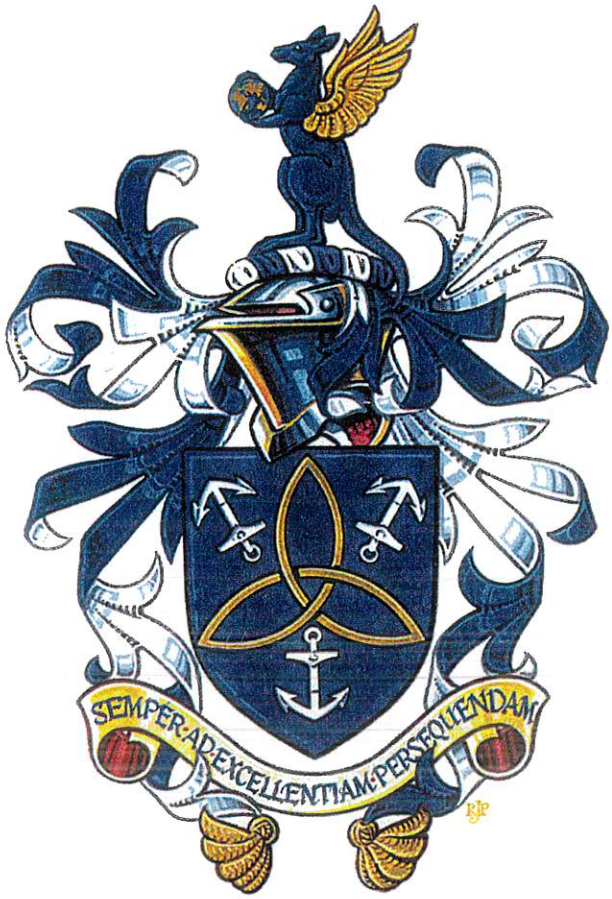
The Garnett Passe and Rodney Williams Memorial Foundation

Frontiers in Otorhinolaryngology 2000

August 11-13, 2000
The Stamford Plaza
150 North Terrace, Adelaide
Australia



The Garnett Passe and Rodney Williams Memorial Foundation

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Frontiers in Otorhinolaryngology 2000
Programme

Friday, August 11

5.30 pm - 6.15 pm	Registration – Terrace Ballroom Foyer	
6.15 pm - 9.00 pm	Drinks/Buffet Dinner	
	Welcome	Dr Peter Freeman Chairman of Trustees The Garnett Passe and Rodney Williams Memorial Foundation

Saturday, August 12

8.10 am - 8.15 am	Opening Remarks	Professor Anne Cunningham

Olfactory Sciences Session

Chair	***Anne Cunningham***
8.15 am - 9.00 am	Gordon Shepherd, Yale University School of Medicine *How we smell; new insights into old problems*
9.00 am - 9.30 am	Brian Key, University of Queensland *How do olfactory axons grow during development and regeneration?*
9.30 am - 10.15 am	Donald Leopold, University of Nebraska Medical Center *Nasal nuances in human olfactory perception*
10.15 am - 10.45 am	Morning Tea and Informal Poster Viewing
Chair	***Kevin Kane***
10.45 am - 11.30 am	Peter Mombaerts, Rockefeller University *Targeting olfaction: the molecular basis of olfactory perception*
11.30 am - 12 noon	Anne Cunningham, Garvan Institute of Medical Research *A window into neurogenesis: stem cells and growth factors in the olfactory system*
12 noon - 12.30 pm	Alan Mackay-Sim, Griffith University *Developmental neurobiology of human olfactory epithelium*
12.30 pm - 12.45 pm	Open Discussion
12.45 pm - 1.45 pm	Lunch

Otological Sciences Session

Chair	***Peter Freeman***
1.45 pm - 2.30 pm	Ed Rubel, University of Washington *Hair cell regeneration in the inner ear of birds and mammals*
2.30 pm - 3.00 pm	Marcus Atlas, University of Western Australia *Meniere's disease – What would Prosper Meniere think now?*

Chair	**John Funder**
3.00 pm - 4.00 pm	Formal Poster Session and Afternoon Tea
Chair	***Dean Beaumont***
4.00 pm - 4.30 pm	James Pickles, University of Queensland *Acoustic trauma and fibroblast growth factor expression in the mammalian inner ear*
4.30 pm - 5.00 pm	Robert Shepherd, University of Melbourne *Deafness induced changes in the cochlea and central auditory pathway: implications for cochlear implants*
5.00 pm - 5.15 pm	Open Discussion
5.15 pm - 6.00 pm	Drinks
7.00 pm for 7.30 pm	Conference Dinner Special presentation: William Coman

Sunday, August 13

Cancer Biology Session

Chair	***Doug Tracy***
8.30 am - 9.15 am	William Gullick, University of Kent at Canterbury *Growth Factor receptors in human cancer*
9.15 am - 9.45 am	Edouard Nice, Ludwig Institute for Cancer Research *Inhibitors of the EGFR tyrosine kinase: from laboratory to the clinic*
9.45 am - 10.15 am	Roger Daly, Garvan Institute of Medical Research *The roles of cyclin D1 and cortactin in human cancers amplified at chromosome 11q13*
10.15 am - 10.45 am	Andrew Scott, Ludwig Institute for Cancer Research and Austin & Repatriation Medical Centre *Characterisation and targeting of amplified wild-type and mutant epidermal growth factor receptors expressed on tumours in-vivo*
10.45 am - 11.15 am	Morning Tea and Informal Poster Viewing
Chair	***John Funder***
11.15 am - 11.45 am	Tony Burgess, Ludwig Institute for Cancer Research *Signalling from the epidermal growth factor family in normal and neoplastic cells*
11.45 am - 12.15 pm	Andrew Sizeland, Royal Victorian Eye and Ear Hospital *The role of TGF beta in head and neck cancer*
12.15 pm - 1.00 pm	Yosef Yarden, Weizmann Institute of Science *Growth factors and receptor tyrosine kinases in human cancer: signaling mechanisms and therapeutic opportunities*
1.00 pm - 1.15 pm	Open Discussion
1.15 pm - 1.30 pm	Presentation of Poster Prizes and closing remarks

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Abstracts of Invited Speaker Presentations

HOW WE SMELL; NEW INSIGHTS INTO OLD PROBLEMS

Gordon M Shepherd
Section of Neurobiology, Yale Medical School, New Haven,
Connecticut, USA

Among the major senses, the sense of smell has seemed the most enigmatic. The stimuli can't be delivered with precise control as in vision or audition, there is no spatial quality to be mapped, and the odor sensations are difficult to describe. Clinically, the sense of smell seems almost vestigial in the human, and testing for smell is cumbersome and seems to reveal relatively little of clinical importance.

For these and other reasons, the human sense of smell has historically seemed of limited interest to researchers. However, from the point of view of current research on the brain, it is one of the most interesting systems in all of neuroscience. In this lecture we will describe some of the current areas of research, with particular emphasis on those that may be relevant to the clinic.

Odor molecules inhaled into the nasal chamber are initially transduced by interacting with receptor molecules in the fine cilia at the tips of olfactory receptor cells in the olfactory epithelium. The several million cells in the human express several hundred different receptor molecules. Experiments in rodents show that each receptor responds to a specific range of odor molecules. The receptor cells are thus like cone photoreceptors with different peak sensitivities but overlapping spectra. Molecular models show how the functional groups of the odor molecules may interact within a binding pocket in the receptor molecule. Understanding these interactions may enable artificial sensors to be built that can have industrial applications as well as detect hidden drugs, land mines, etc.

The receptor cells send their axons through the cribriform plate to end in multicellular modules called glomeruli in the olfactory bulb. The glomerulus is a functional unit, receiving converging input from the subset of cells expressing a given receptor. The glomeruli are arranged in a sheet. Since odors differentially activate the glomeruli, the glomerular layer transforms the information carried in "odor space" into a two dimensional "odor image" in neural space. This odor image is equivalent to a visual image in the visual pathway and the mapping of frequency in the auditory system.

Each glomerulus is tuned to a different set of odors, reflecting the inputs from the receptor cells. Microcircuits within the olfactory bulb compare the inputs through the parallel glomerular pathways, extracting information about odor molecular identity independent of odor intensity, the same principle used in the retina to extract wavelength information independent of light intensity.

The olfactory receptor cell undergoes continual turnover, perhaps the only neuron in the adult mammalian brain to retain this capacity. How odor specificity is retained and wiring connections re-established is an intriguing subject of current research. It is emerging as an important model for use in brain transplants. In addition, cells within the brain continue to migrate from the subventricular zone into the olfactory bulb to form new interneurons.

Higher processing of smells occurs in the olfactory cortex, whose output goes either to subcortical limbic regions involved in emotion or cortical regions that generate the conscious perception of smell. Current research is revealing the much more widespread appreciation of the importance of smell in nutrition, quality of life, and as an early signal of clinical disorders.

HOW DO OLFACTORY AXONS GROW DURING DEVELOPMENT AND REGENERATION?

Brian Key
Department of Anatomical Sciences, University of Queensland,
Brisbane, Queensland, Australia

In order for our sensory systems to make "sense" of the external environment they often process information with the use of topographical maps. In the olfactory system this spatial segregation of inputs begins in the primary olfactory pathway. Axons arising from olfactory neurons expressing specific odorant receptor proteins converge to form discrete neuronal connections with second-order neurons in structures referred to as glomeruli in the olfactory bulb. The positions of glomeruli appear to be preserved between both olfactory bulbs within the same animal and between different animals. Thus, the formation of glomeruli in their topographically correct position during both development and regeneration is probably critical for normal olfactory sensation. We have been investigating the molecular and cellular basis of olfactory axon guidance and targeting in the olfactory bulb. The olfactory pathway can be divided into two distinct regions, the peripheral olfactory nerve and the central olfactory bulb. Evidence to date indicates that different mechanisms are operating to guide axons within these anatomically distinct regions. We propose that a hierarchy of molecules progressively sorts axons into discrete fascicles and directs them to their topographically correct sites during development. In particular, olfactory axons are uniquely identified by a cell surface glycocode that most likely facilitates their sorting within the nerve fibre layer. Although receptor proteins have been implicated in the subsequent convergence to specific glomeruli the underlying mechanisms are unknown. What are the cues that stimulate axon sorting and convergence to the correct site in the bulb? Analysis of mutant mice lacking an olfactory bulb has suggested that the bulb is not necessary for axon sorting or convergence. In these mice the olfactory nerve and a structure reminiscent of the presumptive nerve fibre layer (the fibrocellular mass) are present whereas the olfactory bulb and second-order olfactory neurons are absent. Axons sort out and converge to form glomerular-like loci even in the absence of the olfactory bulb. These results suggest that axon-axon interactions may be all that is required for axons to sort out and converge. This, however, does not explain why axons do not sort out in the olfactory nerve during normal development. Olfactory axons show no evidence of sorting until they have reached at least the olfactory nerve fibre layer of the bulb. Moreover, axons appear to sort out but do not converge in mice deficient in specific receptor proteins (Wang *et al.*, Cell, 93:47-60, 1998). Thus sorting out and convergence appear to be separate events. Interestingly, during regeneration in the adult, olfactory axons are able to converge and form glomeruli but they fail to sort out appropriately. Instead, they converge to multiple inappropriate sites in the bulb. Taken together it appears that convergence is axon autonomous whereas sorting is dependent on interactions in the nerve fibre layer. Two important questions now need to be addressed. First, can axons sort out in the total absence of the olfactory nerve fibre layer or fibrocellular mass; and second, what are the differences between axon sorting in the nerve fibre layer during development and regeneration?

NASAL NUANCES IN HUMAN OLFACTORY PERCEPTION

Donald Leopold
Department of Otolaryngology, Head and Neck Surgery
University of Nebraska Medical Centre, Omaha, Nebraska USA

The nose is a major participant in the human sense of smell. It provides the airways that allow movement of odorant molecules to the receptors, and it houses the primary olfactory receptors that send axons to the more central olfactory neurons. The initial event in olfaction is the movement of odorant molecules with the nasal airflow to the olfactory cleft. Clinical experience suggests that total nasal airway blockage precludes olfactory ability. We theorized that a partial blockage must have some effect, and from model studies we knew that the nasal anatomy determined airflow patterns. In a group of 19 patients with various levels of nasal airway blockage, we measured olfactory ability and performed CT scans. By correlating the olfactory ability with the anatomy, we were able to determine that increases in size of the nasal cavity around the olfactory cleft generally increase the olfactory ability. The nasal anatomical differences between men and women may also help explain the sex differences in olfactory ability.

Once the odorant molecule reaches the top of the nasal cavity, it must interact with an olfactory receptor on the primary neuron. The location of these receptors is, however, not clearly delineated. Using electrophysiological and histological methods, the olfactory neurons anterior and inferior to the traditional olfactory cleft have been identified. This may have implications in patients with polyps who smell with and obstructed olfactory cleft.

Another issue is whether these nasal neurons always function correctly. A small minority of patients have phantom perceptions of an odor. Histological analysis of olfactory mucosa from these patients shows clear abnormalities with neuromas and decreased large axon bundle neurons.

These studies and others are allowing us to explain the clinical presentations we see, and to recommend therapies based on solid research data.

TARGETING OLFACTION: THE MOLECULAR BASIS OF OLFACTORY PERCEPTION

Peter Mombaerts
Laboratory of Vertebrate Developmental Neurogenetics
The Rockefeller University, New York, New York, USA

Our nose can smell almost any volatile molecule, often at minute concentrations. A good perfumer can use the nose to identify by name hundreds, sometimes thousands of distinct chemical compounds. What makes the olfactory system so sensitive and specific? Odorant receptors are G-protein coupled seven-transmembrane proteins, encoded by the largest gene family known to exist in the mammalian genome. The complexity of the odorant receptor repertoire in mammals is estimated at 1000 genes, representing 1-2% of the estimated total number of genes.

A given odorant receptor gene is expressed in a very small subset of neurons within one of four stripes or "zones" of the olfactory epithelium. It appears that a neuron only expresses a single odorant receptor gene. Using genetic manipulation techniques, we have created strains of mice in which expression of a defined odorant receptor is coupled to that of an axonal marker, either taulacZ or tauGFP. In these mutant mice, labelled axons project typically to either of two glomeruli, which reside at recognizable positions in each bulb. There are ~1800 glomeruli in an adult mouse bulb, and ~1000 odorant receptor genes in the mouse genome; thus, each odorant receptor may correspond to two specific glomeruli. These findings define the glomerulus as a convergent site of axonal projections from neurons that express a given odorant receptor.

The axonal convergence of neurons expressing a given odorant receptor to a pair of spatially defined glomeruli in the bulb creates a daunting wiring problem: 1000 neuronal subsets must be sorted reproducibly onto 1800 targets in the bulb during development. Moreover, because the map stays constant while neurons are being replaced throughout adult life, every day correct synaptic connections must be made by axons of newly generated neurons. We are interested in the mechanisms governing the development of these axonal projections.

A WINDOW INTO NEUROGENESIS: STEM CELLS AND GROWTH FACTORS IN THE OLFACTORY SYSTEM

Anne Cunningham
Sensory Neurobiology Group, The Garvan Institute of Medical Research and School of Paediatrics, University of New South Wales, Sydney, New South Wales, Australia

The olfactory neuroepithelium is unique in maintaining a progenitor population which continues to proliferate and generate new neurones in the adult making it an invaluable system for the study of neurogenesis and axon outgrowth. If we understood the basic cellular and molecular mechanisms controlling olfactory stem cell function, progenitor proliferation and neuronal differentiation we may be able to develop new modalities for treating disorders of the sense of smell. In addition, being able to recapitulate these processes in other less plastic areas of the CNS, is highly relevant to the discovery of new treatments for neuronal injury and degeneration. In this paper we will review some of these areas of current intense interest and focus on our laboratory's contribution.

Studies using retroviral infection of regenerating olfactory neuroepithelium have supported the existence of a multipotent olfactory progenitor in the basal cell region which has the potential to give rise to both sustentacular, or supporting cells, and neurones. This progenitor resides in the globose basal cell (GBC) population but, to date, has remained poorly characterised. A host of growth factors have been now described in the olfactory neuroepithelium and bulb. Our laboratory has described the expression of BDNF, CNTF and GDNF in the neuroepithelium, each with unique patterns of cellular distribution (Buckland & Cunningham, 1999). In particular, BDNF and CNTF appear likely to play a role in progenitor function, while GDNF may be a crucial player in olfactory neuronal differentiation. We are using *in vitro* primary culture systems, such as described in Cunningham *et al*, (1999), to explore the mechanisms of action of these factors.

This work has emphasised the key importance of isolating the true olfactory stem cell for study of its lineage potential and its unique growth factor requirements. Recently, we have been successful in isolating olfactory progenitor cells from neonatal rat olfactory tissue. In primary culture they form large multicellular aggregates, or 'neurospheres', a striking *in vitro* characteristic of neural stem cells isolated from other CNS sites. The cells in the olfactory neurospheres expressed nestin, the intermediate filament protein widely used as marker of proliferating neural stem cells, and also labelled with GBC-1, a monoclonal antibody that recognises GBCs (Goldstein & Schwob, 1996). By addition of specific exogenous growth factors we were able to promote proliferation of neurospheres and differentiation down the neuronal pathway. Molecular analysis was performed by manually collecting single neurospheres for RNA isolation and RT-PCR performed using oligonucleotides specific for nestin, the olfactory neuronal transcription factor Olf-1, GAP-43, β-tubulin isotype 3 and GFAP. This analysis of individual neurospheres confirmed expression of messages for nestin and the neuronal markers and we were able to examine dynamic alterations in these gene transcripts on exogenous application of specific trophic factors. This *in vitro* model of neurogenesis will allow us to further define the molecular characteristics of olfactory progenitor cells, explore their pluripotentiality, and search for novel stem cell and neurogenesis genes. These experimental approaches are unravelling the secrets of the olfactory neurogenic process.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation and the NHMRC of Australia

DEVELOPMENTAL NEUROBIOLOGY OF HUMAN OLFACTORY EPITHELIUM

Alan Mackay-Sim
Centre for Molecular Neurobiology, Griffith University, Nathan, Queensland, Australia

The olfactory epithelium is one of a few places in the nervous system in which new neurons are produced throughout adult life. This unusual regenerative power, called "neurogenesis", is probably maintained because the sensory neurons are directly exposed to, and can be damaged by, microorganisms and toxins in the inspired air. Our research is focussed on understanding human olfactory neurogenesis at the cellular and molecular level. Knowledge of this fundamental biology is now also leading to clinical applications far beyond the olfactory organ.

In animal studies we have identified the functions of several growth factors in olfactory neurogenesis *in vitro* and have identified autocrine and paracrine signalling pathways via which these growth factors could regulate olfactory neurogenesis *in vivo*. The goal of this systematic analysis is to develop a model which describes the passage from undifferentiated stem cell to mature sensory neurons and the growth factors which regulate this passage via proliferation, differentiation and cell death. We have defined the roles for a number of signaling factors including fibroblast growth factor (precursor proliferation), transforming growth factorβ2 (precursor differentiation), platelet-derived growth factor (neuron survival), insulin-like growth factor (neuron differentiation), and dopamine (neuron differentiation). We are currently investigating the roles of the neurotrophins, and several growth factors in the transforming growth factorβ family. The goal of this work is to achieve an understanding of olfactory neurogenesis similar to that achieved for hematopoiesis. This will lead to better treatments for anosmias caused by damage to the olfactory epithelium and nerve.

We have developed new techniques for biopsy and culture of the human olfactory epithelium *in vitro*. This work showed that olfactory epithelium is distributed in the nasal cavity much more widely than previously thought. Additionally, neurogenesis was observed in tissues taken 25 hours post mortem and in tissues from patients as old as 72 years. These observations suggest that human olfactory neurogenesis is very robust and might be useful for studying human neurological diseases. Human olfactory epithelium can be biopsied using a nasal endoscope and giraffe forceps under local anesthesia and does not damage the sense of smell.

Olfactory epithelium is helping us understand the aetiology of schizophrenia. Schizophrenia affects 1% of the population and places large social and economic costs on the community. We have observed significant abnormalities in neurogenesis in patients with schizophrenia. These differences were in cell attachment, proliferation, and cell death - all of which are consisted with the hypothesis that schizophrenia is a disease of brain development. This work is currently being repeated and extended to patients with bipolar disorder and to unaffected family members. DNA microarray technology will be used to identify gene expression differences in the olfactory epithelium of patients and controls.

Olfactory ensheathing cells are special glial cells which guide and assist the developing olfactory axons as they find their way to the brain. Recent publications indicate that olfactory ensheathing cells isolated from the brain can promote repair of the damaged spinal cord and peripheral nerves. We are investigating whether olfactory ensheathing cells from the nose could be used for autologous repair of the nervous system. Our recent results indicate that

nasal olfactory ensheathing cells were able to induce recovery after transplantation into the transected spine of the rat. Paraplegic animals regained movement in the legs and regained inhibition of spinal reflexes. Regrowth of spinal nerves across the transection was confirmed histologically. Current research is focussed on the biology of olfactory ensheathing cells - how to purify them and how to proliferate them in culture with the aim of developing human therapies based on autologous transplantation.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation, Queensland Health, the Stanley Foundation and the Rebecca L Cooper Medical Foundation.

HAIR CELL REGENERATION IN THE INNER EAR OF BIRDS AND MAMMALS

Edwin W Rubel
Virginia Merrill Bloedel Hearing Research Center, University of Washington,
Seattle, Washington USA

Sensorineural hearing loss results primarily from the loss of hair cells due to genetic anomalies, aging, noise, environmental toxins and ototoxic drugs, or from an interaction of these factors. The only therapies are amplification or electrical stimulation (cochlear implants). The 1987-8 discovery that mature birds can regenerate new hair cells in the cochlea and balance organs of the inner ear inspired the hope that regeneration can be induced in the human organ of Corti and vestibular organs, and form the basis for new therapies for hearing and balance disorders.

This presentation will review progress in the field of hair cell regeneration during the past decade. Two principal questions are posed: What can we learn from studies of hair cell regeneration in birds that might help induce this process in mammals? And, can we use modern molecular biology to "trick" the mammalian inner ear into initiating this process?

Studies of birds have identified the source of newly regenerated hair cells and discovered the sequence of events leading to hair cell regeneration, including some of the molecular interactions between cells that both limit and promote hair cell regeneration. In addition, we show that hair cell regeneration enables the restoration of both hearing and vestibular reflexes following damage due to aminoglycoside ototoxicity. Work on mammals has yet to show functional levels of hair cell regeneration in the cochlea or vestibular organs of mature animals. However, recent studies using a variety of approaches are providing encouraging results. Results will be presented from *in vivo* and *in vitro* studies on the mammalian inner ear which show that the early stages of regeneration can be induced by extrinsic application of growth factors or altered gene expression.

Research supported by NIH and the Oberkotter Foundation.

ACOUSTIC TRAUMA AND FIBROBLAST GROWTH FACTOR EXPRESSION IN THE MAMMALIAN INNER EAR

James O Pickles
Vision Touch and Hearing Research Centre, University of Queensland
Brisbane, Queensland. Australia

Anatomical effects of acoustic trauma were studied in guinea pigs, immediately after the end of short acoustic exposures which caused only small increases in auditory thresholds, thus imitating the exposures commonly experienced in everyday life. The anatomical changes in hair cells included slight splaying of the outer hair cell stereocilia, and severe bending and separation of the inner hair cell stereocilia, with many of the inter-stereociliary links being severed. It is known that auditory thresholds eventually completely recover from the degrees of hearing loss measured in these experiments. The results suggest that hair cells are able to repair the degrees of anatomical damage that were observed after such exposures.

The response to acoustic trauma includes enhanced expression of fibroblast growth factor (FGF) receptor 3 in hair cells, possibly as part of the damage repair mechanism. However, it is not known which ligand could stimulate the receptor. Therefore, the expression of messenger RNA coding for fibroblast growth factors, their receptors, and the receptor splice variants were investigated in dissected fractions of the mouse inner ear. The results suggest that the sensory/nerve ganglion area of the inner ear expresses high levels of FGF8b. This FGF is an effective ligand for FGF receptor 3c, also expressed at high levels in the area, including in outer hair cells. The neural/sensory area also expresses high levels of FGF3, which is an effective ligand for FGF receptors 1b and 2b, also expressed at high levels in the area. The lateral wall of the cochlea (including stria vascularis and spiral ligament) expressed high levels of FGF2, which is an effective ligand for FGF receptor 1c, expressed heavily in that area. The results suggest which ligand/receptor combinations might be involved in development, maintenance and repair in different parts of the cochlea, and points to the possible importance of FGF8b in hair cell repair.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation.

DEAFNESS INDUCED CHANGES IN THE COCHLEA AND CENTRAL AUDITORY PATHWAY: IMPLICATIONS FOR COCHLEAR IMPLANTS

Robert Shepherd on behalf of
Robert K. Shepherd, Natalie A. Hardie
Department of Otolaryngology, University of Melbourne, Parkville, Victoria, Australia

Cochlear implants electrically stimulate discrete populations of residual auditory nerve fibres in profound and severely deaf patients in order to provide important temporal and pitch cues for speech perception. A sensorineural hearing loss can result in significant atrophic and degenerative changes within the cochlea and central auditory pathways. These changes may contribute to the strong negative correlation between duration of deafness and speech perception evident among cochlear implant patients. In this paper, we will review the effects of a neonatal hearing loss on both the cochlea and central auditory pathway and discuss their implications for cochlear implants.

A sensorineural hearing loss initiates a gradual, ongoing degeneration of auditory nerve fibers. Associated with these degenerative changes are loss of peripheral processes and demyelinization of the soma; changes that are likely to affect neural response properties to electrical stimulation. Neurons within the auditory brainstem exhibit a rapid reduction in soma area. The extent of these changes depends on age; loss of auditory nerve fibers prior to the onset of hearing can result in widespread degeneration of central auditory neurones. Concomitant with rapid reductions in soma area are significant reductions in the volume of auditory brainstem nuclei, together with a deafness related reduction in synaptogenesis within the auditory midbrain.

Physiological studies reveal that the central auditory pathway can be readily activated via electrical stimulation of the auditory nerve, and that basic response properties appear unaffected by long-term auditory deprivation. Moreover, animals with no prior auditory experience show evidence of a rudimentary cochleotopic organisation in central auditory nuclei. This organisation is considered important for pitch discrimination based on electrode location within the cochlea. However, deafened animals show increased response latencies and significant reductions in temporal resolution compared with normal animals.

These studies illustrate the functional status of the auditory pathway following a sensorineural hearing loss. Evidence suggests that the central auditory pathway has the potential to undergo plastic change in response to behaviourally relevant stimulation via a cochlear implant; this plasticity may play a significant role in the improved clinical performance observed among cochlear implant patients with device use.

This work was funded by The Garnett Passe & Rodney Williams Memorial Foundation.

GROWTH FACTOR RECEPTORS IN HUMAN CANCER

William Gullick
Department of Biosciences, University of Kent, Canterbury, England, United Kingdom

Growth factors are small proteins made by cells which are secreted into the intracellular space, lymph or bloodstream which then act on other cells locally or at a distance. The concentration of the growth factor is sensed by receptors present on the cell surface. These are proteins which span the cell membrane whose extracellular regions are responsible for ligand recognition. Ligand binding causes receptor dimerisation, which is followed by oligomerisation into patches on the cell surface. These interactions result in phosphorylation of the intracellular region of the molecule via its own intrinsic tyrosine kinase activity. One family of receptors, which seems to have a particularly important role in human cancer, is the Type 1 or EGF family. These consist of four genes specifying receptor proteins (EGFR, c-erbB2, c-erbB3 and c-erbB-4, also known as HER 1-4) and ten genes encoding ligands (EGF, TGF alpha, Amphiregulin, Heparin-binding EGF, Betacellulin, Epiregulin and the four NRG genes). The receptors are induced to dimerise in various combinations by particular ligands thereby also recruiting different selections of second messengers and thus stimulating different intracellular pathways.

Since human cancer is characterised by a loss of normal growth regulation much work has examined the possibility that these systems may be altered and thus responsible for at least part of this transformation. Three non-exclusive types of alterations have been found. Receptors with a normal structure are often found to be expressed at an unnaturally high level in certain common solid tumours. In some tumour types the receptor genes have been found to possess activating mutations. Finally, some tumours contain excessive amounts of one or more growth factors which activate aberrantly receptor signaling. In each case the end result is too much information traffic through the system. The mechanism by which this occurs however is important as it determines possible therapeutic strategies. Alterations to the EGF receptor family have now been catalogued in many human tumour types, including head and neck cancer.

These systems are a target for designed inhibitors which can be evaluated as anti-cancer treatments. Two basic strategies have been developed to target excessive receptor signaling. The first exploits receptor overexpression on the tumour cells relative to lower levels on normal tissues. A well known example is the drug Herceptin which is a humanised monoclonal antibody to the c-erbB-2/HER2 receptor which has shown promise in the treatment of breast cancer patients whose tumours overexpress this protein. The other available strategy is to make small molecule tyrosine kinase inhibitors. These have broader applications, as they would be appropriate for use whatever the mechanism underlying receptor overactivity. An early example of such a drug is IRESSA which is directed to the EGF receptor and has a low toxicity in Phase I trials and is being studied now in Phase II/III trials for efficacy.

It has become evident that surrogate markers for drug activity evaluation are urgently required, not least as with these relatively non-toxic drugs the Maximum Tolerated Dose is not the correct model for choice of the most active dose and schedule. It is important therefore that these low toxicity designed drugs are developed in parallel with a practical method of determining the correct patients to treat and with surrogate makers for drug development and ideally individual patient dose optimisation.

INHIBITORS OF THE EGFR TYROSINE KINASE: FROM LABORATORY TO THE CLINIC

Edouard Nice
Ludwig Institute for Cancer Research and CRC for Cellular Growth Factors
Parkville, Victoria, Australia

The signalling pathways controlled by the EGF family of growth factors and their receptors (the ErbB family of receptor tyrosine kinases) are capable of regulating the proliferation and differentiation of many tissue types. Uncontrolled activation of the EGF receptor has been implicated in a number of human cancers including brain, squamous cell tumours of the head and neck, lung, breast, ovary, pancreas, colon and prostate and is associated with poor clinical prognosis, non-responsiveness to chemotherapy and decreased survival. The EGFR family therefore is an important target for anti-cancer therapeutics capable of inhibiting signalling from these receptors.

Specific and effective small molecule inhibitors of the EGFR tyrosine kinase based on pyrimidine, tyrphostin and substituted quinazoline structures have been described. The inhibitory action of all these compounds is due to competition with ATP for binding to the kinase domain of the receptor. The Ludwig Institute for Cancer Research is developing the EGFR inhibitor, AG1478 (4-(3-chloroanilino)-6,7-dimethoxyquinaziniline) for use in the clinic early in 2001. In the first instance, patients with advanced glioblastoma, who over express the EGFR or who express the mutant activated form of the EGFR (delta 2-7 EGFR) will be given the opportunity to participate in a Phase 1 trial. AG1478 is highly specific for the EGFR (and delta 2-7 EGFR), and has an IC50 of approximately 3nM using *in vitro* kinase assays or 60 – 80nM as an inhibitor of mitogenesis in cells dependent on the activation of the EGFR. Our pre-clinical studies have addressed formulation, duration of inhibitory signal *in vitro*, pharmacokinetics, bio-distribution, *in vivo* efficacy and potential synergy with other treatment regimes (eg monoclonal antobodies or cytotoxic drugs). Our recent data using the Cytosensor, a cell based biosensor that uses a light-addressable potentiometric sensor to measure rapid (<30sec) changes in pH (sensitivity 0.01pH units per minute), has been used to develop a rapid and sensitive method to investigate the specificity, potency and duration of inhibitor action on cells. We hope to use the cytosensor results to optimise the AG1478 treatment protocols.

MENIERE'S DISEASE – WHAT WOULD PROSPER MENIERE THINK NOW?

Marcus Atlas, Department of Otolaryngology, University of Western Australia, Perth, Western Australia, Australia

Meniere's disease has fascinated generations of doctors who have struggled to understand and explain the various features of this disease. Unfortunately, proven scientific knowledge is embarrassingly scarce and this has led to varying views regarding its nature, pathophysiology, diagnosis and treatment. This presentation features major controversies in Meniere's disease and highlights the role of the immune system raising the possibility of new treatments.

Since Prosper Meniere described this condition in 1861 there has been great controversy with regard to terminology in Meniere's disease. The principal pathological feature of Meniere's disease is endolymphatic hydrops but this is an unacceptable term for the disease because it may be present in many other conditions. The presence of Meniere's disease variants and the clinical and sub-clinical features of bilateral Meniere's disease play a major role in clinical management. Bilateral Meniere's disease is found increasingly as follow-up lengthens and a study utilising electrocochleography indicates "sub-clinical" involvement in 35% of patients with unilateral involvement.

The aetiological features of Meniere's disease remain uncertain but the role of the immune system is increasingly recognised as being important. Immunohistochemical and animal model studies confirm an autoimmune attack directed against the endolymphatic sac and systemic immune-mediated diseases are associated with a Meniere's-type condition. The presence of circulating autoantibodies directed against crude inner ear antigens also indicate an immune basis for inner ear disease and are present frequently in Meniere's disease. Heat shock protein 70 appears to be similar to the antigen detected in these studies.

The controversial history of Meniere's disease surgery is highlighted including vestibular nerve section. New drug treatments utilising novel drug delivery systems may be the next exciting stage of management.

THE ROLES OF CYCLIN D1 AND CORTACTIN IN HUMAN CANCERS AMPLIFIED AT CHROMOSOME 11q13

Roger Daly on behalf of
Roger J. Daly, Douglas H. Campbell, Ronaldo J. Bova, Rina Hui and Robert L. Sutherland
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Human cancers arise as a consequence of a multistep process involving activation or overexpression of oncogenes and inactivation and deletion of tumour suppressor genes. Gene amplification is often utilized by cancer cells as a mechanism to increase expression of particular genes and thereby provide a selective advantage in terms of proliferation, survival, invasion and/or metastasis. Specific chromosomal amplification events characterize certain cancers. For example, chromosome 11 band q13 (11q13) is commonly amplified in a variety of human cancers including 30-50 % of head and neck squamous cell carcinomas (HNSCC) and approximately 15 % of breast cancers. The 11q13 amplicon spans 2.5-5 Mb and harbours several genes whose products may contribute to tumour progression: *CCND1*, which encodes the cell cycle regulatory protein cyclin D1; *HST-1/FGF4* and *INT2/FGF3*, which encode two members of the fibroblast growth factor family; and *EMS1*, which encodes the actin-binding protein cortactin. However, since *HST-1/FGF4* and *INT2/FGF3* are rarely expressed in 11q13-amplified cancers, *CCND1* and *EMS1* have attracted the most recent attention regarding their role in HNSCC and breast cancer, as illustrated below.

Cyclin D1 is the regulatory subunit of cyclin-dependent kinases 4 and 6. Complexes of cyclin D1 with these kinases phosphorylate the retinoblastoma gene product pRb and thereby promote S phase entry and progression. These complexes are inhibited by the tumour suppressor gene product $p16^{INK4A}$. In order to investigate the role of cyclin D1 in HNSCC at a specific anatomical site, immunohistochemistry for this protein and $p16^{INK4A}$ was performed on 148 squamous cell carcinomas of the anterior tongue. Overexpression of cyclin D1 or loss of $p16^{INK4A}$ were both significantly associated with reduced disease-free and overall survival. Multivariate analysis confirmed these parameters as independent predictors of death from tongue cancer. Also, analysis of four subgroups based on positivity or negativity for these two antigens revealed that loss of $p16^{INK4A}$ in the presence of cyclin D1 overexpression conferred the worst disease-free and overall survival.

The protein encoded by *EMS1*, cortactin, consists of an N-terminal actin-binding repeat region, a helical domain followed by a stretch of amino acids rich in serine, threonine and proline residues (together designated the HP region), and a C-terminal src homology (SH)3 domain. Cortactin couples signalling events to cytoskeletal reorganization, and its overexpression due to gene amplification may influence the invasive and/or metastatic properties of cancer cells. We recently investigated the prognostic significance of *EMS1* amplification in a series of 961 primary breast carcinomas. *EMS1* was amplified in 15% of samples and there was no correlation between amplification of *EMS1* and *INT2/FGF3* or *CCND1*. *EMS1* amplification was associated with increased risk of relapse and death in estrogen receptor (ER)-negative, but not ER-positive, tumours. This is in contrast to *CCND1* amplification, which was associated with poor prognosis in ER-positive patients. Consequently, in breast cancer, *EMS1* and *CCND1* amplification can occur independently and are associated with different disease phenotypes.

It is therefore evident that analysis of particular 11q13 markers and their expression in HNSCC and breast cancer may identify patient subgroups with increased risk of relapse

leading to optimization of treatment regimens. These studies also raise interesting issues regarding the biology of these cancers. For example, the poor prognosis associated with *EMS1* amplification in ER-negative breast cancer may be related to the high expression of the epidermal growth factor receptor (EGFR) in this patient subgroup. Signalling via this receptor leads to tyrosine phosphorylation of cortactin, and we have recently demonstrated that the HP region of this protein is a target for EGF-induced serine phosphorylation by the extracellular signal-regulated kinases (Erks). These modifications lead to altered activity and subcellular distribution of cortactin, respectively. Since both the EGFR and cortactin are overexpressed in HNSCC, this suggests that these signalling events may also play a key role in progression of this disease.

CHARACTERISATION AND TARGETING OF AMPLIFIED WILD-TYPE AND MUTANT EPIDERMAL GROWTH FACTOR RECEPTORS EXPRESSED ON TUMOURS *IN-VIVO*

Andrew Scott, on behalf of
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The Epidermal Growth Factor Receptor (EGFR) is an attractive target for tumour-targeted antibody therapy in view of its over-expression in many types of epithelial tumours. Binding of antibody to the EGFR may result in inhibition of signaling leading to an anti-tumour effect, and additive effects of antibody therapy combined with chemotherapy in animal models, and in human trials in patients with head and neck and lung cancers, have been reported. However, the use of EGFR specific antibodies may be limited by uptake in organs that have high endogenous levels of the wild type EGFR, such as the liver. Antibodies directed to a tumour specific variant of the EGFR (de2-7 EGFR or EGFRvIII) provide an alternative targeting strategy, although the lower proportion of tumors that express the de2-7 EGFR restricts this approach. The de2-7 EGFR is a naturally occurring extracellular truncation of the EGFR found in a number of tumour types including glioma, prostate, breast and lung but not in normal tissue. While this truncated receptor does not bind ligand, it does possess low constitutive activity and imparts a significant growth advantage to glioma cells grown as tumour xenografts in nude mice and is able to transform NIH3T3 fibroblasts. We have developed a novel monoclonal antibody (D806) that potentially overcomes the difficulties associated with targeting the EGFR expressed on the surface of tumour cells. The D806 antibody bound to de2-7EGFR transfected U87MG glioma cells (U87MG.Δ2-7) with high affinity (~ 1 x 10^9 M^{-1}), but did not bind parental cells which express the wild type EGFR. In contrast, D806 showed excellent saturating and dose response binding to an immobilized recombinant extracellular fragment of the wild type EGFR. Significantly, the D806 antibody did not bind to the EGFR fragment when it was in solution, suggesting it can only bind the wild type receptor under certain conditions. D806 also bound to the surface of A431 cells, which due to an amplification of the EGFR gene over-expresses the wild type EGFR. Interestingly, the D806 antibody only recognised 10% of the total EGFR molecules expressed by A431 cells and the binding affinity was lower than that determined for the de2-7 EGFR. The D806 antibody specifically targeted U87MG.Δ2-7 and A431 xenografts grown in nude mice with peak levels in U87MG. Δ2-7 xenografts detected 8 h after injection. No specific targeting of parental U87MG xenografts was observed. Following binding to U87MG.Δ2-7 cells, the D806 antibody was rapidly internalized by macropinocytosis and subsequently transported to lysosomes, a process that probably contributes to the early targeting peak observed in the xenografts. Thus, the D806 antibody can be used to target tumour cells containing amplification of the EGFR gene or de2-7 EGFR but does not bind to the wild type EGFR when expressed on the cell surface.

SIGNALLING FROM THE EPIDERMAL GROWTH FACTOR FAMILY IN NORMAL AND NEOPLASTIC CELLS

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The epidermal growth factor receptor family members (EGFR, erbB2, erbB3 and erbB4) mediate the biological effects of ligands such as EGF, transforming growth factor alpha and the neuregulins. The EGFR system is involved in regulating normal cell proliferation, movement, differentiation and survival. Mutation, overexpression and/or autocrine stimulation of an EGFR family member is a frequent occurrence in many carcinomas. Indeed in conjunction with other cell surface regulatory systems such as the integrins, the perturbation of EGFR signalling appears to be a major component of oncogenesis. It has been known for many years that ligand binding to the extracellular domain induces dimerization of the receptor, with consequential activation of the intracellular tyrosine kinase domain. At present our understanding of the mechanism of ligand binding, the 3-dimensional structure of the EGFR and the activation mechanism for the kinase are far from complete, but information is increasing rapidly. Our recent studies on the kinetics of ligand binding to the EGFR extracellular domain indicate that there are two forms of the EGFR in solution: one form binds EGF with 2nM affinity the other with a K_d ~500nM. In an attempt to understand the structure and function of the EGFR we have constructed molecular models of both the extracellular domain and the kinase domain. These models have been useful for studying the ligand binding characteristics of EGFR, the dimerization mechanism and the design of potential inhibitors of the receptor kinase. Various mutant forms of the EGFR have been useful for providing information about the dimerization and activation processes as well as mitogenic signalling from the EGFR homodimer. It is clear that stimulation of the EGFR can modulate many different biological processes, but we are particularly interested in the control of cell proliferation and cell movement. There appears to be a strong interaction between signalling from the EGFR system and integrin signalling in the formation of filapodia. The EGFR is known to stimulate quiescent cells as well as cells transiting the G1 phase of the cell cycle. Our recent studies provide evidence that the EGFR also prevents apoptotic death during S-phase. In conjunction with agents which induce apoptosis, EGFR inhibitors can retard, the growth of human tumour xenografts. Consequently, inhibitors of signalling from the EGFR family offer opportunities for the development of novel strategies for tumour therapy.

THE ROLE OF TGF BETA IN HEAD AND NECK CANCER

Andrew Sizeland on behalf of
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Transforming growth factor β (TGFβ) is a potent regulator of squamous epithelial cell proliferation, differentiation, migration, organization and death. TGFβ signals through a heteromeric receptor complex of two distinct type I and type II serine/threonine kinase receptors, TbRI and TbRII. Using a series of transmembrane mutated receptors, we demonstrated that the transmembrane domain plays a pivotal role in TGFβ receptor activation. Furthermore, we established a model in which the receptor activation occurs via a relative orientational rotation. In accordance with this rotation model and using truncated receptors, we further established that the extracellular domain of the receptor negatively regulates ligand-independent receptor activation. Head and Neck tumors often display diminished TGFβ responsiveness and attenuated TGFβ signaling. Certain Head and Neck cancer derived cell lines show a loss of growth inhibition when exposed to TGFβ. While this is partly explained by a decrease of type II receptor expression in 87% of head and neck cancers, it is unusual that certain TGFβ signaling events are not affected in the cell lines. To study how the expression level of the receptor affects the signaling pathways, we generated cell lines, in which the expression of TGFβ receptor is inducible. Using these cell lines, we demonstrated the importance of expression level of the receptor in selective TGFβ signaling. Low expression levels of the type I receptor are sufficient to activate specifically a TGFβ induced growth inhibition pathway. High level of the type I receptor is required for induction of one of the extracellular matrix gene, plasminogen activator inhibitor-1 (PAI-1). Furthermore, we found that the nuclear localization of Smad2 specifies TGFβ mediated growth inhibition but not the PAI-1 activation while nuclear localization of Smad3 is required for the PAI-1 activation but do not mediate growth inhibition. We also demonstrated that Smad7 negatively regulates TGFβ mediated PAI-1 pathway but has little effect on the growth pathway.

GROWTH FACTORS AND RECEPTOR TYROSINE KINASES IN HUMAN CANCER: SIGNALING MECHANISMS AND THERAPEUTIC OPPORTUNITIES

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Growth factors and their transmembrane receptor tyrosine kinases play important roles in the control of cell proliferation, migration, and differentiation. One group of growth factors, comprising epidermal growth factor- (EGF-) like proteins and neuregulins (NRGs), stimulates cells to divide by activating a group of related receptors of the ErbB family. Oncogenic animal viruses frequently activate ErbB proteins. Moreover, sustained activation of ErbBs by secreted growth factors (autocrine loops) occurs in several types of human cancer. Amplification and rearrangements of the erbB-1 gene occur in a significant fraction of glioblastoma and correlate with reduced patient survival. Similarly, amplification of the erbB-2 gene correlates with shorter time of relapse of breast cancer, shorter overall patient survival and resistance to hormonal therapy and chemotherapy.

Consistent with their pivotal role in inductive signaling to cell proliferation, blocking ErbB function results in retarded tumor growth. Examples include the clinically approved anti-ErbB-2 monoclonal antibody (Herceptin), similar antagonists of ErbB-1, and low molecular weight inhibitors of tyrosine kinases. Comprehensive understanding of ErbB signaling may provide more opportunities for drug development. Our studies imply the existence of a richly interactive, layered signaling network, which involves ErbB-2, a ligandless co-receptor, and its major partner, the kinase-defective ErbB-3. The network will be discussed in terms of robustness, diversification of signal transduction, added control and complex machineries of signal termination.

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Abstracts for Poster Presentations

POSTER 1: GENETIC CAUSES OF HEARING IMPAIRMENT

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With an incidence of >1/1000 infants, prelingual severe or profound hearing impairment is the major sensory defect in humans. In Australia and other developed countries, more than 60% of cases are believed to be of genetic origin. For most children, the hearing impairment will profoundly affect their social and personal development. The costs to affected families and the society are significant, both financially and socially.

Hereditary deafness may be syndromic or non-syndromic. Non-syndromic neurosensory deafness (NSND) accounts for 70% of genetic cases, most of these being autosomal recessive (~80%). Despite the high incidence of NSND, relatively little is known about the genes involved. This is due to the large genetic heterogeneity, the difficulties in classifying patients according to the affected gene, and the high frequency of deaf people intermarrying. It is estimated that defects in one of more than 100 different genes can cause non-syndromic deafness. Within the last few years, linkage studies in selected families have led to the mapping of over 60 loci for NSND. So far 14 genes for NSND have been identified. The gene products include structural proteins, ion transport proteins and transcription factors. Of special interest is the recently identified connexin 26 (Cx26) gene. Cx26 is a member of a family of gap junction proteins involved in cell communication. Mutations in Cx26 cause deafness in nearly 1 in 5 children with congenital hearing impairment. A specific mutation (35delG) accounts for approximately 3/4 of the Cx26 mutant alleles. We have also characterised other Cx26 mutations in the Australian population, including M34T, V37I and L90P. Cx26 mutations cause hearing losses ranging from mild to profound, but we have shown it to be associated with a characteristic high frequency hearing loss.

We will discuss the genetic aspects of NSND hearing impairment, especially our analysis of the Cx26 gene and of families with dominant hearing loss. We will also discuss the potential benefits and problems of using genetic information in the screening, detection and management of hearing loss. Understanding the aetiology of deafness is of immediate importance for the counselling of affected families and for the development of novel therapies and may therefore, in the future, influence management strategies.

POSTER 2: DEAFNESS INDUCED CHANGES IN NEURAL PROCESSING IN THE VENTRAL COCHLEAR NUCLEUS

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A goal of cochlear implant research has been to attempt to provide replication of neural signals in response to auditory stimuli in normally hearing individuals. However, profoundly deaf patients possess abnormal auditory systems, with morphological and physiological alterations in neural structures. The aim of this investigation was to use intracellular recordings in normal and deafened rats to assess the impact of deafness on neural processing of peripheral information in the ventral cochlear nucleus (VCN). Three groups of rats were examined: normal, short-term (ST) deaf (~3 months) and long-term (LT) deaf (~1 year). Rats were deafened at 10 days of age with gentamycin (250 mg/kg i.m) and frusemide (125 mg/kg i.m). Processing of auditory nerve input was assessed by examining the pattern of the excitatory post-synaptic potentials (EPSPs) and action potential (AP) generation in the VCN following intracochlear electrical stimulation in rats anaesthetised with urethane (1.3g/kg i.p). Intracellular recordings were made in 114 neurons: 44 in control, 62 ST deaf, and 8 LT deaf animals (deafness was verified by an absence of an evoked auditory brainstem response to clicks). Three EPSP summation patterns were established reflecting the timing in underlying inputs: synchronised (no delay in EPSP arrival times), composite (appearance of two or more EPSPs summating) and multiple EPSP (limited summation between EPSPs). Synchronised EPSPs were seen most in control animals (64%, 6% and 12.5% normal, ST and LT deaf respectively). Composite and multiple responses, however, were seen in greatest proportions in deafened animals (composite - 34%, 84% and 62.5%; multiple - 2%, 10% and 25%). In normal animals, APs and the subsequent level of hyperpolarisation could also be used to define three response types[1] reflecting the level of recurrent inhibition. These response types could no longer be distinguished in deafened rats with most neurons displaying short duration or no hyperpolarisation. These results indicate that the arrival of synchronised inputs is compromised in deafened rats. This has implications for temporal processing, which relies on coincident arrival of converging input and may explain the reduction observed in temporal processing of auditory midbrain neurons in LT deafened animals compared with normal[2].

1. Paolini A.G. and Clark G.M., Brain Research Bulletin, 46: 317-327, 1998
2. Shepherd R.K., Baxi J.H. and Hardie N.A. J. Neurophysiol. 82: 1363-1380, 1999

POSTER 3: NEURAL CONNECTIVITY IN THE CENTRAL AUDITORY SYSTEM OF NEONATALLY DEAFENED CATS

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The retrograde transport of a fluorescent dye (Fast Blue; FB) was used to compare the afferent projection pattern in the auditory brainstem of normal cats with that in animals with a profound hearing loss induced early in development using ototoxic drugs. A total of 11 adult cats were used: two normal hearing controls; nine animals deafened at 10 days after birth - three bilaterally and six unilaterally. Dye injections were made into the inferior colliculus (IC) under anaesthesia (ketamine, 20 mg/kg, im; xylazine, 3.8 mg/kg, im), using a glass micropipette connected to a microsyringe. Three of the unilaterally deaf animals received injections in the IC contralateral to the deaf ear, and three in the IC ipsilateral to the deaf ear. Following a survival period of one week, animals were given a lethal injection of pentobarbitone and transcardially perfused with 4% paraformaldehyde. The brainstem was removed and 50 μm coronal sections were cut on a cryostat. Sections were examined with an excitation light of 360 nm for fluorescence. The location of all retrogradely labelled neurones was recorded using a computer-linked digitising system. Results demonstrate that the main afferent projection patterns in bilaterally deaf cats, as revealed by FB labelling, do not differ greatly from control. In unilaterally deaf animals injected in the IC contralateral to the deaf ear, there was an increase in the proportion of labelled cells in the cochlear nucleus on the intact side of the brain, suggesting altered patterns of neural connectivity. There was evidence of nucleotopic and tonotopic organisation in the auditory system of all deafened animals, with afferent projections showing different local concentrations within the IC. These findings suggest that the segregation of neural input to the IC from lower brainstem regions is maintained in animals profoundly deafened very early in development.

POSTER 4: SPEECH PERCEPTION BY COCHLEAR IMPLANTEES: EFFECTS OF CHANGES IN FREQUENCY-TO-ELECTRODE ALLOCATION

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This study investigated effects of altering the pattern of allocation of frequencies to electrodes within the cochlea. In cochlear implants, sound is filtered into a number of frequency bands, and the outputs of these bands are used to control the electrical signals on the same number of electrodes. The current Cochlear Ltd implant devices utilise up to 20 filters and up to 20 electrodes which are spaced 0.75 mm apart. Earlier work (Henry *et al.*, J. Acoust. Soc Am. in press) has shown that the ability to discriminate stimulation on adjacent electrodes is highly correlated with the amount of speech information perceived within frequencies allocated to the same electrodes. However, this correlation is significant only for frequencies up to 2.6 kHz. This low-frequency range contains formant frequencies essential for vowel identification and cues for consonant identification. The hypothesis that arose from the earlier work, and which is tested in this study, is that speech perception may be improved by spreading out the frequency-to-place allocation in the low frequencies. Wider electrode spacing was implemented by using a reduced number of electrodes (10) which spanned the full usable array, and two frequency-to-electrode patterns were implemented by allocating 18 filters to the 10 electrodes in two ways. An even frequency allocation was implemented by applying two adjacent filters to each electrode which essentially corresponded to normal frequency allocation. A low-frequency-spread allocation was implemented by applying the first 9 nine filters to 9 electrodes, and the remaining 9 filters (above 2.6 kHz) to the most basal electrode. Seven subjects had take home experience with each map before speech perception was tested. Word tests in quiet showed no mean significant advantage for either map. However, 3 subjects performed significantly better with low-frequency spread and 2 with even allocation. Information transmission analysis indicated that vowel formant information was improved by spreading out the low frequencies, but that for 2 subjects, consonant information deteriorated when only one electrode was used for information above 2.6 kHz. For sentences in noise, there was a significant advantage for low-frequency spread, with no subject performing significantly better with the even allocation. In conclusion, advantages may be gained by allowing flexibility in frequency allocation in future processors.

This study was supported by the Garnett Passe and Rodney Williams Memorial Foundation.

POSTER 5: IMPROVEMENTS IN SPEECH PERCEPTION FOR COCHLEAR IMPLANTEES WITH COMPRESSION OF INPUT SIGNALS

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Ten adult users of hearing prostheses manufactured by Cochlear Limited participated in a trial investigating the potential benefits of amplitude compression of input signals. Five subjects used the CI22/Spectra-22 system, and the other five used the more-recent CI24/SPrint system. For each implant user, a compressor was introduced to process microphone signals before they were passed to the unmodified Spectra-22 or SPrint device. The compressor applied a compression ratio of 2:1 for all input signals above approximately 55 dB SPL, and had attack and release times of 5 and 50 ms, respectively. For each implantee, the overall gain was adjusted to produce similar, comfortable loudness with compression either enabled or disabled for speech presented at a typical conversational level (65 dBA). Scores for recognition of words in sentences presented at three levels showed statistically significant increases with the input compression enabled. At average levels of 45, 55, and 70 dBA, the mean improvements were 20, 17, and 3 percentage points, respectively. A test of speech perception in noise showed that, on average, the compression caused a small but statistically insignificant decrease in performance. It is concluded that moderate compression of input signals can lead to substantial perceptual benefits for users of existing cochlear implant systems, especially when listening to speech at relatively low levels. However, it is also possible that such compression can increase the loudness, and thereby reduce listeners' tolerance, of some low-level background noises. Therefore, if an input compressor is incorporated into future sound processors for cochlear implants, it may be appropriate to provide a manual control so that users can easily enable or disable the compression.

This work was supported by the Garnett Passe and Rodney Williams Memorial Foundation and the Co-operative Research Centre for Cochlear Implant and Hearing Aid Innovation.

POSTER 6: REGENERATION OF THE OLFACTORY SYSTEM AFTER INJURY: HOW ACCURATE IS IT?

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The olfactory system is a unique part of the nervous system; nerve cells are generated throughout life and it can regenerate even after injury. It therefore provides an excellent model for examining the growth, development and maintenance of nerve cells. Information we obtain about how this system develops and regenerates may be useful in treating brain disorders and spinal injuries. In order to progress down the path of treating brain injuries and disease we need to know what signalling molecules are involved in allowing neurons to differentiate, to grow and to target their topographically correct areas. We are interested in determining (1) the mechanisms that allow the olfactory neurons to regenerate, and (2) how accurately the system can be regenerated. We have developed a technique of chemically degrading the entire olfactory neuroepithelium in mice, but which leaves the basal layer of stem cells unharmed. By giving 2 intraperitoneal injections each of dichlobenil and methimazole over 4 days, the olfactory neuroepithelium is totally degraded after one week. Over the next 2 weeks the severed axons within the olfactory nerve pathway degenerate, after which time it becomes apparent that olfactory neuron regeneration has begun to occur. By examining the projection of a subset of neurons whose axons normally terminate in two glomeruli in topographically fixed locations in the olfactory bulb (P2-tau-LacZ mice Mombaerts *et al* 1996) we can determine whether the regenerating neurons are able to target their correct position in the olfactory bulb. To determine which cells and signalling molecules are important for regenerating neurons, we have performed immunohistochemistry using a range of antibodies against markers specific for various olfactory cell types and against axon guidance molecules.

POSTER 7: OLFACTORY GLOMERULAR TARGETING IN MICE LACKING OLFACTORY BULBS

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The olfactory sensory neurons which reside in the epithelium lining the nasal cavity each express one out of a possible thousand odorant receptors. Sensory neurons expressing specific odorant receptors typically project their axons to at least one topographically-fixed glomerular target in each olfactory bulb. Therefore, the bulb provides a spatial map whereby the quality of an olfactory stimulus is encoded by a distinct spatial pattern of activity defined by the specific combination of glomeruli activated by a given odorant. We are interested in the cellular and molecular cues involved in guiding these axons to their precise glomerular targets. Although we know that the odorant receptors themselves are involved, the role of the target olfactory bulb cells in providing cues for the guidance and mapping of these axons is unclear. We have investigated the role of olfactory bulb neurons in the targeting of axons in Gli3 deficient mice expressing LacZ under the control of the P2 odorant receptor gene. In Gli3 deficient mice olfactory bulbs fail to develop and instead primary olfactory axons form a fibrocellular mass lacking laminar organization. In the absence of normal postsynaptic targets, P2 axons continued to sort out and specifically converge onto discrete loci in the fibrocellular mass. Targeting of axons to loci was also observed for a larger subpopulation of olfactory axons expressing cell surface carbohydrates recognised by the plant lectin *Dolichos biflorus* agglutinin. In conclusion, although the precise topography of the olfactory spatial map appears to be dependent on cues derived from the olfactory bulb, olfactory axon subsets can sort out and converge in the absence of their normal synaptic partners.

POSTER 8: EXPRESSION OF THE INTERMEDIATE FILAMENT, NESTIN, IN THE MATURE OLFACTORY NEUROEPITHELIUM

Kharen Doyle, M Khan and A Cunningham (presented by Kerry Nichol)
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The nestin gene encodes for a predicted protein which forms a distinct sixth class of intermediate filament proteins (Lendahl *et al.*, 1990). Intermediate filaments are highly diverse cytoskeletal proteins which exhibit cell type specificity of expression. A growing body of evidence suggests that they may be involved in the complex cellular processes controlling alterations in cell morphology, adhesion and proliferation. Nestin has been widely used as a marker for proliferating neural progenitor cells in the developing nervous system. Recently, neural stem cells have been isolated from several regions of the adult CNS and these have all been characterised by nestin expression. Nestin expression is not exclusive to progenitor cells, however, as during embryogenesis nestin protein first appears in the endfeet that contact the basal lamina on the pial surface of the neural tube in a specialised class of cells known as radial glial cells (Marvin *et al.*, 1998). Later in development, nestin becomes abundant in radial processes which span the cell from ventricle to pia and these cells are known to play an important role in directing the migration of differentiating neurones (Rakic, 1971).

The peripheral olfactory neuroepithelium is a proliferative zone where neurogenesis and cell migration is ongoing in the adult and hence we were interested in whether it expressed nestin. Using immunohistochemistry, we examined nestin expression in the mature rat olfactory neuroepithelium and found it to be restricted to the basal compartment, the area where the neuronal progenitor cells reside. However, the pattern of immunoreactivity was consistent with expression of nestin by the endfeet and inferior processes of the sustentacular cells, rather than the adjacent basal cells. Using a bank of antibody markers, we confirmed nestin's pattern of distribution to be different to that of cytokeratin (a marker of horizontal basal cells), the GBC-1 antigen (used to mark globose basal cells), GAP43 (a marker of immature neurones), and carnosine (a marker of mature olfactory neurones). We compared its pattern of immunoreactivity to that of SUS-4 which is a marker of sustentacular cells. Following unilateral surgical bulbectomy, which causes a wave of neuronal cell death followed by proliferation of immature neurones, nestin immunoreactivity became slightly upregulated bilaterally and appeared to span the neuroepithelium from apical to basal regions in a pattern consistent with sustentacular cell expression. We propose that nestin may play a role in the migration of immature olfactory neurones on the scaffolding of sustentacular cells, in a manner analogous to its role in radial glial cells during embryonic development of the central nervous system. The upregulation in sustentacular cells postbulbectomy might reflect an intense requirement for mobility and remodelling in the regenerating neuroepithelium.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation and the NH&MRC of Australia

POSTER 9: LOOKING INSIDE OLFACTORY PROGENITOR NEUROSPHERES USING CONFOCAL MICROSCOPY

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The peripheral olfactory system is unique in maintaining a neuronal progenitor population which continues to proliferate and generate new neurones in the adult. The progenitor cell is believed to reside in the globose basal cell (GBC) population and it is likely that this stem cell might differ significantly from the comparatively dormant pools of stem cells found in other areas of CNS, eg. the subventricular zone.

Our laboratory is interested in olfactory neurogenesis and the processes of proliferation and neuronal differentiation. We have isolated olfactory stem cells *in vitro* using selective filtration and a modification of the olfactory neurone culture method previously described (Cunningham *et al*, 1999) and have generated neurospheres - large, multicellular, clusters of cells. Using fluorescent immunocytochemistry and a Leica TCS NT confocal system we have been able to look "inside" the neurospheres and begin to characterise the morphological and immunoreactive features of the stem cells. BrdU incorporation assays confirmed that the cells within the spheres were proliferating. The progenitor cells expressed immunoreactivity for nestin, the intermediate filament protein which has been used extensively to characterise proliferating neural stem cells from other sites. Many of the neurospheres labelled fairly homogeneously with GBC-1, an antibody marker for globose basal cells *in vivo*. In contrast, the cells in the neurospheres were negative for β-tubulin isotype 3 immunoreactivity, a marker for neurones. It was striking, however, that there was an association of tiny β-tubulin positive neurones with the neurospheres, and in many instances they could be observed migrating around the edges of the neurospheres. Double staining for β-tubulin and S100 confirmed that immature neurones apparently migrated away from the spheres in close association with an olfactory glial cell. This is the first description of robust proliferation of olfactory progenitor cells *in vitro* and the generation of olfactory progenitor neurospheres. Our current studies are focussed on understanding the unique characteristics and trophic factor requirements of the olfactory neuronal stem cell.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation and the NHMRC of Australia

POSTER 10: GROWTH FACTOR CONTROL OF NEUROGENESIS IN ADULT OLFACTORY EPITHELIUM

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Neurones are produced throughout adult life in the olfactory epithelium, even in humans, making this tissue a convenient and accessible model for studying the autocrine and paracrine factors which regulate proliferation, differentiation and cell death during neurogenesis. We investigated the effects of growth factors in serum-free media in olfactory epithelium cultures comprising only basal cells and supporting cells. With this well-defined culture system we examined the effects of several growth factors on proliferation, differentiation and survival of the basal cells and neurons. Three growth factors exerted separate effects on cells at different stages of the neuronal lineage: 1) basic fibroblast growth factor stimulated proliferation of globose basal cells, the neuronal precursors, 2) transforming growth factor-β2 induced these precursors to differentiate into neurons and 3) platelet-derived growth factor promoted survival of these immature neurons. We conclude that basic fibroblast growth factor, transforming growth factor-β2 and platelet-derived growth factor act sequentially on neuronal precursors and immature neurons during olfactory neurogenesis and may act similarly during neurogenesis in other parts of the nervous system.

POSTER 11: KINASE CASCADES INVOLVED IN OLFACTORY RECEPTOR NEURONAL DEVELOPMENT

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In this study, differential display was used to identify gene transcripts which were up-regulated during the differentiation of a clonal olfactory neuronal cell line (OLF442) by serum deprivation for 48 hr. We identified focal adhesion kinase (FAK) and mammalian STE20p-like kinase (MST) up-regulation by this method and link the expression of these proteins to the maturation of olfactory receptor neurons.

FAK is a non-receptor protein tyrosine kinase, and as such plays a central role in morphogenetic processes that occur during development. Here we demonstrate that FAK and FRNK (FAK related non-kinase) protein levels are upregulated and that FRNK is not a caspase-3 cleavage product of FAK but most likely an alternatively spliced FAK gene product. The primary role of FRNK expression is to act as an inhibitor of FAK by transiently blocking the formation of focal adhesions and reducing the phosphorylation of both FAK and other proteins bound in the focal adhesion complex[1]. How this process effects the ERK cascade and OLF442 differentiation is currently being investigated.

Additionally, mouse MST1/KRS2 and MST2/KRS1 mRNAs have been sequenced for the first time. MST1/2 are serine/threonine protein kinases that have been shown to be activated by a subset of stress conditions or apoptotic agents but are not activated by commonly used mitogenic stimuli[2]. Thus, the biological role of MST remains elusive as it is activated by several different agents. However it appears that it may function at an early step in the phosphorylation events that are specific responses to particular forms of stress. It is important to note that stress pathways have been shown to mediate effects in diverse biological processes including apoptosis, development and adaptation to environmental changes. We are investigating the role that MST plays during neuronal differentiation and the signalling cascades that traffic extracellular signals to the nucleus.

1. Richardson, A. and Parsons, T. (1996) Nature 380:538-540.
2. Creasy, C.L. and Chernoff, J. (1995) Gene 167:303-306.

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POSTER 12: NEURITE EXTENSION IS INDUCED BY INSULIN-LIKE GROWTH FACTOR I IN ADULT MOUSE OLFACTORY EPITHELIUM EXPLANT CULTURE.

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Insulin-Like Growth Factor I (IGF-I) regulates neuronal differentiation and induces neurite extension in adult subventricular zone stem cells (Brooker *et al.*, 2000). It also stimulates neuronal precursor proliferation when infused into the nose (Pixley *et al.*, 1998). The aim of the current study is to define the role of IGF-I in olfactory neurogenesis. Using immunohistochemistry on frozen sections of adult mouse olfactory epithelium, we first localized IGF-IRα on olfactory neurons and precursor cells. Then using *in vitro* techniques, olfactory epithelium explants were grown in DMEM-ITS in the presence of IGF-I, α-IGF-I, α-IGF-IRα or media alone. Cultures were assessed for total neuron number and neurite length. Neurons were identified by a bipolar morphology and β-tubulin expression. We observed: 1) the optimal differentiating effect of IGF-I occurred at a concentration of 5 ng/ml, 2) neurite extension was increased 2-fold by IGF-I, 3) antibodies to IGF-I blocked this effect and, 4) the IGF-I effect on neurite extension was facilitated by a growth stimulatory monoclonal antibody to IGF-IRα. IGF-I has not yet been reported to be present in the olfactory epithelium, but as it is produced in the olfactory bulb (Garcia-Segura *et al.*, 1991), found in the olfactory mucus (Federico *et al.*, 1999) and its receptor is present on olfactory precursors (Pixley *et al.*, 1998). We conclude that IGF-I may act as a maturation factor to induce neurite outgrowth in immature olfactory neurons.

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POSTER 13: OLFACTORY ENSHEATHING CELL TRANSPLANTS IN PERIPHERAL NERVE REPAIR

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Repair of a facial nerve gap presents a technical challenge to the surgeon and, at best, a moderate degree of recovery for the patient. It is usually done with a nerve("cable") graft, generally a sensory nerve and modified to fit the dimensions of the facial nerve gap. This technique is associated with donor site morbidity, anaesthesia and neuroma at the site of donation, and a high degree of technical difficulty in suturing the nerve graft into the gap. Alternatives to nerve grafting include the use of autologous muscle or vein, biodegradable polymer tubes, silicone tubes, collagenous tubes, and fibrin glue, sometimes including Schwann cells or growth factors. Numerous studies on these conduits and substrates have produced mixed results thus far. Recently olfactory ensheathing cells, isolated from the olfactory bulbs, were grafted into silicone tubes to assist axon regeneration across a 12-15 mm gap in the rat sciatic nerve (Verdu *et al* 1999). These are unique glia which surround the olfactory nerve with properties of both Schwann cells and astrocytes. Olfactory ensheathing cell grafts from the olfactory bulbs are surgically difficult to obtain and an inappropriate source for autologous grafting. In contrast, olfactory ensheathing cells can be relatively easily taken from the olfactory mucosa in the nose. The aim of this study is to investigate whether nasal olfactory ensheathing cells can stimulate axon regeneration in severed peripheral nerve. Rats were anesthetised with halothane and the sciatic nerve exposed from the dorsal side. The nerve was sectioned and a 15 mm gap created. The gap was bridged with a silicone tube sutured to the epineurium of the cut ends of the nerve. Olfactory ensheathing cells isolated from donor animals were grafted into the silicone tube and/or into the nerve stumps; a control group received no cells. 12 weeks later the animals were assessed for their responses to pin prick stimulus then re-anesthetised. Conduction across the nerve gap was assessed electrophysiologically using the evoked electromyograph and the compound action potential. At the end of these experiments, the rats were euthanised. The sciatic nerve was then removed, fixed and sectioned for histological analysis of axonal regeneration.

Verdu, E *et al.* (1999) Olfactory bulb ensheathing cells enhance peripheral nerve regeneration. Neuroreport **10**: 1097-101.

POSTER 14: TREATMENT OF AIRWAY INFLAMMATION WITH MACROLIDE ANTIBIOTICS

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Introduction Macrolides have been used for decades as an important chemoterapeutic in the treatment of infectious diseases. The last ten years has also seen an increasing interest in the interaction between macrolide antibiotics and the immune system. Recently, clinical reports have shown that long-term, low-dose treatment of chronic sinusitis with macrolides is effective. However, the mechanisms behind the effect have not been clarified. The overall aim of the present project is to investigate the anti-inflammatory effect of macrolide treatment from the molecular level to its impact on the quality of life of patients with chronic airway disease.

Results A preliminary clinical study (in collaboration with Dr Sven Lindberg, Lund University, Sweden) 14 patients with chronic sinusitis not responding to surgery or steroids has shown that 11 patients responded to erythromycin 500 mg daily. Saccharine transport time was improved from 51±9.6 min to 41±9.9 min. The symptoms of nasal congestion was markedly reduced $p<0.001$, as well as the amount of nasal secretions, $p<0.01$. and the viscosity of the secretions, $p<0.05$. Overall the patien'ts quality of life was greatly improved.

A cell culture system for nasal polyps has been developed. In the cell cultures we will compare the effects of erythromycin and prednisone on cytokines expressed in polyp tissue. Preliminary results show that both erythromycin and prednisone reduced the expression of Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF).

Conclusion There is increasing evidence that macrolide antibiotics has immuno-modulating properties and may prove beneficial in the treatment of various diseases causing chronic airway inflammation, such as chronic sinusitis, nasal polyps, asthma, bronchiectasis and cystic fibrosis.