearis. *Acta Anat. (Basel)* **29**, 53–90.

WHITEHEAD, M. C. & MOREST, D. K. (1985). The development of innervation patterns in the avian cochlea. *Neurosci.* **14**, 255–276.

YNTEMA, C. L. (1937). An experimental study of the origin of the cells which constitute the VIIth and VIIIth cranial ganglia and nerves in the embryo of *Amblystoma punctatum*. *J. exp. Zool.* **75**, 75–101.

ZHOU, X-N. & VAN DE WATER, T. R. (1987*a*). The effect of target tissues on survival and differentiation of mammalian statoacoustic ganglion neurons in organ culture. *Acta Otolaryngol.* (Stockh) **104**, 90–98.

ZHOU, X-N. & VAN DE WATER, T. R. (1987*b*). Hema hydrogels as a substratum for culture of inner ear and statoacoustic ganglion explants. *Hearing Res.* **27**, 183–191.