

Introduction:

The different research groups within the Save Sight Institute approach the problems of blindness from a clinical, laboratory and epidemiological perspective. Integrating with those exploring digital media, education and service, the groups work in close collaboration and are listed alphabetically.

Table of Contents

Introduction:	i
Table of Contents:	i
Clinical Epidemiology Research Group	i
Cornea Research Group	ii
Digital Media Research Group	iv
Electrophysiology & Glaucoma Research Group	v
Lens Research Group	vi
Retinal Research Groups	ix
Retinal Development, Aging & Eye Cancer Research Group	ix
Retinal Vascular Biology Research Group	xiii
Retinal Dystrophy Research Group	xv



Community, Clinic and Laboratory Research
– a powerful interface

Clinical Epidemiology Research Group

This group's research focuses on the causes, impacts, frequency and risk factors for common age-related eye diseases such as AMD, cataract, glaucoma, diabetic eye disease and other vascular problems affecting the eye. Visual impairment caused by these diseases, together with under-corrected refractive error, exerts a substantial impact on the independence, aged care service utilization and quality of life of older Australians. It also contributes to premature nursing home admission and mortality. Because of increasing life expectancy, age-related sensory loss now represents one of our most important medical challenges. Generation of accurate data on the prevalence, incidence and risk factors is a critical step in responding to this challenge.

Group Members

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Ronald Klein, MD MPH, *Research Associate. (USA)*

Karen Cruickshanks, BSc MPH PhD, *Research Associate (USA)*

Research Activity

The Blue Mountains Eye Study (BMES) was the first large population-based assessment of visual impairment and common eye diseases in a representative older Australian community. This project has now provided reliable Australian estimates of the frequency (prevalence) of eye disease, refractive error and visual impairment, and has assessed their principal risk factors.

The study also provided the first large-scale Australian data on the frequency and risk factors for age-related hearing loss through a separate project, the Blue Mountains Hearing Study, conducted in collaboration with members of the Department of Linguistics at Macquarie University.

We also collaborated with researchers from the University of Wisconsin-Madison, USA, to align key measures of eye disease and hearing loss with a similar US study, conducted in Beaver Dam, Wisconsin. A first paper, pooling data on age-related maculopathy from our study with data from the US and the Netherlands, is now in press.

BMES has extensively studied the diet of this older population to assess nutrition and diet-eye disease links. Longitudinal assessments of fractures, vascular events and mortality in this community, including links with sensory loss, are also underway.

Projects in 2001

Blue Mountains Study:

- Completing a number of phases of the main study.
- Reporting data from the 5-year examinations of participants initially seen during 1992-4 & reviewed during 1997-9.
- Reporting data from the Hearing Study.
- Completing an extension project involving assessing new residents to

the area and those who had recently turned 50.

Cataract & Memory Study: Designed a NHMRC-funded trial aimed at determining whether there are cognitive benefits from cataract surgery. It is being conducted in association with Prince of Wales Medical Research Institute and is recruiting patients from both Prince of Wales and Westmead Hospitals.

Retinal Vein Bypass Study: This NHMRC-funded collaborative trial across 3 sites (Perth, Sydney & Melbourne) aims to establish whether a laser-induced artery-vein bypass will benefit people who develop central retinal vein occlusion, a largely untreatable cause of vision loss in the older age group.

Highlights in 2001

The group was awarded 2 new NHMRC grants for 2001-3, titled: "Retinal vascular signs to predict stroke" & "Mobile phone effects on vision & hearing" plus an ORIA grant for 2001, titled: "Risk factors for incident age-related maculopathy: data pooling across 3 continents". Funding for these 3 grants totals \$761,000.

Cornea Research Group

Corneal research in the Institute remains focused on investigating the pathogenesis of infectious keratitis and keratoconus, which remain important causes of corneal blindness both within our Australian communities and worldwide.



Corneas are stored in specially prepared media

Group Members

Kathy McClellan, MBBS PhD FRACO FRACS, *Unit Head*

Simon Collier, BSc PhD, *Postdoctoral Scientist*

Raj Devasahayam, BAppSc, *Eye Bank Laboratory Manager*

Research Activity

The cornea is that part of the anterior eye wall, modified for light transmission via the lens to the retina. The cornea contributes significant refractive power to the eye and must remain transparent and regularly shaped to fulfill these functions.

The corneal surface is covered by stratified non-keratinising epithelium, which is uniquely placed in an external mucosal environment. This location must be maintained by an adequate circulation of tears from the lacrimal gland as well as by a healthy conjunctiva and eyelids that can close completely and facilitate tear drainage via the lacrimal canalicular system. In addition the cornea must have an intact sensory nerve supply to activate these mucosal defences and maintain an intact surface epithelium.

We have shown that older age predisposes the cornea to more severe infections and have defined those enzymes that are activated within the cornea in keratoconus. These findings will provide a basis for the development of better treatments for these blinding eye conditions.

Projects in 2001

Infectious Keratitis in the Older Adult: McClellan. An understanding of the mechanisms of corneal infection remains a priority if blindness from this condition is to be prevented. In 2001 Dr Kathy McClellan continued her research in the Save Sight Institute to determine risk factors for development of corneal infection, monitor causative organisms and antibiotic sensitivities and review the length of hospital stay required for optimal treatment of corneal infections in older patients.

A retrospective study was conducted of patients 55 years of age or older admitted to Sydney Eye Hospital over a 5-year interval with a diagnosis of keratitis, corneal ulcer or corneal abscess. There were 211 admissions for infectious keratitis of which medical records were available for 196 patients (100 female and 96 male). Important ocular risk factors for infectious keratitis included previous eye surgery (42%), previous cataract surgery (40%), pseudophakia (36%), glaucoma (21%), corneal graft (19%), bullous keratopathy (14%), previous herpes simplex keratitis

(14%) and use of topical steroids (11%). Concurrent systemic diseases included diabetes mellitus (13%), systemic immunosuppression (6.6%), alcohol consumption > 20 g/week (5.6%) and rheumatoid arthritis (5.6%).

Corneal scrapings for culture and sensitivity testing were done for 133 (68%) patients with positive cultures returned from 85 (64%). The most common bacterial isolates were coagulase-negative staphylococci (41%), other staphylococci including *Staphylococcus aureus* (24%) and *Pseudomonas* sp (8.2%). All organisms were sensitive to at least one of the commonly used topical fortified antibiotics (cephalothin or gentamicin). Medical management with frequent topical fortified antibiotic eye drops was effective in saving the eyes of two thirds of these patients. Additional surgical treatment was required by 35% of patients, including corneal transplantation in 11%.

This study shows that any loss of the corneal epithelial barrier function constitutes an important risk factor for the development of infective keratitis in older patients. It gives clinicians guidance in the management of the at-risk older patient, indicating that careful post-operative follow up, timely removal of corneal sutures and management of other conditions that may cause instability of the corneal epithelium, will prevent the development of corneal infection. The work also confirms that the bacteria that commonly cause corneal infections remain sensitive to available antibiotics. Widespread antibiotic resistance does not currently threaten management of surface eye infections, in contrast to systemic infectious diseases.

Mechanisms of Development of Keratoconus: Collier. Keratoconus is a bilateral corneal thinning disorder, characterised by progressive corneal thinning, protrusion and scarring. It generally affects young adults and has an incidence of about 1 in 2000 in the general population. Keratoconus is most commonly an isolated disorder, but may be associated with a variety of factors including atopy, connective tissue disease, Down's syndrome, contact lens wear and eye rubbing. If the disease progresses beyond the point where the refractive errors can be corrected with contact lenses, corneal transplantation is the only treatment. Keratoconus is the most common indication for penetrating keratoplasty.

The thinning and ectasia of the cornea in keratoconus are suggestive of a degraded extracellular matrix and research has concentrated on the possible role of the matrix metalloproteinase (MMP) family of enzymes in the corneal degradation in keratoconus. Studies attempting to determine whether keratoconus corneas contain more MMPs, or more activated MMPs than normals, have yielded conflicting results. In normal corneas, MMPs are thought to be expressed only at low levels or not at all, but they can be induced by inflammatory stimuli resulting from injury or disease, with dramatic and devastating consequences in the case, eg., of corneal melting. Present studies in the SSI laboratory, using normal corneas, are investigating the expression of most known MMPs, using RT-PCR, in *ex vivo* human corneal epithelial cells, and in primary cultures of keratocytes. Surprisingly, it was found that mRNA for only MMP-14, MT1-MMP, was expressed in 13 of 13 corneal epithelia. Next most prominent were MMPs 10, 1, 2, 3, and 9, in that order. Historically, studies have focused on the gelatinases, MMP-2 and -9. *In situ* hybridisation was used to localise MT1-MMP mRNA mainly to the basal epithelial cells, and protein expression was confirmed in the same locations by immunohistochemistry. Thus MT1-MMP might be involved in the normal maintenance and turnover of the extracellular matrix components of the basement membrane.

Digital Media Research Group

Research in this group is focused on exploiting the potential of the digital media, information technology (IT) and the communications revolution to –

- Create and implement state of the art outreach ophthalmic services for remote and rural communities.
- Develop cutting edge ophthalmic teaching services to provide excellence in education of future eye doctors interfacing Rural Clinical School in Dubbo and the University of Sydney.
- Empower the community through the internet by providing interactive access to information about eye care and lifestyle choices which can help prevent eye disease.

Group Members

Frank Billson, FRACO FRACS FACS, *Unit Head*

Nitin Verma, MD (Oph) FRACO

Ivan Ho, MBBS

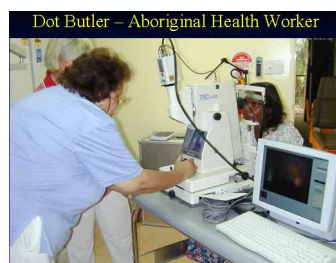
Max Conway, PhD FRACO FRACS

Research Activity

- **Teleophthalmology Initiative:** The challenge of providing specialist eye care services outside the metropolitan centres of Australia is a significant one. This group is working to develop more effective ways of screening for eye disease in remote and rural areas. A digitally equipped mobile screening unit is being trialed in NT as a model for a similar initiative in Dubbo and remote regions of Australia.
- **Virtual Teaching Initiatives:** The unit is researching initiatives to provide distance learning services and developing innovative ways for students to have clinical trial experience in a *virtual* arena before engaging in patient practice.
- **Interactive Community Services:** As digital media increasingly become a delivery tool for community information and access to services, this group is focusing on improving ways of delivering Sight Saving Information to the community and developing simple interactive eye services to be accessed via e-media.

Projects in 2001

Tele-ophthalmic Diabetic Eye Screening Program:



Aboriginal Health worker Dot Butler using digital media to screen for Diabetic Retinopathy in NT.

In 2001, postgraduate student Ivan Ho having completed the implementation and cost-effective analysis of the Tele-ophthalmic Diabetic Eye Screening Program in the remote Aboriginal communities of the Top End of Northern Territory will run the training program at Sydney Eye Hospital.

This program centres around a Mobile Diabetic Eye Screening Unit comprised of an ophthalmically trained Aboriginal Health Worker and Registered Nurse and equipped with a Digital Non-Mydriatic Fundus Camera with archiving and Tele-ophthalmic capabilities. The capturing and transmitting capability of the unit allowed all retinal images to be electronically sent to Darwin for assessment of the need for specialist treatment as well as confirmation of diagnosis by ophthalmologists based there.

The team conducted a detailed accuracy analysis which demonstrated that the Digital Fundus Imaging system is comparable to conventional 35mm Slide Fundus Photography for screening and grading diabetic retinopathy. The analysis confirmed that the digital technology is highly sensitive and specific in detecting sight threatening retinopathies.

The Mobile Tele-Ophthalmic Team covered over 20,000km to provide diabetic eye screening services in 19 out of 32 remote Top End communities. It reviewed 370 out of 584 diabetic patients in remote communities and significantly improved the diabetic eye screening coverage rates from 44% to 76% across the population, with several communities achieving 100% screening coverage rate.

The Mobile Diabetic Eye Screening Team have developed the most updated and extensive database on all the diabetics in the Rural Aboriginal communities of the Top End. They are now expanding and providing a more comprehensive and *holistic* service to the communities by integrating the database with the Territory Health Service Community Diabetic Register.

The mobile eye team now works in close partnership with Nutritionists, Diabetic Educators, Endocrinologists, General Surgeons and Renal Physicians, in the form of Specialist Outreach Services, to provide comprehensive diabetic care for the patients and establish an efficient screening and primary referral unit for specialist diabetic treatment.

From The data collected to date, Tele-Ophthalmology holds promise to be the future in delivery of Ophthalmic Services to rural and remote Australia. With completion of the collection of data from all 32 communities in the study in the coming

year it is hoped not only to further confirm this, but also to demonstrate the cost effectiveness of the program.

Virtual Ophthalmology Clinic: 2001 saw further education trials using the unit's virtual ophthalmology program which allows medical students to gain skills in history taking, making use of "virtual" clinical patients. This computer-based program mimics real interactions with patients, in that students have complete control of the order in which they ask questions and gather information, to make a diagnosis before an examination. The information can then be emailed to the supervisors and mentors of the program and when a provisional diagnosis is made, the student is then allowed to continue their examination and suggest investigations. The program has the potential to be delivered via the Internet and further work has been done towards achieving this. The strength of the program is that it allows the candidate to develop skills in interviewing and forming a diagnosis, sparing both the patient and the student the stress of learning these skills in a live situation. This is seen as an adjunct or prelude to patient contact for the students.

Electrophysiology & Glaucoma Research Group

Glaucoma causes painless irreversible peripheral vision loss and is a major cause of blindness in developed countries. This group has developed a technique for objective perimetry that assesses peripheral vision objectively and has the potential to detect glaucoma at an earlier stage than previously possible. Further research will investigate its application to optic neuritis and testing visual fields in children.

Group Members

Alexander Klistorner, BMed PhD, *Unit Co-Head*

Stuart Graham, FRACO, *Unit Co-Head*

Frank Billson, FRACO, FRACS

John Grigg, FRACO, FRACS

Lisa Feldman, *Electrophysiology Technician*

Chandra Balanchandran, BscMed, MBBS, *Postgraduate Student*



Objective mapping of the visual field using technology developed by SSI.

Research Activity

The electrophysiology group has been further developing techniques for detecting glaucoma and other causes of visual loss including optic neuritis (multiple sclerosis) and optic gliomas in children using the multifocal pattern VEP (MPVEP). This involves the use of an electrode array oriented around the occipital region of the skull recording multiple channels of data from the visual cortex, and utilises a new stimulating and recording algorithm, to test all parts of the visual field simultaneously.

The MPVEP is able to objectively identify the extent of glaucomatous damage and may be able to detect changes before subjective field loss occurs. Dr Balachandran has tested a large number of normal children and has shown its utility in detecting field loss in several cases with optic gliomas.

Highlights in 2001

ObjectiVision Pty LTD (University and SSI are major shareholders) has been joined by a major commercial partner MedCorp. A commercial objective perimeter the AccuMap, for the detection of the visual field defects has just been released.

The AccuMap System recently won 2 Australian Design Awards. Alex Klistorner & Stuart Graham patented the use of virtual reality goggles as a future method of applying this test.

International Glaucoma Society research award – top 10 finalists.

Lens Research Group



Research in this group is directed at identifying molecules and mechanisms that govern the behaviour of lens cells in health and disease.

Our studies have identified molecules that play key roles in both normal and pathological lens development. Currently we are working to gain a better understanding of how these molecules are regulated in the eye. This is fundamental to identifying new therapeutics for retarding or preventing cataract, one of the most common (and costly) diseases of ageing.

Group Members

John McAvoy, BSc PhD, *Unit Co-Head*
 Frank Lovicu, BSc PhD, *Unit Co-Head*
 Robbert de longh, BSc PhD, *Unit Co-Head*
 Richard Stump, BSc PhD, *Lab Manager*
 Angela Hales, BSc PhD, *Postdoctoral Scientist*
 Oonagh Lynch, BSc PhD, *Postdoctoral Scientist*
 Heidi Brown, BSc PhD, *Postdoctoral Scientist*
 Jessica Boros, BSc, *Research Assistant*
 Sally Hawthorn, *Technical Officer*
 Tatiana von Bahr, *Research Associate (University of Uppsala)*
 Michael O'Connor, BSc, *Postgraduate Student*
 Elizabeth Wederell, BSc, *Postgraduate Student*
 Sharyn Ang, BSc, *Postgraduate Student*
 Yongjuan Chen, BSc, *Postgraduate Student*
 Nagalaxmi Iyengar, *Honours Student*

Research Activity

The lens transmits and focuses light onto the retina. To do this it needs to be crystal clear and to have appropriate refractive properties. This depends on the development and maintenance of highly ordered cellular architecture.

The lens consists of two forms of cells encapsulated within a basement membrane; (i) elongated fibre cells, grow to several

millimetres in length, and are precisely aligned to form a regularly packed spheroidal mass, and (ii) cuboidal epithelial cells form a single-layered sheet that covers the anterior surface of the fibres.

Whilst the fibre cells make up the bulk of the lens and mostly determine its optical properties, epithelial cells play a key role in maintaining an appropriate physiological environment within the lens. In addition, the epithelium contains the stem cells that proliferate, migrate and differentiate into the new fibres that are progressively added to the fibre mass throughout life.

We have focussed our attention on growth factors because of their importance in regulating cell fates in developmental systems. Our studies have identified members of the FGF growth factor family as inducers of lens cell proliferation, migration & differentiation; responses that are induced in a progressive dose-dependent manner. We have proposed that an anterior-posterior gradient of FGF determines lens polarity and growth patterns. A major thrust of research activity in our laboratory is aimed at testing this hypothesis.

Insights into the molecular basis of the major lens pathology, cataract, have also arisen from our growth factor studies. We have shown that members of the transforming growth factor beta (TGF β) family, induce aberrant growth and differentiation of lens cells. This progressively leads to disruption of normal cellular architecture and opacification of the lens.

Cataract is the most common cause of blindness in the world today. Although surgery is generally effective, in many countries it cannot keep pace with the growing demand. Moreover, complications such as aberrant growth and differentiation of lens cells left behind after cataract surgery, require further treatment and add to the cost of cataract management. Because of its clinical significance it is vital to understand how TGF β is regulated in the eye and how it induces cataractous effects on the lens. This information is fundamental to understanding the molecular basis of cataract and devising strategies for prevention.

Projects in 2001

FGF signalling: Lovicu, Boros. These studies were aimed at elucidating how FGF induces different cellular responses. We investigated the intracellular signalling cascades that are activated by FGF. We showed that the ERK1/2 (MAPK1/2) signalling cascade is involved in both proliferation and differentiation. Inhibition of ERK1/2 blocks cell proliferation; however, it blocks only the morphological (elongation), not the molecular (beta-and gamma-crystallin gene expression) aspects of differentiation. This indicates that fibre differentiation depends on activation of several intracellular signalling cascades.

PDGF/IGF signalling: Lovicu, Lynch, Iyengar. IGF/insulin and PDGF growth factor families can induce lens epithelial cells to undergo cell division. To determine if IGF and PDGF utilise the same signalling pathway(s) as FGF, we examined the role of ERK1/2 signalling in IGF- and PDGF-induced lens cell proliferation. Similar to FGF, both IGF and PDGF can induce lens cell proliferation, in an ERK-dependent fashion. However, PDGF activates ERK within 10 minutes compared to 20 minutes for IGF. Differences in the temporal activation of lens cell proliferation by IGF and PDGF may be due to differences in the mode by which these growth factors activate ERK1/2. Overall, our results support an important role for ERK signalling in growth factor-induced lens cell proliferation.

FGF receptor 3 and fibre differentiation: de Longh, Chen, Deng, (National Institutes of Health, Bethesda, USA). The importance for normal fibre differentiation of FGF signalling through the FGFR3 receptor was indicated from results of studies on *fgfr3* null mice. These mice have a null mutation of the *fgfr3* gene and show abnormalities in fibre maturation. The denucleation process that occurs in the lens cortex is disturbed and the elimination of nuclei appears to be delayed compared with the wild type. TUNEL-positive pyknotic nuclei are retained deep into the lens cortex. These results indicate the FGFR3 signalling plays an important role in fibre maturation in the lens cortex.

BMP/Activin receptors and lens development: de Longh, Chen. The TGF β superfamily includes TGF β , BMPs and activins/inhibins. Our studies have shown that signalling via TGF β receptors is important for lens fibre differentiation. BMPs have also been shown to play a role in lens induction. In this study we investigated the expression and signalling potential of BMP and activin receptors during lens

development. The results suggest that during lens development there is signalling by several members of the TGF β superfamily. Up-regulation of the type I BMP receptor, Alk3, in fibres bereft of TGF β receptor signalling, is suggestive of a compensatory mechanism and further reinforces the notion that signalling by TGF β family members is required for terminal fiber differentiation

Microarray analysis of lenses of transgenic mice deficient in TGF β signalling: *de longh, McAvoy*

Previously we showed that expression of dominant-negative forms of either type I or type II TGF β receptors in the lens, so as to inhibit TGF β signalling, resulted in degeneration of cortical and nuclear lens fibres. We used Gene chip analysis to investigate the effects of inhibiting TGF β signalling on gene expression in lenses of these transgenic mice. The results indicate that during terminal differentiation of lens fibres TGF β signalling plays a major role in modulating expression of cytoskeletal proteins. Disruption of TGF β signalling in lens fibres results in disruption of the cytoskeleton, intracellular transport processes and lipid metabolism and activation of apoptotic pathways.

Altered integrin expression during TGF β -induced cataract formation: *Wederell, Brown, Lovicu, de longh*. Previous studies with cultured rat lenses and transgenic mice have shown that TGF β -induced cataract involves an epithelial-mesenchymal transition (EMT), resulting in the formation of subcapsular fibrotic plaques. This EMT involves dramatic changes in cell morphology, gene expression and the aberrant accumulation of extracellular matrix (ECM) proteins (e.g. fibronectin, collagen I and III and tenascin). To further characterize this TGF β -induced EMT, we examined the expression of integrin subunits that bind these matrix components, during the formation of cataractous plaques. Consistent with the aberrant expression of ECM molecules in TGF β -induced anterior subcapsular plaques (fibronectin, collagen I and III), this study indicates that lens epithelial cells undergo major changes in integrin gene expression during this cataractous process.

Lens regeneration: *O'Connor, Lovicu, McAvoy*. FGF is a potent inducer of fibre differentiation in lens epithelial explants. However, elongating cells in FGF-treated explants do not show the same degree of alignment with their neighbours as do

elongating fibres in vivo. There is growing evidence to suggest that other, as yet unidentified factors, are involved in coordinating the elongation and alignment of fibre cells. Recent developments in tissue culture techniques pioneered by our laboratory are enabling a more rigorous investigation of the intraocular fluids, particularly the vitreous humour, in search of these factors.

Crim 1 expression: *Lovicu, little (University of Queensland), McAvoy*. We localised a new gene, *Crim1* (initially identified by colleagues at University of Queensland), during eye morphogenesis. *Crim1* is particularly strongly expressed in the lens epithelium. During postnatal growth, expression is reduced in all ocular tissues and eventually it is only expressed in the lens. Investigating the functional significance of *Crim1* expression in the lens forms the basis of an ongoing collaborative study with colleagues at the University of Queensland.

Wnt signalling: *Stump, von Bahr, Ang, Pinson (University of California, Berkeley, USA), Lovicu, de longh McAvoy*. We studied the expression of members of the Wnt growth factor family and its receptors, the Frizzleds (Fzs). Wnts are commonly involved in regulating expression of molecules involved in cell proliferation, communication and adhesion. We have shown that several Wnts and Frzs are expressed in the lens. A mouse mutant that is deficient in a co-factor required for Wnt signaling does not develop a lens epithelium. This supports our hypothesis that Wnt signaling plays a key role in the formation and maintenance of the lens epithelial sheet.

Oestrogen protects against TGF β -induced cataract: *Hales, von Bahr, Davis (Cedars-Sinai Medical Centre, USA)*. We previously showed that oestrogen protects the lens from the cataractous effects of TGF β . To gain a more detailed understanding of the role of oestrogen in the lens, we initiated studies of adult mice with a mutation in the oestrogen receptor. Histological analysis showed that the lens cytoarchitecture is severely disrupted in these mutant mice. This is consistent with a role for oestrogen in lens cell differentiation and maintenance.

Highlights in 2001

Frank Lovicu took up the Sydney Foundation for Medical Research Lectureship, held jointly

in the SSI and the Dept of Anatomy and Histology.

John McAvoy and Angela Hales organized the third meeting of the NSW Society for Cell and Developmental Biology held at the Save Sight Institute in March.

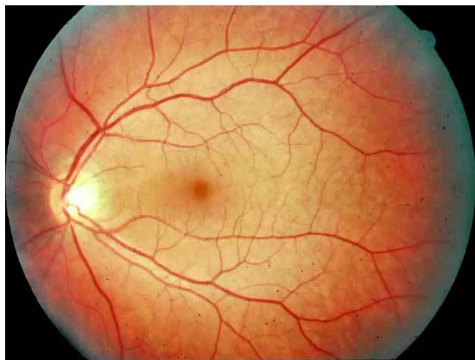
Rob de longh set up a new DNA microarray workstation in the SSI.

Elizabeth Wederell was awarded the prize for Best Oral presentation at the IBR 1st Annual Postgraduate/Postdoctoral Conference, University of Sydney.

Oonagh Lynch was awarded the prize for Best poster presentation at the IBR 1st Annual Postgraduate/Postdoctoral Conference, University of Sydney.

Retinal Research Groups

The retina, which detects light like the film of a camera, is the most sophisticated and delicate part of the eye. Retinal diseases, such as diabetic retinopathy and age-related macular degeneration, are now the commonest causes of untreatable blindness in Australia.



The SSI has 3 research groups working on various aspects of the retinal biology and pathology –

- 1) Retinal Development, Aging & Cancer
- 2) Retinal Vascular System
- 3) Retinal cell death and survival

The groups work in close association – sharing information, insights and frequently joint publications.

Age-related Macular Degeneration is a common thread of research in terms of:

- basic biology, development and changes in associated vasculature.
- the importance of therapy for sub-retinal new vessels in the blinding stage of this disease.
- the fact that genetic factors may be important in susceptibility to the disease.

Retinal Development, Aging & Eye Cancer Research Group

Research aims to shed light in 3 individual areas and explore the connections between them –

- Retinal Development
- Age-related Macular Degeneration
- Eye Cancers

Group Members

Jan Provis, BSc PhD, *Unit Co-Head*
Philip Penfold, BSc PhD, *Unit Co-Head*
Michele Madigan, BSc PhD, *Unit Co-Head*
Diana van Driel, BSc, *Senior Research Assist.*
Li Wen, Bmed, MMed, *Research Assistant*
Riccardo Natoli, BSc (Hons), *Research Assistant*
Elisa Cornish, BSc, *Postgraduate Student*
Trent Sandercoe, BSc, *Postgraduate Student*
Pierre Georges, BSc, *Postgraduate Student*
Van Pham, MD, *Postgraduate Student*
Kathy Wu, MBBS, *Postgraduate Student*
James Wong, MBBS, *Postgraduate Student*

Members advising on clinical aspects

Frank Billson, FRACO, FRACS, FACS
Max Conway, PhD, FRACO, FRACS
Matthew Healey, BSc, *Honours Student*
Andrew Chang, FRACO
Meidong Zhu, PhD, *Research Fellow*

Research Activity

Retinal Development:

The human 'macula' is a region of the retina that includes several specialisations not present in non-primate retina and which together enable us to see detail. Understanding the development and aging of the macula and how this unique structure makes humans vulnerable to degenerative disease is the principle aim of our research into development and aging.

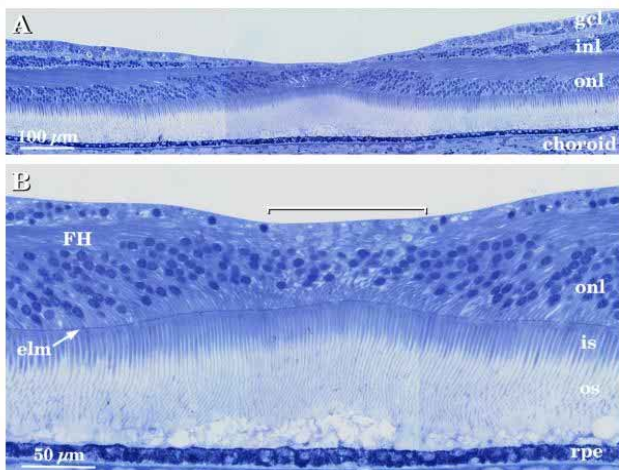
The fovea is at the geometric center of the macula and is identifiable by a number of structural modifications including: (1) an absence of a direct blood supply from the retinal circulation, (2) the presence of a pit which thins the retina locally, (3) a focal concentration of a class of colour sensitive photoreceptors ('cones'), and (4) the inclusion of specialized circuitry which conserves the electrical responses of individual cones.

Few groups internationally are applying modern molecular tools to the investigation of primate retinal development and human retinal disease. During 2001 we have developed new approaches to investigate the biology of the macula. Our focus is to identify unique molecular profiles associated with the unique anatomy and physiology of the macula and fovea.

Projects in 2001:

Growth Factors in Retinal Development:

Cornish, Hales, Natoli, Provis. Previously we found that the blood vessel-free area in which the fovea forms is established before structural modification of the retina commences (see Provis et al., 2000). Recent findings suggest that Fibroblast Growth Factor-2 (FGF-2) and two of its receptors (R1 & R4) are 'switched off' in cone photoreceptors while the retina structurally reorganizes at the fovea. Our present aim is to identify the factors that bind these receptors in the retina and to establish an organ culture technique to test their effects.



High power microscopic view of the foveal region of the adult human fovea – the part of the retina that enables us to resolve fine details.

Blood Vessel Growth During Foveal

Development: *Sandercoe, Hendrickson (University of Washington, Seattle), Penfold, Provis.* Our previous studies showed that a factor expressed at the macula prevents blood vessels growing into the foveal region before the foveal depression forms. Recently we have found that before the fovea forms, cells occupying the avascular area are hypoxic and express a factor, vascular endothelial growth factor, that normally would promote blood vessel growth. Despite this expression the macula remains avascular, except in pathology like macular degeneration. The findings suggest that loss of the (yet unidentified) anti-angiogenic factor expressed at the macula may result in formation of new blood vessels at the macula.

Metabolic Activity in the Developing Primate

Retina: *Georges, Madigan, Provis.* Labeling of a range of transporter proteins and enzymes expressed by glial cells in the retina is used as an indication for the onset of transmitter mediated cell-cell signalling in the retina. Results show that while synapses form very early in the retina, and the enzymes needed to fuel oxidative metabolism are in place early in development, the onset of excitatory neurotransmission - indicated by the presence of the proteins needed to transport neurotransmitter between the synapse and the glial cells - is delayed. These findings help us to understand how and when signals originating in the retina are involved in establishing the retinal blood supply.

Macular Degeneration:

AMD occurring in Dry and Wet forms, involves disruption of the barriers which normally protect retinal tissue from outside influences. Blood-retinal barriers (BRB) exist at two principal sites; an inner barrier consisting of retinal vascular endothelial cells & glia limitans, and an outer barrier represented by a monolayer of retinal pigment epithelial cells.

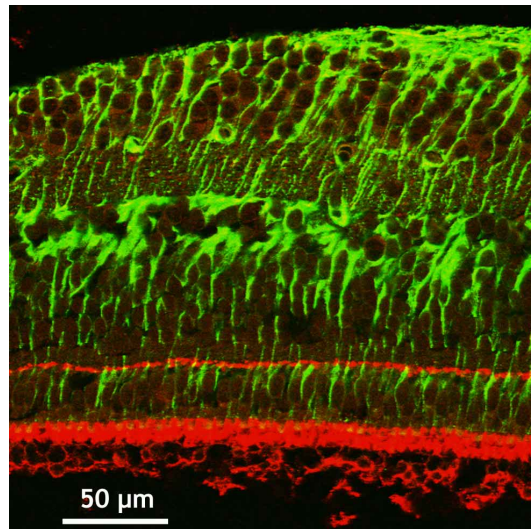
Our studies aim to determine the distinguishing features of Wet and Dry AMD and provide an improved rationale for clinical management of the disease, for which treatment options are extremely limited; presently no recognised therapy is available for the Dry form.

Projects in 2001:

Smoking & AMD: Penfold, Provis, Billson. We have reported, in an ABC Radio Health Report program, that AMD is causally linked to smoking. In the adult macula there is a critical balance between limited blood supply and high metabolic demand such that even minor perturbations of circulation, as may occur in incipient vascular disease or as a consequence of smoking, lead to metabolic stress in foveal neurons and/or glia. Such perturbations and the resultant physiological 'stress' may be the origin of signals which induce macular degeneration.

Previously SSI staff Norris Tsang, Philip Penfold, and Frank Billson looked at risk factors of macular degeneration, in particular the oxidative damage caused by smoking to the retina. Their investigation demonstrated that smokers were three times more likely to develop macular degeneration when compared to non smokers. This observation has been confirmed by the Blue Mountains Eye Study (Eye Sight August 1997). The study estimates that approximately 100,000 people in Australia suffer late stage AMD. It was estimated that 20% of these cases were directly linked to smoking.

Steroids & AMD: Penfold, Wen, Madigan Provis. A number of clinical pilot studies indicate that 'wet' retinal diseases, particularly AMD, may be treated by intravitreal administration of triamcinolone acetonide (TA), a corticosteroid. Although this class of drugs is known to display differential capacities to mediate anti-inflammatory and permeability effects, the modes of action of TA have not been defined. We have developed *in vitro* assays using isolated human vascular endothelial cells to assess the effects of TA on expression of inflammatory proteins and the receptors known to have a role in neovascularization in AMD. Our results show that TA down-regulates inflammatory markers as well as the receptors involved in neovascularization.



The developing macula showing FGF receptor 1 (in red) being expressed by cone photoreceptors.

Glial Changes in Aged and AMD Retinas:

Penfold, Wu, Billson and Madigan.

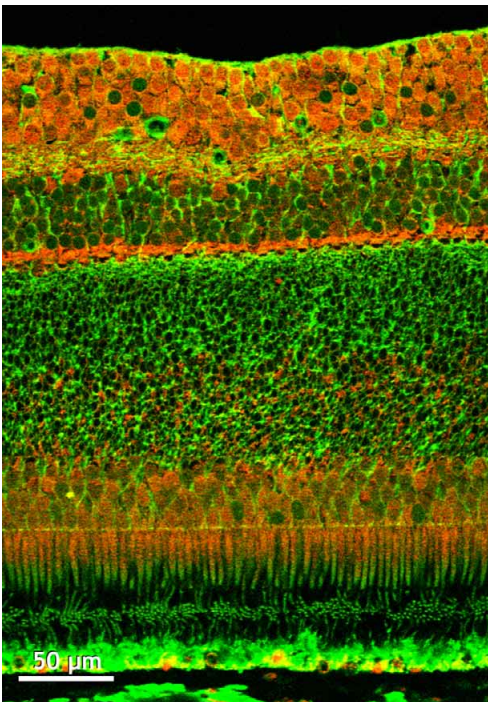
We have previously established that glial cells markers, in particular glial fibrillary acidic protein (GFAP), are indicators of retinal stress. In this context we have been investigating changes in GFAP expression in normal aged and AMD retinas.

Neuronal transporters & glutamate

homeostasis: Penfold, Pow [Uni. of Queensland]). Exposure of isolated retinas to 30 micro molar D-aspartate, which is a substrate for all high affinity glutamate transporters, resulted in the accumulation of D-aspartate into Müller glial cells but not glutamatergic neurons as evinced by immunocytochemistry for D-aspartate. Further incubation of such loaded retinas in physiological media, in the absence of D-aspartate, resulted in the slow release of accumulated D-aspartate from the Müller cells and its accumulation into populations of photoreceptors & bipolar cells.

This result indicates that after initial transport into Müller cells, reversal of direction of transport of D-aspartate, and thus by inference glutamate, by GLAST, readily occurs. D-aspartate released by Müller cells was strongly accumulated into cone photoreceptors which are known to express GLT-1, and into rod photoreceptors which we demonstrate here to express the retina specific glutamate transporter EAAT5 (excitatory amino transporter 5). Populations of glutamatergic bipolar cells, which express GLT-1 also exhibited avid uptake of D-aspartate. We conclude that the Müller cell glutamate transporter GLAST is responsible for

most of the initial glutamate clearance in the retina after its release from neurones. However, some glutamate is also returned from Müller cells, to neurons expressing GLT-1 and EAAT5, albeit at a slow rate. These data suggest that the role of neuronal glutamate transporters in the retina may be to facilitate a slow process of recycling glutamate back from Müller cells to neurons after its initial clearance from perisynaptic regions by GLAST.



The fovea of an adult retina showing selective expression of FGF receptor 3

Quantitative Analysis of Leucocytes in AMD: Penfold, Wong. Animal models, *in vitro* assays and pilot clinical studies suggest that intravitreal triamcinolone acetonide may be useful in the treatment of age-related macular degeneration. We have reported the effects of intravitreal triamcinolone acetonide injection on a subretinal neovascular lesion, microglial morphology and quantitative expression of MHC-II antigens. Triamcinolone acetonide significantly decreased MHC-II expression consistent with immunocytochemical observations which revealed condensed microglial morphology. The modulation of subretinal oedema and microglial morphology correlates with *in vitro* observations suggesting that down regulation of inflammatory markers and endothelial cell permeability are significant features of the mode of action of triamcinolone acetonide.

Eye Cancers:

Retinoblastoma (Rb) and Uveal Melanoma are the most common primary intraocular eye cancers in children and adults respectively. Better understanding of the pathogenesis of these tumours involves understanding the normal processes of development and aging in the retina and choroid, as well as the mechanisms controlling proliferation and cell death within tumours. Combined clinical and laboratory research provides understanding of the biology of these tumours and improves the rationale for treatment. This is important given the morbidity associated with enucleation, the side effects of therapies, particularly radiation, and the high incidence of metastases in melanoma.

Projects in 2001

Ocular Tumours: Retinoblastoma and Melanoma: Clinical study of incidence and treatments of Rb in NSW 1975-2001: Conway, Billson, Madigan. This study is a major review of children with Rb referred to the New Childrens Hospital, Westmead over the past 25 years. Chemotherapy and focal therapy (usually laser therapy) have emerged as the therapy of choice for children with smaller tumours or bilateral Rb. In unilateral cases, where the tumour usually fills the eye before detection, enucleation remains the most appropriate management. The importance of early detection and intervention for Rb are again emphasised.

Clinical studies of uveal melanoma: Billson, Conway. This study involved evaluation and development of microsurgical techniques for tumour removal. The study found important diagnostic criteria for the successful microsurgical removal of tumours involving the iris and ciliary body, as well as describing innovations in microsurgical technique for repairing the iris and ocular tissues after tumour excision. A new infra-red diode laser has become available to supplement local surgery, especially for smaller tumours and is currently being trialed at the SSI. This treatment involves heating the tumour (hyperthermia); as the energy is absorbed within the tumour, it ultimately results in death of the cells, with minimal damage to surrounding tissues. This laser is regarded as a critical addition for the complete management of ocular melanomas with preservation of the eye and vision.

Effects of biological modifiers on Rb growth and expression of immunofunctional markers:

Conway, Penfold, King (Pathology, Uni. Syd), Madigan. Available treatments for Rb are associated with significant morbidity and side-effects. We have investigated the effects of several biological modifiers including sodium butyrate and all-trans retinoic acid that can induce differentiation and/or cell death (apoptosis) of Rb cells, and modulate expression of immune molecules, and proteins important for inhibiting cell death and controlling cell cycle. Initial studies indicate that these compounds may also enhance the effects of chemotherapy. The potential for biological modifiers to control Rb growth (including growth of the tumour blood supply) continues to be investigated.

In vivo effects of retinoids and sodium butyrate on tumour growth in an Rb mouse model: Ibarra, Uusitalo, O'Brien (Ocular Oncology, UCSF, San Francisco), Madigan. In collaboration with Prof O'Brien, Ocular Oncology UCSF, San Francisco, these studies are investigating the efficacy of various retinoids and sodium butyrate in controlling intraocular tumor growth in a mouse model of hereditary Rb.

Effects of radiation on Rb: Madigan, Zhang & Stevens. Rb cells are very sensitive to ionising radiation. Ongoing studies are using Rb and Rb-reconstituted cell lines to investigate some of the pathways important for Rb cell survival and death. For example, while most malignancies express a p53 gene mutation, both p53 (and p21 downstream) are upregulated following radiation of Rb cells, consistent with functional p53 in Rb.

Targeted radiotherapy of ocular melanoma: Conway, Billson, Li, Lai, Madigan, Allen (St George Hospital, Uni. of NSW). Laboratory research has focused on targeting radiotherapy more precisely to melanoma cells to deal with both primary and metastatic disease. We have recently been studying a melanoma specific antigen (Mab 9.2.27) which may have potential as a target for radiotherapy treatment. Preliminary studies show that choroidal melanomas and choroidal melanoma cell lines (from Prof Jager, Netherlands) express 9.2.27 immunoreactivity.

A role for matrix metalloproteinases in uveal melanoma and retinoblastoma? Lai, Conway, Billson, Provis, Crouch and Madigan. Matrix metalloproteinases (MMPs) are a family of enzymes involved in degradation of extracellular matrix

components such as collagen, laminin and proteoglycans. MMPs are involved in normal development, wound healing, new blood vessel growth and tumour invasion and metastases. To date, the role(s) of MMPs ocular melanoma and particularly Rb have not been fully established. Our studies in ocular melanoma indicate that MMP-2 and MT1-MMP are involved in tumour angiogenesis; MMP-2 expression by fibroblasts within tumours may also implicate EMMPRIN, an inducer of MMP activity produced by neighbouring tumour cells, in melanoma growth. These studies are continuing and will be extended to include Rb.

Interestingly, we have also observed intense expression of MMP-2 in glial cells and neurons in detached retina overlying large choroidal melanomas. In normal retina, MMP-2 expression is only seen associated with blood vessels. These observations suggest a role for MMP-2 in glial hypertrophy, and perhaps in neurite outgrowth observed with long-term retinal detachment.

Highlights in 2001

A/Prof Provis was co-convenor of 8th Australasian Ophthalmic & Visual Science Meeting held at University of Sydney, Dec 7-9, 2001. There were over 150 presentations during the weekend, including specialist lectures and symposia. The meeting will be held in Sydney and convened by the same team until 2003.

Retinal Vascular Biology Research Group

This group aims to identify new and improved treatments for retinal diseases by studying the role of retinal vascular biology in diabetic retinopathy and acute age-related macular degeneration (AMD).

Group Members

Mark Gilles, MB BS PhD FRACO, Unit Head

Wei Luo, MD MPH, Clinical Research Officer

Marina Tretiach, BSc, Postgraduate student, Laboratory Research

Svetlana Cherepanoff, BMed Sci MB BS, Postgraduate student, Laboratory Research

Jenny Wyndham, BSc Dip Ed MS MPH, Postgraduate student, Laboratory Research

William Chua, MB BS, FRACO, Postgraduate student, Clinical Research

David Van Reyk, BSc, PhD, (Assoc. Lecturer, UTS), Research Associate

Florian Sutter, MB BS, Research Fellow

Research Activity

Laboratory Group

Retinal vascular diseases with impaired blood supply to the retina and leakage of fluid through small blood vessel walls (capillaries) are among the commonest causes of blindness – especially diabetic retinopathy, retinal vascular occlusive disease, ocular inflammation and the wet form of AMD. Swelling of the macula, or "macular oedema", causes impairment of vision which deteriorates with time. To investigate the molecular basis of macular oedema the Gillies group has developed a model to study retinal vascular permeability.

Projects in 2001

The role of 'matrix metalloproteinase (MMPs) in the control of retinal vascular permeability: Wyndham, Gillies, Collier, Wakefield, DiGirolamo. The role of matrix metalloproteinases in diabetes-induced leak of retinal capillaries was confirmed. Studies are now directed towards whether the matrix metalloproteinases are critical in the development of macular oedema, and, if so, whether the pathway is open to drug inhibition.

Tyrosine phosphorylation of junctional complex proteins in macular oedemas: Gillies. We have proposed that chemical modification loosens the junctions holding vascular cells together resulting in increased leak. We have shown that the increased leak that is induced by the cytokines VEGF and TGF beta is associated with increased phosphorylation at zones of cell-cell contact. We have now demonstrated that these changes can be inhibited by tyrosine kinase inhibitors. We will now determine more precisely which proteins and enzymes are involved in this process in order to facilitate the search for potential drug treatments.

The effect of paravascular cells on retinal vascular permeability: Tretiach, Gillies. It is likely that cells situated near the retinal blood vessels can influence permeability. In this project, Müller cells and pericytes have been co-cultured in the permeability assay system. We have identified a critical

role for Muller cells in the control of the blood retinal barrier. Muller cells tighten the barrier under normal oxygen tensions, but they secrete factors that lead to leakiness when oxygen levels fall.

The effect of retinal laser treatment on the permeability of retinal vessels: Gillies, Tretiach, McAvoy, Provis, Davies. While laser treatment of retinal swelling is often a very effective treatment, its mode of action is poorly understood. We propose that laser treatment induces the secretion of a protein that stabilises the "blood-retinal barrier". Preliminary laboratory experiments in which various retinal cell types are added to the vascular permeability assay suggest that this may indeed be the case. The identification of a barrier-restoring factor might have enormous therapeutic potential. An animal model of diabetic macular oedema has been characterized in order to study this phenomenon further.

Autoantibodies in macular degeneration: Cherepanoff, Gillies, Penfold. Antibodies directed against the retina are known to occur in age related macular degeneration. This project has demonstrated that the presence of retinal autoantibodies in a patient's serum is associated with an increased risk of loss of vision. A range of patient sera is now being screened to determine whether different types of antibodies are associated with different types of immune response in "wet" and "dry" macular degeneration.

Clinical Group

The clinical group was established to test interventions developed by laboratory research conducted by this and other groups within the Institute by means of "randomised clinical trials".

Projects in 2001

Intravitreal triamcinolone for age-related macular degeneration: Gillies, Luo, Simpson, Penfold, Hunyor, Billson. The Gillies clinical research group has analysed the results from a 4 year randomised clinical trial, funded by the NHMRC, to test whether an injection into the eye of the steroid, triamcinolone, can reduce the risk of blindness in patients with AMD. From this trial we have data on the safety of steroids injected into the eye, a treatment with wide potential application, that is the most comprehensive ever published. Disappointingly, the intervention

had no effect on the final visual outcome. Photographic outcomes, however, revealed an encouraging anti-angiogenic effect of the drug (see below).

Photographic outcomes in the intravitreal triamcinolone study: *Chua, Gillies*. In order to understand how intravitreal triamcinolone works, the retinal angiograms and colour photographs of the macula were being analysed with respect to the nature & size of the lesions & their changes in both size & leak after treatment. It was found that treatment with intravitreal triamcinolone significantly inhibited the growth of the abnormal vessels invading the macula. This provides the foundation for studies which we propose that intravitreal triamcinolone be tested for earlier ("occult") forms of wet AMD, or in conjunction with other forms of treatment such as photodynamic therapy (which is also available at the Institute).

Retinal bypass study: *McAllister, Gillies, Mitchell*. This is an NHMRC supported randomised clinical trial of a "retinal bypass" procedure for central retinal vein occlusion, a common, often devastating condition. A high powered laser is used to create a new route of venous drainage out of the eye in suitable patients. The study is a multicentre Australian trial undertaken in collaboration with Dr. Ian McAllister of Lions Eye Institute, Perth. Our center has the second largest group of patients of the 5 sites involved.

Triamcinolone for Diabetic Macular Oedema Study: *Gillies, Sutter*. Based on encouraging data from the Intravitreal Triamcinolone Study for wet AMD, the Retinal Clinical Research Unit has embarked on exploratory randomized clinical trial of intravitreal triamcinolone for diabetic macular oedema. We are particularly interested in whether the treatment is effective when visual acuity deteriorates despite appropriate laser treatment, which is unfortunately all too common. Early, anecdotal experience of the treatment used in this situation is encouraging, but it is yet to be tested scientifically. With our internationally recognized laboratory and clinical experience with intravitreal triamcinolone, our Unit is well positioned to spearhead research of this promising new treatment for a common cause of blindness

Highlights in 2001

Mark Gilles, Wei Luo, Marina Tretiach and Svetlana Cherepanoff traveled to Florida to present their work at the Association for Research in Vision and Ophthalmology, the largest meeting of its type in the world. Mark Gillies participated in a Juvenile Diabetes Research Foundation roundtable discussion on improving the analysis of results from clinical trials in diabetic retinopathy.

Dr. Florian Sutter, a Swiss ophthalmologist with an interest in retinal diseases, joined the group to begin the TDMO study. Dr. Sutter is also performing research under the supervision of Prof. Billson.

Mark Gillies was Co-Scientific Chair of the Australian Ophthalmic and Visual Science Conference in Sydney in December. He also gave a key note lecture on diabetic eye disease at a postgraduate seminar for endocrinologists in Queensland.

Retinal Dystrophy Research Group

In 2001 the Retinal Dystrophy Research Group led by Professor Jonathan Stone, based in the Department of Anatomy and Histology on the main University of Sydney campus, consolidated their links with SSI and continued to make progress on collaborative projects at the Sydney Hospital and Eye Hospital campus.

Group members

Jonathan Stone, BScMed PhD, *Unit Head*
Krisztina Valter MBBS PhD, *Postdoctoral Scientist*
Donald Lee MBBS, *Postgraduate Student*
Natalie Walsh, BSc, *Postgraduate Student*

Research Activity

Research concerns the mechanisms which control the death and survival of neurones in degenerative retinal disease. Clearly there are many areas of common interest, both in laboratory and clinical research, with the other SSI research groups. It is planned to expand the presence and activity of this group at the SSI in the coming years.

Projects in 2001

Light Restriction in Retinitis Pigmentosa(RP) Trial: *Stone, Graham, Klistorner*. This trial is a joint project between the Retinal Dystrophy Group and Stuart Graham and Alex Klistorner. It is a phase I trial of the effect of reducing the retina's exposure to rod-exciting (blue)

wavelengths on the progress of RP. Our initial findings are limited but encouraging. The trial will continue for some time, to gain sufficient patients and follow them for sufficient time. It has the potential to provide RP sufferers with a manageable way of slowing the progress of the degeneration which blinds them. Success would be a major breakthrough in treating this disease.

Ultrastructural Analysis of the Outer Plexiform

Layer: Stone van Driel. This study, in collaboration with Diana van Driel, centred on the analysis of synapse distribution within the rat retina (outer plexiform layer). A classical EM study was completed in 2001 and a paper on this work is currently under review.

Measurement of Outer Segment Length:

Walsh, van Driel. This study, in collaboration with Diana van Driel, has concentrated on preparing retinas in plastic embedding media with fixation of sufficient quality to allow the measurement of the length of outer segments of photoreceptors. This measurement has become important in the interpretation of our ERG studies.

In Situ Hybridisation Studies of retinal models:

Walsh, Valter, Natoli. Both Natalie Walsh and Krisztina Valter are working with Riccardo Natoli to improve their in-situ hybridisation work on our retinal models. This is in recognition of Riccardo Natoli's considerable skills with this technology.