CELL THERAPY LIMITS LOSS OF VISION IN AN ANIMAL MODEL OF RETINAL DEGENERATIVE DISEASE

TREVOR MCGILL
Bachelor of Science, University of Lethbridge, 2002

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the Degree

MASTER OF SCIENCE

Psychology and Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

Abstract

The Royal College of Surgeons (RCS) rat was used as a model of human retinal degenerative disease, and for studying the efficacy of cell transplantation treatments. In order to characterize the spatial vision of the RCS strain, the visual acuity and contrast sensitivity of adult non-dystrophic RCS rats was measured. The acuity and contrast sensitivity of these rats was normal. The acuity of dystrophic RCS rats was also characterized to determine how photoreceptor degeneration affects vision. These rats progressively lost visual acuity from one month of age until eleven months of age when they were judged to be blind. The degeneration of vision in these animals was more protracted than would be predicted from previous anatomical and electrophysiological measures. Subretinal transplantation of human-derived Retinal Pigment Epithelial (RPE) cells and human Schwann cells into the dystrophic RCS rat significantly delayed the loss of visual acuity. These studies show that cell transplantation may be a viable method of limiting loss of vision in humans with retinal degenerative blinding diseases.

Acknowledgements

The author would like to thank the many people who provided support and encouragement throughout the pursuit of this project. In particular: my supervisor, Glen Prusky for providing inspiration and guiding my efforts on this project, and always having an open and optimistic mind; Ray Lund for his continued willingness to provide insight, and suggestions; and Rob Douglas for helping me with technical issues. Thanks also to my parents who provided me with the most important thing of all, opportunity. Finally, to my fiancée, Charity Kopeck, who along with my parents, stood by me through all my decisions in my educational career.

Contents:

List of Figures

List of Abbreviations

RCS = Royal College of Surgeons

RPE = Retinal Pigment Epithelium

AMD = Age-related Macular Degeneration

RP = Retinitis Pigmentosa

VWT = Visual Water Task

CNS = Central Nervous System

AREDS = Age Related Eye Disease Study

c/d = cycles per degree

PETH = Pink Eye Tan Hooded

CCAC = Canadian Council on Animal Care

CCBN = Canadian Center for Behavioural Neuroscience

ERG = Electroretinogram

ATCC = American Type Culture Collection

IACUC = Institutional Animal Care and Use Committee

NTF = Neurotrophic Factor

bFGF = basic Fibrillary Growth Factor

CNTF = Ciliary Neurotrophic Factor

GDNF = Glial Derived Neurotrophic Factor

rdy +, *p*+ = non dystrophic, pigmented

rdy -, *p*+ = dystrophic, pigmented

AAV = Adeno-Associated Virus

Chapter 1-Introduction

Millions of people around the world suffer varying degrees of vision loss due to diseases that irreversibly degenerate components of the visual system. In particular, degenerative diseases such as Age-related Macular Degeneration (AMD) and Retinitis Pigmentosa (RP) affect the retina, the delicate layer of tissue that lines the back of the eye. These two retinal degenerative diseases are thought to be the leading direct causes of blindness in North America; AMD typically affects elderly people with approximately one-third of humans over the age of 75 being afflicted with the disease (Sommer *et al.*, 1991; Klein *et al.*, 1992; Mitchell *et al.*, 1995); while RP affects approximately 1 in 3500 people (Bundy and Crews, 1984; Bunker *et al.*, 1984; Kaplan *et al.*, 1990).

1.1 Age-related Macular Degeneration

There are two forms of AMD: wet AMD and dry AMD. The wet form of AMD constitutes only 10% of the total cases of AMD, but it also causes the most significant amount of damage to the retina in the shortest amount of time (Ferris *et al.*, 1984; Mohan *et al.*, 2003). Leaky, newly growing blood vessels grow through a weak Bruch's membrane, the membrane dividing the choroid and the Retinal Pigment Epithelial (RPE) cell layer (Simpson, 1986). The RPE cell layer is responsible for providing nutrition and structural support to the photoreceptors, the light sensitive cells in the retina. The leaky vessels also disrupt the RPE cell layer and eventually cause a detachment of the macula, the area of the retina with the highest concentration of cone photoreceptors which is also responsible for central vision. This retinal detachment causes the photoreceptors, particularly within the macula, to become malnourished and eventually die (Alder *et al.*, 1999). Along with the death of photoreceptors, there is scar tissue formation from the

leaky blood vessels and a fluid layer develops that impedes the exchange of oxygen and nutrients (Alder *et al.*, 1999), introducing more factors that impede normal vision.

The dry form of AMD comprises the remaining 90% of cases. Clinically, dry AMD is characterized by the loss of vision, the lack of obvious vascular abnormalities, and the accumulation of drusen (Sunness *et al.*, 1989; Bird *et al.*, 1995; Eisner *et al.*, 1992; Curcio and Millican, 1999). Drusen deposits appear as small yellow spots within the macula and are typically found between the RPE cell layer and Bruch's membrane (Figure 1). These deposits are thought to be formed by the waste products of photoreceptors and RPE cells and contain complex lipids (Haimovici *et al.*, 2001), calcium (Ulshafer *et al.*, 1990), and amyloid-beta (Dentchev *et al.*, 2003) which is the major constituent of Alzheimer's plaques. The accumulation of drusen progresses with age (Klein *et al.*, 2002), and it is thought that eventually the drusen or its constituents interfere with the function of RPE cells and other retinal cells (Sarks *et al.*, 1982; Johnson *et al.*, 2003). The progressive loss of RPE function likely causes a progressive loss of photoreceptors and subsequent loss of central vision (Johnson *et al.*, 2003; Curcio *et al.*, 1996). The severity of dry AMD varies with the most severe form eventually developing into wet AMD. Both the wet and dry forms of AMD result in a dramatic loss of central vision and eventually legal blindness, which is characterized by a visual acuity of less than 20/200.

According to Suner and colleagues (2004), nicotine from cigarette smoking is a very strong environmental risk factor for developing wet AMD. Experimentally, nicotine has been found to increase the size and severity of vascularization of the choroid (Suner *et al*,. 2004). In addition, there may also be a hereditary component of AMD (Yates and

Moore, 2000; Allikmets *et al.,* 1999; Gorin *et al.,* 1999) and according to a recent review, (Klaver *et al.*, 2003) there have been a number of genes that have been implicated in having an association with the development of AMD, although no specific genetic causes of the disease have been identified.

1.2 Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is the name given to a category of genetic eye diseases that cause gradual deterioration of the retina (Rivolta *et al.*, 2002). The name itself refers to pigment changes in the retina which are usually due to the movement of Retinal Pigment Epithelial (RPE) cells. In all cases of RP, a distinguishing feature used for diagnosis is the drastic discoloration of the retina due to the movement of pigmented RPE cells (Gartner and Henkind, 1982; Kalloniatis and Fletcher, 2004) (Figure 1). In most cases of RP, a defect causes the death of photoreceptor cells which is followed by the detachment of RPE cells from Bruch's membrane and their migration into the inner retina where they accumulate around the thin-walled blood vessels (Milam *et al.,* 1998).

RPE cells and photoreceptors form a functional unit responsible for the conversion of light to electrical signals which are sent from the retina to the visual cortex (Figure 2). RPE cells and photoreceptors also have a trophic relationship in which the RPE cells provide nutrients as well as physical support to the photoreceptors (Kalloniatis and Fletcher, 2004). Destabilization of this relationship because of a mutation or change in the surrounding environment, often results in a loss of photoreceptors and a progressive loss of vision. One mutation, which affects the *MERTK* gene in the RPE cells, renders the RPE cells unable to phagocytose photoreceptor outer-segments at a normal rate (Edwards and Szamier, 1977; Gal *et al.,* 2000).

RP is usually associated with changes in the retinal pigment epithelium (RPE) which may be primary or secondary to photoreceptor loss (Gu *et al.,* 1997; Marlhens *et al.,* 1997; Maw *et al.,* 1997; Petrukhin *et al.,* 1998). Either photoreceptor type (rods or cones) may be predominantly affected with the RPE being affected either centrally or peripherally. Given the number and distribution of rods and cones in the retina, prognostic information about the patient's vision loss depends on whether the process is primarily a rod-cone dystrophy, where the rods are affected before cones; or a cone-rod dystrophy, where the cones are affected before rods. The highest concentration of cone photoreceptors is found in the fovea, an area of the retina within the macula. These areas of the retina are responsible for the high visual acuity of humans. The peripheral retina contains both rod and cone photoreceptors, however, rods out number cones approximately 20 to 1. Most cases of RP are primary photoreceptor defects causing a rod-cone dystrophy in which the rods are affected first. Patients with the rod-cone form of RP are presented with tunnel vision and night vision problems which progresses to a loss of all peripheral vision. Other cases consist of cone-rod or pure-cone dystrophies in which the cones are affected either first or only. Patients with this form of RP are presented with visual acuity loss, color discrimination loss, and eventually a complete loss of central vision.

1.3 Current treatments for AMD and RP

There are many treatments that are directed toward limiting the progression of retinal degenerative disease. One of these techniques is the administration of growth factors directly. A number of growth factors including bFGF, CNTF, and GDNF have been identified to be of benefit to cells in the retina (Carwile *et al.*, 1998). Some of these

growth factors have been used in attempts to prevent photoreceptor loss in animal models which will be discussed later. To achieve photoreceptor rescue, these factors have to be injected into either the vitreous or the subretinal space, however, this treatment method may require multiple injections for long term maintenance because some growth factors only remain in the vitreous for up to 24 hours. As well, the appropriate dosage levels and frequency of dosages are unknown at this point.

The second method is the delivery of growth factors by gene therapy. Gene therapy using adenovirus vectors expressing appropriate wild-type enzymes may be an effective method to slow photoreceptor loss. Gene therapy with an adenovirus carrying bFGF has been shown to promote photoreceptor survival in Royal College of Surgeons rats for up to fifty-six days when compared with controls (Akimoto *et al.,* 1999). Unfortunately, there have been a few safety concerns with infecting human patients with a virus, and recently there have been a few deaths cautioning the use of this method (Lehrman 1999; Marshall, 1999). In 2001, Rasmussen and colleagues completed the first clinical trial of gene therapy for human AMD, however, this was primarily a safety study directed towards tolerance, feasibility, and dose and not designed for prevention of the disease. Supposing safe and long-term beneficial effects of AAV gene therapy, candidate genes for transfection might be MERTK (Smith *et al.*, 2003), CNTF (Liang *et al.*, 2001) and GDNF (McGee Sanftner *et* al., 2001).

Thirdly, dietary and drug treatments may be useful in several forms of retinal dystrophy. In 2001, the National Eye Institute reported (AREDS report; AREDSRG, 2001) that a high-dose combination nutritional supplement with vitamin C, vitamin E, beta-carotene, and zinc could reduce the risk of advanced AMD by about 25%. Seddon

and colleagues (1994) examined the relationship between dietary intake of vitamins A, C, and E and AMD, and found no significant effects of Vitamin A or E in relation to developing wet AMD, although there was some evidence that a higher intake of Vitamin C may marginally reduce the risk of developing the disease. In addition, Seddon and colleagues (2003) showed that a diet with a high fat intake increases the risk of developing advanced AMD, although consumption of fish and nuts appears to decrease this risk. As well, consumption of dark green leafy vegetables, which contain antioxidants, may decrease the risk of developing wet AMD (Seddon *et al.*, 1994; Smith, 1999). Another study (Berson *et al.*, 1993) suggested a high dose vitamin A treatment for patients with RP; however, there are some safety concerns with this treatment (Sibulesky *et al.*, 1999). Although there seems to be a beneficial effect of high levels of vitamin intake, in the last few years there has been significant concern with the validity and safety with which the AREDS study was carried out (Seigel, 2002; Gaynes, 2003) suggesting caution when employing these strategies.

Fourthly, laser treatment can be used to coagulate leaky blood vessels in the retina, and has been used with limited success. There are two forms of the laser treatment: hot and cold. The hot treatment technique is used to cauterize abnormally growing blood vessels in the retina, but in the process can produce irreversible scar tissue formation leaving the patient with a damaged retina and corresponding visual loss. Because of the damage caused to the retina, this technique is only performed in the peripheral retina and the macula, but preferably not in the fovea. The laser treatment is intended to prevent blood vessels from growing into the macula, and especially the fovea, in an attempt to prevent the loss of central vision. It is not uncommon, however, that the

blood vessels will re-grow, or that the treatment may not be successful in its first attempt, and therefore treatment may need to be repeated numerous times (Miller *et al.*, 1999; Bressler *et al.*, 2001; Schmidt-Erfurth *et al.*, 1999).

The cold laser treatment involves injecting a light-sensitive chemical (Visudyne, www.qlt-pdt.com) that generates oxygen radicals when excited by a particular wavelength of laser light (689 nm) in the presence of oxygen (Yang, 2004). This laser treatment has been shown to not only reduce neovascularization of the retina but also to improve visual function (Miller *et al.*, 1999; Bressler *et al.*, 1999; Yang, 2004). This treatment results in local damage to neovascular endothelium, resulting in vascular occlusion. Visudyne appears to accumulate in neovasculature, including choroidal neovasculature (van den Bergh *et al.*, 2004), and therefore there may be damage to retinal structures including the retinal pigmented epithelium and outer nuclear layer of the retina following photoactivation. Both laser treatments are for wet AMD and although they delay the progression of the disease, this treatment is only beneficial in 10% of the total cases of AMD, and is only successful approximately in half of those treated (Bressler *et al.*, 1999). Even with successful treatment, it can take up to two years and is not guaranteed to eliminate blood vessel formation.

Another method of preventing the progression of the disease is by way of cell transplantation. Animal studies have shown that cell transplantation into the subretinal space before significant photoreceptor loss can limit the progress of the disease any many of these will be discussed throughout the thesis. The results of cell transplantation studies in humans have not been promising (Algvere *et al.*, 1999; Kaplan *et al.*, 1997), raising a number of concerns when considering this treatment technique for clinical

application. First the dystrophy needs to be identified early enough for the treatment to have a chance at success. Ophthalmologists suggest annual eye and vision exams allowing any visual dysfunction to be detected at a very early stage. If this recommendation was followed and the dystrophy was identified early, cell transplantation might be able to limit the loss of photoreceptors and consequently vision for a significant period of time. Secondly, the donor cells and the surgery technique itself must be safe and not put the patient at risk. Animal studies have shown that subretinal transplantation is a relatively safe, quick, and effective procedure for delivering donor cells without injuring the retina. Donor cells must be non-tumorogenic and must not activate the host immune system. Although there have been reports of immune system rejection of cells, there are a number of methods of circumventing the immune reaction which include immune suppression or maybe transplantation of syngeneic cells (e.g. cells harvested from a patient and transplanted back into that patient). Thirdly, the treatment must replace the lost function for a significant period of time. Currently, the results of cell transplantation in humans have not been encouraging most likely due to late intervention, at a stage of severe photoreceptor degeneration. Early cell transplantation in animal models has been shown to be of benefit for extended periods of time; however, late transplantation in animal models has not studied and is the focus of future studies.

A different approach to treat retinal degenerative disease is reversal of the disease. One method used in this approach is the transplantation of retinal sheets into the degenerating retina in an attempt to replace the lost cells. The retinal sheets are required to be easily accessible, tissue-matched, disease free, and of appropriate age. Retinal sheet transplantation has been shown to be of benefit to threshold sensitivity tests when

transplanted into rd mice (Kwan *et al.,* 1999); however, attempts at retinal sheet transplantation into the human eye have provided no evidence of functional improvement (Kaplan *et al.*, 1997; Das *et al.*, 1999).

Microelectrode prosthesis is another method of attempting to reverse retinal degenerative disease. Microelectrode prostheses are implanted microphoto-diodes that carry visual signals to the inner retina. This treatment has been studied in various model systems where the transplants were placed either subretinally (Peyman *et al.*, 1998) or adjacent to the vitreal surface (Eckmiller, 1997). These transplants were successful and were found to conduct electrical signals in response to light. The subretinal transplants were expected to stimulate bipolar cells and the vitreal surface transplants were expected to stimulate ganglion cells. However, much work needs to be done to ensure that appropriate visual signals are presented to the retinal cells. It also remains to be seen how the CNS interprets these signals. At present, the best that might be expected from these endeavors may be a crude image, which would be a drastic improvement in the visual function of patients with severe central vision loss.

With the growth and development of new technology, it is now possible to image photoreceptors at high-resolution, non-invasively, in living humans (Liang *et al.*, 1997). This technological advance (adaptive optics) has given insight into the function of photoreceptors in real time, however, implementing technology such as this for evaluating treatments or for diagnosing retinal disease is not possible, yet. In the future, this technology may provide a beneficial tool in diagnosing retinal diseases, and answers to many of the current research questions regarding retinal degenerative disease and possible treatments or cures.

In summary, there are currently few effective treatments available for AMD and RP, and the only useful treatments apply to a small proportion of the patients with AMD. In addition, the treatments that are available are inadequate in their capacity to stop retinal degeneration. Clearly, a new effective treatment for these diseases is needed.

Chapter 2-Characterization of spatial vision in a rat model of human retinal degenerative disease

2.1 Use of Animal models

Although the symptoms and some of the genetic causes of retinal degeneration in RP are known, there is very little known about the mechanisms of retinal degeneration itself. For example, in patients with AMD it is not known what causes the disease or why the photoreceptors begin to deteriorate, although once degeneration begins, the consequences have been well characterized. In RP, although the genetic mutations in many cases have been identified, the specific causes of RPE and photoreceptor dysfunction are largely unknown. It is, therefore, vitally important to understand the fundamentals of how retinal degeneration affects spatial vision before appropriate treatments for such diseases can be developed.

In order to develop new treatments for AMD and RP, an appropriate animal model system must be used to identify the causes and consequences of retinal degeneration. More specifically: the cellular functions or dysfunctions, the effects imparted on different systems such as the retina and the cortex, and most importantly, the functional consequences of retinal degeneration on spatial vision. Because spatial vision is the tool used to classify these diseases, diagnose patients, and assess the efficacy of treatment, it is vital that the effects of retinal degeneration and therapeutic interventions on spatial vision be explored.

There are a number of important considerations in determining an appropriate animal model. First, it is important to use a model in which the gene mutation responsible for retinal degeneration has been identified. The identification of the

mutation allows for further investigation into the cell biology and gene expression. As well, once the gene is identified in animals with retinal degenerative disease, humans can be screened for the gene. Second, the model must show progressive retinal degeneration. By studying the pathology of the mutation on retinal morphology in an animal with a lifetime shorter than a human, researchers should be able to characterize the fundamental mechanisms of cell death and degeneration more quickly. Third, the animal model must be available for the visual capabilities to be tested quickly, efficiently, and repeatedly over the course of the retinal degeneration. Previous studies of visual capacities have used electroretinograms or other electrophysiological measurements in various parts of the visual system to determine how retinal cells respond to various visual stimuli. Researchers have also been able to study retinal receptive fields, cortical receptive fields, and other visual functions such as contrast detection and square wave grating detection. Although much has been learnt from these studies, none of the methods used have provided quantitative measures of spatial vision. Fourthly, the animal model must also be available for testing treatment regimens for efficacy and for safety which usually requires numerous trials. Because treatments for human retinal degenerative disease are the goal of these studies, treatments effective in preventing the loss of vision and retinal cells will inevitably be used in multiple models of retinal degenerative disease before moving to clinical trials. Finally, similarity to common forms of human retinal degenerative disease is an important criterion. AMD is caused by a subretinal dysfunction that causes the eventual secondary death of photoreceptors and corresponding loss of vision. Although most forms of RP are caused from a primary photoreceptor defect, some forms are caused from RPE cell dysfunction causing a secondary loss of

photoreceptors. Therefore some animal models may be homologous to some forms of retinal degenerative disease while being analogous to others at the same time.

2.2 Candidates

Fortunately for researchers, there are a number of animal models available for studying retinal degenerative diseases (see recent review Chader, 2002), and many of the genes found to be mutated in animals have been found to be similarly abnormal in humans with retinal degenerative disease. Although many of the models discussed here do not have direct correlates identified in humans, the pathology of these deficiencies is often very similar. Some models of retinal degeneration are: a rhesus monkey with cone-rod photoreceptor defect (Imaki *et al.,* 1987), the Briard dog with an RPE 65 gene defect (Veske *et al.,* 1998), a transgenic pig model with a rhodopsin mutation (Li *et al.*, 1998), and the Abyssinian cat with a rod-cone photoreceptor defect (Narfstrom and Nilsson, 1987). There are also many rodent models including the rd mouse (Sidman and Green, 1965) and the RCS rat (Bourne *et al.,* 1938; Dowling and Sidman, 1962).

Non-human primates might provide a good model of the human visual system because they have visual systems similar to humans, relatively high visual acuity, can be trained on tests of vision similar to those used in humans, and have large eyes which would make surgery techniques less complicated. There is a primate model using the rhesus monkey that has shown evidence of drusen build-up similar to dry AMD in humans (Engel *et al.*, 1998; Ulshafer *et al.*, 1987). There is also a laser induced primate model (Husein *et al.*, 1999; Miller *et al.*, 1986; Virdi *et al.*, 1982), however, training is very time-consuming which effectively limits the number of animals used in a study, and they are not the best animal in which to understand the fundamentals due to their

complicated visual systems. Primates are also high cost, low availability, and there are serious moral, ethical, and political issues involved when using them as subjects.

Pigs might make a good model for studying retinal degenerative disease because the pig retina is very similar to the human retina, containing a large number of both rod and cone photoreceptors (Li *et al.*, 1998). In addition, the pig eye is well suited for experimental, surgical and/or therapeutic treatments. There has also been transgenic model of RP developed (Li *et al.*, 1998), in which, like RP patients with the same mutation, there is early and severe rod loss with the surviving cones slowly degenerating. However, there are limited pig models of retinal degeneration, breeding pigs is labor intensive, and behavior measurements of visual system capabilities are difficult to obtain.

Dogs are a good model system because they have relatively good vision and large eyes making surgery techniques less troublesome. There are a number of natural mutations which cause retinal degeneration in dogs which result in abnormalities ranging from photoreceptor defects to RPE cell defects (Chader *et al.*, 2002). Dogs are easily trainable; however there are currently no tasks available for behaviourally measuring the visual capabilities of dogs. Also, canine vision is dichromatic unlike the trichromatic vision of humans. In addition, the major disadvantage of using dogs is that the time course of known forms of retinal degeneration in canines is 12-15 years, far too long for characterizing the fundamentals of treatments for retinal degeneration.

Cats also make a good model for studying retinal degeneration because felines have relatively large eyes and the visual system in felines has been studied quite extensively. It is also possible to measure behaviorally the visual capabilities of cats (Muir and Mitchell, 1975). Unfortunately, the Abyssinian cat is currently the only known

feline model of retinal degeneration and the genetic mutations responsible for retinal degeneration in the cat have not yet been characterized. In addition, the course of retinal degeneration in cats, although far more rapid than in dogs and primates, it is still too slow for rapidly characterizing the mechanisms of treatments for retinal degeneration.

Mice have also been used in the research of retinal degenerative diseases. There are many different mouse models and the mutation responsible has been identified in many of them (Chader, 2002). Also, the mouse genome has been characterized allowing transgenic mice models with specific mutations to be generated on demand. Mice have also had a great deal of anatomical and cell biological studies performed on them, and the visual capabilities of mice have been characterized behaviorally. However, a significant disadvantage to the mouse model is that even though the visual capabilities have been characterized, mice have relatively poor vision (Prusky, West, *et al.,* 2000a; Prusky *et al.*, 2003). This makes it difficult to discriminate between treatment and control groups in experimental studies. In addition mice have small eyes which can complicate eye surgery techniques.

With the exception of mice, most of the previously discussed animal models of retinal degenerative disease have never had vision directly measured, but rather inferred by ERG, recordings of thresholds in the superior colliculus, or single unit responses in the cortex. Recently, however, rats have been used for a number of studies in which their visual capabilities have been characterized in a fashion similar to that used in humans (Prusky *et al.*, 2000a; Prusky *et al.,* 2000b; Prusky *et al.*, 2000c; Prusky *et al.*, 2002; McGill *et al.*, 2004). Despite previous misconceptions, rats have a typical mammalian visual system with visual functions that are easily characterized using The Visual Water

Task (Figure 3) (Prusky *et al.*, 2000a). Rats have small eyes compared to those of humans, but recent technological advances have allowed researchers to modify a subretinal transplantation procedure developed in rabbits (Lopez *et al.*, 1987) for use in rats (Li *et al.*, 1988a). Although the rat retina appears to lack a fovea or area centralis, it is very similar to human peripheral retina, which can provide quite detailed vision at close distances. The rat retina contains both rods and cones with most of the behavioral testing being done under photopic conditions in which rods are fully saturated. Previous work has also shown that rats are capable of making color discriminations (Jacobs *et al.*, 2001).

The rat visual system evolved to specialize in low light and low resolution vision, however many of the psychophysical measurements that can be obtained in humans can also be quantified in rats. Furthermore, visual functions such as acuity and contrast sensitivity can be characterized in rats in a similar fashion as in humans. Normal laboratory rats have a visual acuity of approximately 1.0-1.2 cycles per degree (c/d) (Dean, 1981a; Prusky *et al.*, 2000; McGill *et al.*, 2004), and although this is far lower than the 30 c/d acuity of humans, it is a quantifiable measurement of vision. Rats also have a characteristic inverted "U" shaped contrast sensitivity function (Keller *et al.*, 2000; McGill *et al.*, 2004) as found in humans, albeit the peak sensitivity is lower in rats than in humans. It has also been shown that rats can discriminate between gratings that differ in orientation by as little as 3 degrees (Bowden *et al.*, 2002), and that motion coherence, thought to be an extra-striate property, can be measured in rats (Neve *et al.*, 2002). Even though the rat's visual capabilities are not specialized for a high degree of

central vision, they have a visual system that is fundamentally similar to that of humans with similar measurable visual functions that can be assessed behaviourally.

There are many rat models (Chader, 2002) of retinal degenerative disease; however, the Royal College of Surgeons rat is of particular interest because the RCS rat has a recessive mutation in the *Mertk* gene expressed in the RPE cells (D'Cruz *et al.*, 2000). This mutation renders the RPE cells unable to perform their normal supportive role of phagocytosing photoreceptor outer-segments at a normal rate (Mullen and LaVail, 1976; Edwards and Szamier, 1977) resulting in progressive photoreceptor death. This mutation is also the orthalogue to the mutation (*MERTK)* found in a proportion of human patients with RP (Gal *et al.,* 2000), with both the rat and human forms following the same degenerative profile. Because vision and photoreceptor loss in AMD may be attributable in many cases to RPE cell dysfunction, the RCS rat serves as an indirect model when contemplating non-genetic interventions such as cell transplantation to alleviate the condition.

The development of the Royal College of Surgeons rat model began in 1938, when Bourne and colleagues (1938) recognized a dystrophy in a pink-eyed tan hooded (PETH) rat. Dowling and Sidman (1962), cross-bred the PETH strain to develop both pigmented and pink-eyed rats both dubbed the Royal College of Surgeons (RCS) rat; the breeding pairs of which were originally housed at the Royal College of Surgeons in London. The strain has since been out-bred to produce a pigmented non-dystrophic line as well (Lavail *et al.,* 1975; Lavail *et al.,* 1981).

Previous reports of anatomical changes in the retina of pigmented dystrophic RCS rats have shown that by three weeks of age, there is already significant disruption of the

outer segments (Lue *et al.,* 1994; Sauve *et al.,* 2001). By one month of age, this process is even more evident and in addition, the outer nuclear layer is beginning to thin. By three months of age, this layer has been reduced to a single layer of cell bodies with the debris zone occupying the former outer-segment area; by six months the inner nuclear layer is in direct contact with the RPE cell layer with very few photoreceptors surviving (Figure 4, adapted with permission from Sauve *et al.*, 2002). Secondary changes in the inner retina, including the loss of retinal ganglion cells from whole quadrant segments of retina, as well as laminar disruption and changes in the inner plexiform layer have also been shown in animals more than 6 months old (Villegas-Perez *et al.,* 1996; Marc *et al.,* 2003; Jones *et al.,* 2003).

2.3 Quantification of RCS rat spatial vision.

Although many studies have characterized the effects of retinal degeneration on anatomical and physiological components of the visual system in the RCS rat, spatial vision has been poorly studied. Two previous studies have shown that there is deterioration in spatial vision from that of non-dystrophic pigmented animals, but neither examined the progress over time (Coffey *et al.,* 2002; Hetherington *et al.,* 2000). Furthermore, these researchers were using testing regimens in which the maximum performance was considerably below the visual thresholds of rodents (Dean, 1981). Recent work (Prusky *et el.,* 2000) developed a new test, the Visual Water Task, in which it is possible to achieve high resolution performance in rodents with a limited training regimen, such that one can study changes in spatial vision over time from an early age, quantify the effects of treatments on performance, and identify small variance in performance among animals and groups.

Preceding experimental treatment studies in the RCS rat, it is important to establish the animal's background visual capabilities for a few reasons. First, characterization of the visual capabilities of the non-dystrophic RCS rat will provide a comparison to other commonly used laboratory rats, and in the process, evaluate the effects of strain differences on the visual system function in these animals. Secondly, it is important to identify the mutated *Mertk* gene as the specific cause of any visual dysfunction found in the dystrophic animals and not an inherent photoreceptor defect, implicating a possible mutation in the *MERTK* gene as responsible for retinal degeneration in humans. Thirdly, characterizing the visual dysfunction in the dystrophic RCS animals will determine how the retinal morphology and physiological measures of the retina relate to conscious spatial vision, as well as supplying the appropriate controls for examining the effects of experimental treatments on spatial vision. Here, the Visual Water Task was used first to examine the visual capabilities of non-dystrophic RCS rats, and second, determine how spatial vision deteriorates with time in dystrophic RCS animals.

Methods

Animals

The animals were housed and handled with the authorization of the Canadian Council on Animals Care (CCAC) and supervision of the animal care committee at the University of Lethbridge.

Four Long Evans hooded rats, six non-dystrophic RCS rats (rdy^+, p^+), and six dystrophic RCS rats (rdy^-, p^+) were used in this study. Long Evans rats were bred from stock originally obtained from Charles River and raised in the Canadian Centre for

Behavioural Neuroscience (CCBN) vivarium. RCS breeding pairs were obtained from the Lund laboratory at the University of Utah and their offspring were born and raised at the CCBN. Long-Evans rats and non-dystrophic RCS rats of both sexes were tested as adults, at 6-12 months of age. Dystrophic RCS rats of both sexes were tested monthly beginning at one month of age. For the duration of the experiment, all animals were housed in the same room with an ambient temperature of 21°C, 35% relative humidity, 12/12 light/dark cycle, where food and water were available ad libitum. The housing consisted of transparent Plexiglas cages (35cm L x 20cm W x 13cm H) hanging on a rack with other cages.

Visual Water Task

The Visual Water Task has been described in detail previously (Prusky *et al.*, 2000a, Prusky *et al.*, 2000b, Prusky *et al.*, 2000c, Prusky *et al.*, 2002, McGill *et al.*, 2004). The apparatus consisted of a trapezoidal tank containing water, with two computer monitors facing through a clear glass wall into the wide end of the pool (Figure 3). Visual stimuli (sine wave gratings or uniform gray) were generated and projected on the screens using a computer program (Vista©; CerebralMechanics). On the screens, the black level was 0.05 cd/m^2 and the white level was 72.8 cd/m^2, as measured using a Minolta LS-110 light meter positioned at the point where the animal makes a choice. This point was defined by a 46 cm long midline divider which extended into the pool from between the monitors, creating a Y-maze with a stem and two arms. A moveable, transparent Plexiglas escape platform (37cm L x 13cm W x 14 cm H) was always submerged directly below whichever monitor displayed the grating. A LRLLRLRR sequence, a pattern the animals could not memorize, was used for the location of the gratings.

Rats are instinctive swimmers and the Visual Water Task capitalizes on their natural inclination to escape from water to a solid substrate, the location of which was directly paired with a specific visual stimulus-in this case, a sine wave grating. The other arm of the maze had no platform, and the monitor displayed a uniform gray of the same mean luminance. Animals were released into the pool from the wall opposite the monitors, and the end of the divider within the pool set the choice point for the rats that was as close as they could get to the visual stimuli without entering one of the two arms. The length of the divider, therefore, set for the animals the effective spatial frequency of the visual stimuli. The animals usually stopped at the end of the barrier and inspected both screens before choosing a side. If the animals swam to the platform below the grating without entering the arm with the monitor showing a uniform gray, the trial was considered correct; if they swam into the arm of the maze that contained the gray stimulus, the trial was recorded as an error. Animals were first trained on the apparatus, with the simple task to discriminate between a low spatial frequency (~0.1 c/d), vertical sine wave grating (+ stimulus; 100% contrast), and uniform gray of the same mean luminance (36.2 cd/m^2 at the choice point). The animals were tested in groups of 5 or 6, with 15-20 interleaved trials each, with each session lasting 45-60 minutes. No more than two sessions, separated by at least one hour, were performed in a single day. All trials were run with the room lights off. Once animals achieved near-perfect performance (90% or better over at least 40 trials), they were judged to be trained in the procedure and ready for their visual acuity to be measured. A flexible method-of-limits procedure was used in which incremental changes in the spatial frequency of the sine wave grating were made until choice accuracy fell below 70%. Accuracy for a given frequency was

measured in blocks of ten trials when near threshold, and shorter blocks at the low spatial frequencies, thereby minimizing the number of trials far away from threshold. A preliminary grating threshold was established when animals failed to achieve 70% accuracy at a spatial frequency. In order to assess the validity of this estimate, the spatial frequency of the grating was reduced by 3-4 cycles, and the experimental procedures described above were repeated until a stable pattern of performance was established. The performance at each spatial frequency was averaged for each animal and a frequency-of-seeing curve was constructed. The point at which the curve intersected 70% accuracy was recorded as the grating acuity. Dystrophic RCS rats were tested until the age at which they could not discriminate a black screen from a white screen (0.015 c/d), at which point they were judged to be blind.

Contrast sensitivity was assessed using similar procedures, except that the minimal contrast required to differentiate between the screens at different spatial frequencies was measured. Contrast thresholds were measured after grating acuity was assessed, so that minimal re-training was required. Seven spatial frequencies were tested; 0.059, 0.119, 0.208, 0.297, 0.505, 0.712, and 0.890 c/d. At each spatial frequency, trials were initiated at 100% contrast and the contrast was decreased systematically until performance fell below 70% accuracy. The contrast threshold was measured independently at least three times, after which final values were computed from frequency-of-seeing curves of the combined data.

Statistical Analysis

Student's *t*-test was used to compare acuities of the Long-Evans and non dystrophic RCS rats. Repeated measures analyses of variance (ANOVA) were used to

compare the contrast sensitivities of those groups and examine the acuity of the dystrophic RCS rats with time. The probability level at which the null hypothesis was rejected is represented by p; statistical significance was at $p<0.05$.

Results

Training

All rats readily learned to associate swimming to the platform with escape from the water. About 100 trials were required for animals to reach 90% accuracy over 40 trials and there were no obvious group differences in this ability. All animals learned serendipitously to grasp the end of the divider and inspect both screens before making their choice.

Spatial vision of non dystrophic RCS and Long-Evans rats

During the course of testing, both non-dystrophic RCS and Long-Evans rat strains performed the Visual Water Task with near perfect accuracy until the spatial frequency was increased to approximately 0.80 c/d. At around this point, both strains began to make errors in discriminating the stimuli, but still performed above 70% correct until about 1.0 c/d. The average grating acuity of the strains was slightly higher than 1.0 c/d, (Long-Evans=1.03 c/d (SE=0.004), c-RCS=1.01 c/d (SE=0.0009)) and did not differ statistically ($t_{(3)}$= 0.050, P=0.963) (Figure 5). The acuities of the Long-Evans animals were comparable to what we and others have reported previously (Dean, 1981a; Prusky, 2000).

Contrast sensitivity functions of Long-Evans and non-dystrophic RCS rats revealed typical inverted "U"-shaped functions that did not differ significantly, one from the other ($F_{(6,48)}$=1.493, P=0.201)(Figure 6). Both strains had peak sensitivities at 0.208

c/d, and hypothetical intersections of the curves with spatial frequency at around 1.0 c/d.

There was no evidence of strain-related differences in performance in the tests that might reflect different cognitive capabilities.

Acuity of Dystrophic RCS rats with age

The grating acuity of dystrophic RCS rats was tested each month over the course of eleven months. Mean acuities were determined from individual frequency-of-seeing curves (Figure 7). Acuity fell from 0.82 c/d at one month of age to 0.32 c/d by four months of age (Figure 8). The acuity deteriorated thereafter at a slower rate until the tenth month of age when the best animals could only distinguish a black screen from a white screen (one cycle displayed across the two screens, 1 cycle/60° = 0.015 c/d)($F_{(10,50)}$=73.749; $p<0.000$; All SEM<0.057). By the eleventh month, none of the animals were able to distinguish between a black and a white screen and all were thus considered blind. The competence of animals to perform the task did not appear to change as their vision deteriorated; each animal, regardless of its acuity, swam to the barrier (choice point) before making a choice. In addition, the behavior of dystrophic animals in the task was indistinguishable from that of non-dystrophic animals, except that their grating threshold declined progressively with age (Figure 7). However, it was evident at what stage the dystrophic animals were no longer able to discriminate between black and white screens because they no longer swam to the barrier to make their decision. Instead, each animal swam randomly to one side of the pool and followed the perimeter of the pool wall by using its vibrissae until reaching the platform.

Summary

The results show that non-dystrophic RCS rats have acuity and contrast sensitivity measures that are indistinguishable from Long-Evans strain rats, which are known to have normal spatial vision (Prusky *et al.*, 2000b). An acuity of around 1.0 c/d has been achieved in normal pigmented rats in a number of previous behavioral studies using quite different but often more time-consuming testing regimens (Dean, 1981a), not suitable for the studies undertaken here. In addition, physiological recording from cells in the primary visual cortex of normal pigmented rats has given spatial resolution thresholds of around 1.2 c/d, suggesting that this is the true optimal performance that can be expected in rodents. The contrast sensitivity curve of the RCS rats was essentially identical to that of Long-Evans. A similar "U"-shaped function has also been previously characterized (Keller *et al.*, 2000), but the low frequency fall-off in both strains at 0.059 c/d may have been exaggerated by the small number (2) of cycles on the screen.

Previous studies have shown that genetic mutations, such as those of albinism, can produce large changes in visual acuity (Prusky *et al.*, 2002; Birch *et al.*, 1979). With respect to the present studies, abnormalities in the visual function of dystrophic RCS rats (rdy^-, p^+) are exclusively the consequence of the RPE abnormality and not due to irrelevant background effects within the RCS line. The normality of the non-dystrophic RCS rat demonstrated here is important because it can now serve as a suitable control in many experiments. These results also indicate that there are no inherent photoreceptor defects, and that any visual dysfunction found in the dystrophic RCS rats is caused solely from the mutation in the *Mertk* gene.

This study also shows that spatial vision deteriorates in dystrophic RCS rats in two phases – 1) a phase of rapid deterioration already underway by one month, when the

acuity is 0.82 c/d, and continuing until four months of age, by which age the acuity has reached 0.32 c/d, 2) a phase of slow deterioration of responsiveness, which begins at four months and gradually increases in slope leading to eventual total loss of spatial vision by eleven months. This sequence and the levels of performance possible at even six months of age might not be predicted from previous anatomical and functional studies. Anatomical investigation has shown that while a near complete photoreceptor complement is present at one month of age; there is nevertheless considerable disruption of outer segments. Over the next months, there is a fall in photoreceptor density from 300 photoreceptor cell nuclei per midsagittal section at two months of age (about two nuclei thick) to 100 at three months (a single cell layer)(Sauve *et al.*, 2001; DiLoreto *et al.*, 1998). After that, only a discontinuous layer of single cells is seen, although a few photoreceptors are still present at one year of age and these appear to be cones. Because rods account for more than 95% of photoreceptors in the rat, any raw count of photoreceptors without distinction of type is likely to bias towards rods. There has at this point been no systematic study of changes in cone numbers with age. With time, there are changes in the inner retina (Villegas-Perez *et al.*, 1996; Marc *et al.*, 2003; Jones *et al.*, 2003). Some of the changes result from abnormal vascular formations and from 6 months onwards, there is progressive loss of retinal ganglion cells.

ERG studies indicate that the a wave, an indicator of rod function, is lost by day 55 and the remaining b wave, which is largely cone-dependent, disappears by 80-100 days (Sauve *et* al., 2003). Adaptation studies (Girman *et al.,* 2003) indicate that rod function is severely compromised as early as 3 weeks of age, although there is indication that a slowly adapting response is still present up to 3 months, but this would be unlikely

to play any role in discriminations under the testing conditions used here. Physiological studies (Girman *et al.*, 2003) of single-unit responses in the cerebral cortex of RCS rats show that with time, units become less well tuned to specific stimuli, and by 7 months, units can no longer be isolated that respond to visual stimulation. Multiunit recording studies from the superior colliculus under mesopic conditions (background luminance of 0.02 cd/m^2) show that thresholds increase with age and that by 6 months it is hard to get responses to focal stimulation although responses can still be elicited to full-field stimulation (Girman *et al.*, 2003). Certain reflex responses such as the pupillary light reflex can be elicited albeit with higher threshold levels up to at least 12 months of age (Lucas *et al.*, 2003). However, it has been shown that such responses may be driven by melanopsin-containing ganglion cells and may not need rod and cone photoreceptors (Gooley *et al.*, 2003).

Head tracking to moving stripes is lost in untreated dystrophic rats by 8 weeks (Coffey *et al.*, 2002), whereas at the same age normal rats can track at better than 0.5 c/d. Previous work (Coffey *et al.*, 2002) testing acuity in dystrophic RCS rats also showed deterioration in performance with time, but under the testing conditions used, best performance in non-dystrophics was around 0.38 c/d and by 6 months, dystrophic rats from the same strain as that used here were unable to discriminate stripes of 0.1 c/d. The present test gives optimal performance in non-dystrophics at similar levels to those found in other experiments (Dean, 1981a; Girman *et al.*, 1999) and at 6 months dystrophic rats can still perform at 0.28 c/d. The increased sensitivity of the present method allows better analysis of change with time and titration of the dynamics of such change.

It appears then that there are several variables that affect measures of performance. First is the question of whether rods, cones, or some other cell type such as the melanopsin-containing ganglion cells are responsible for behavior. The present study was conducted under photopic conditions in which rods are likely to be saturated; furthermore, adaptation studies (Girman *et al.,* 2003) and the age at which the ERG a wave is lost suggest that much of the testing in dystrophics is done under conditions in which the rods are likely to be severely dysfunctional or non-functional. The luminance levels that drive melanopsin-mediated responses are much higher than those used here. For these reasons, it is most likely that the test is reflecting the abilities of cones. The second variable is the sensitivity of the particular test. It is known, for example, that in patients with retinal disease, the ERG response can be flat, but the patients may still have a considerable degree of vision (Seiving *et al.,* 1999). In dystrophic RCS rats, the cone-based ERG fails at around 14 weeks which is close to the end of the first phase of deterioration in acuity. Comparisons between our results and those attained previously (Coffey *et al.*, 2002) show that even minor changes in methodology and testing conditions can influence performance outcomes considerably. A third potential variable is the condition of the inner retina, whether changes in cell patterns and synaptic details and, after 6 months of age, the loss of retinal ganglion cells, might all contribute to the decline over time. Finally there is the issue of where in the CNS the input signals are being processed to affect the behavior. Based on previous observations (Lashley *et al.*, 1930), it is likely that high resolution responses are cortically mediated. However it cannot be excluded that at lower spatial frequencies, non-cortical mechanisms may also play a role.

The results from this study also provide a suitable background for assessing the efficacy of techniques aimed at preventing the loss of vision due to retinal disease. The work done here shows that visual dysfunction in dystrophic RCS rats is evident at an early age and progresses in two stages. This work also provides the RCS rat model with quantified measures of spatial vision which is a most important tool when assessing experimental treatment regimens for human retinal degenerative disease.

Chapter 3-Limiting retinal degeneration

3.1 Experimental Treatment Techniques used in Humans

There have been a number of attempts to limit retinal degeneration through cell transplantation in humans with RP and AMD. At least 18 people with RP have received sub-retinal fetal (Das *et al.*, 1999; Radtke *et al.*, 999) or neuroretinal transplants (Kaplan *et al.*, 1997). Another 24 people with AMD have received subretinal RPE transplants (Peyman *et al.*, 1991; Gouras *et al.*, 1996; Algvere *et al.*, 1994, 1997, 1999; Weisz *et al.*, 1999). These techniques used in humans have been successful in determining that cells transplanted sub-retinally can live in the retina without any evidence of rejection or abnormalities; however, none of the transplants has been shown to preserve the retina or spatial vision. There could be a number of reasons why vision wasn't preserved in these patients. First, it is possible that the cells that were transplanted died shortly after the procedure. It is also possible that the area the cells were transplanted into may not be optimal for this type of procedure or that injuring the eye may have detrimental effects that might have counteracted any beneficial effects. Another possibility is that the stage at which these patients had surgery was late in the degeneration of the retina. At this advanced stage there are presumably a limited number of photoreceptors left surviving and functioning in the retina and unfortunately, there was no method employed by the researchers to determine the extent of retinal deterioration (i.e. number of photoreceptors surviving) before the procedure. However, the most important indicator of the procedures success is vision, and the best visual acuity of any of the patients before the procedure was 20/200, which is considered legally blind (an order of magnitude worse

than normal) supporting the notion that the retina was severely compromised even before the surgery.

The transplants used in humans were ineffective as the fetal or neuroretinal transplants were probably unable to establish appropriate connections with the adjacent cell layers. The RPE cell transplants were designed (as a preventative measure) to preserve the photoreceptors still alive and may have had a larger effect had the procedure been done at an earlier stage of retinal degeneration. Therefore, even if the transplants were working to preserve vision, none of the patients had enough spatial vision to preserve in order to see a quantitative difference between patients with transplants and control patients. Another reason the transplants may have appeared to not preserve visual function is that the measures of visual performance which included hand-waving, finger-counting and light-perception (Das *et al.,* 1999), may not have been appropriate (quantitative) or sensitive enough. Any positive effects of the treatment within individuals could have been a sham effect, or more likely, a placebo effect.

3.2 Treatment techniques used in the RCS rat

Laser treatment in the RCS rat has been done in an attempt to eliminate collecting debris in the dead zone, the area of the outer retina that the degenerated outer-segments used to occupy. This treatment is intended to reduce the retinal cell degeneration by eliminating excessive debris buildup. This treatment was shown (Behbehani *et al.*, 1984) to improve ERG recordings up to 25 days of age, and morphological study of the retina showed a significant reduction in the debris accumulation in the treated site. Although the ERG recordings showed improvement of retinal function, the improvement lasted for

only a limited period of time. This short duration of the improvements limits the usefulness of this therapy for human studies.

Gene therapy in rats has been shown to reduce significantly the loss of photoreceptors in the treated eye up to two months of age (Dunaief *et al.*, 1995; Vollrath *et al.*, 2001; Akimoto *et al.*, 1999). However, fatalities in human gene therapy experiments (Lehrman *et al.*, 1999; Marshall *et al.*, 1999) have raised safety concerns with this type of therapy.

A number of growth factors have been shown to reduce the loss of photoreceptors in the degenerating retina. These include bFGF, CNTF, and GDNF (Carwile *et al.*, 1998). The use of trophic factors delivered subretinally has been shown to preserve photoreceptors for up to two months after injection; and intra-vitreal injection resulted in even more widespread preservation, across almost the entire retina (Faktorovich *et al.*, 1990). While growth factor delivery may be a viable technique, some growth factors remain in the vitreous for as little as 24 hours requiring other methods of long term delivery to be devised.

A promising technique (being developed) is subretinal cell transplantation. Previous studies have shown that retinal degeneration in the RCS rat can be delayed by the transplantation of an alternative RPE cell type in an attempt to replace the lost function of the defective cells (Lavail *et al.*, 1992). This and other studies have shown that when transplanted, various RPE cell types survive and act to reduce photoreceptor loss, improve ERG recordings and preserve single unit recordings in the superior colliculus (DiLoreto *et al.*, 1998; Yamamoto *et al.*, 1993; Sauve *et al.*, 1998). ARPE19 is an immortalized RPE cell line that exemplifies many features typical of normal RPE cells

(Dunn *et al.*, 1996). There have been a number of recent studies showing the beneficial effects of transplanting ARPE19 cells into the retina of young RCS rats. These studies showed that transplantation of these cells slows the progress of photoreceptor loss (Lund *et al.*, 2001) and the deterioration of retinal sensitivity to light (Yamamoto *et al.*, 1993; Sauve *et al.*, 2002). Subretinal transplantation of ARPE19 cells has also been shown to retain the physiological response specificity of single units in the primary visual cortex of the RCS rat (Girman *et al.*, 2003).

3.3 Effects of ARPE19 transplantation on spatial vision

The majority of studies using the cell transplantation method in the RCS rat have focused on assessing outcomes (rescue or restoration of visual function) by means of ERG and automatic responses such as pupillary light reflex, avoidance behaviors, opto-motor tracking responses (Lawrence *et al.*, 2000), as well as physiological measures such as adaptation and threshold responses recorded in the brain. However, little attention has been devoted to the effects of retinal degeneration on spatial vision. This is surprising given that progressive loss of spatial vision is the most insidious and debilitating consequence of retinal degeneration, and preventing its decline would be the ultimate measure of a successful treatment. Because the effects of cell transplantation on spatial vision have received so little attention, the goal of the present study was designed to evaluate whether the subretinal transplantation of ARPE19 cells can limit the progressive loss of visual acuity in the RCS rat. If this treatment is successful, it will provide essential validation for using cell-based therapies to treat retinal degenerative disease.

Methods

Animals

Thirty pigmented dystrophic (*rdy*$^-$, *p*$^+$) RCS rats were used in this study. The animals were housed and handled with the authorization and supervision of the Institutional Animal Care and Use Committee (IACUC) at the University of Utah, and the University of Lethbridge animal care committee. Every procedure conformed to guidelines of the National Institutes of Health (NIH) and the Canadian Council on Animal Care (CCAC).

Animals were housed in isolated rooms with a 12 hour dark/light cycle, a constant temperature of 22°C, with food and water available ad libitum. They were maintained on the immunosuppressant agent cyclosporin A administered in the drinking water (210mg/l; resulting blood concentration of around 300 μg/liter; Coffey *et al.*, 2002), from 2-3 days prior to transplantation until they were sacrificed after all behavioral testing.

Transplantation

The cell type transplanted was the extended-life human ARPE19 cell line (ATCC; Dunn *et al.*, 1996), which was stored in liquid nitrogen prior to removing, culturing and preparing for transplantation as described elsewhere (Lund *et al.*, 2001). A control group was used in which sham-operated animals received only injection of vehicle. In addition, unoperated non-dystrophic (*rdy+p+*) and dystrophic (*rdy-p+*) rats provided reference material.

All cell transplantations were done in the Ray Lund laboratory and followed the procedure described in Lund *et al.*, 2001. Briefly, 12 rats aged PN21 were anesthetized with 2,2,2 tribromoethanol (230mg/kg) and the eyes were further anesthetized locally with ophthaine. The pupil was dilated with tropicamide and the eye was proptosed slightly using a thread. Cell cultures were trypsinized, washed and delivered as a

suspension in 2µl of Ham's F10 medium through a fine glass pipette (internal diameter 75-150µm) attached by tubing to a 10µl Hamilton syringe into the eye through a small scleral incision (Figure 2). The cell suspension contained about $2x10^5$ ARPE19 cells. The cornea was punctured to reduce intraocular pressure and to limit the efflux of cells. Immediately after injection, the fundus was examined to check for retinal damage or signs of vascular distress. Any animals showing such problems were removed from the study. Donor cells were checked for viability at the beginning and end of a transplantation session using dye exclusion. A typical figure of greater than 95 % intact cells was obtained at both time points. Eighteen sham-injected animals received injection of carrier medium and were otherwise treated identically to the cell transplant group, including receiving cyclosporin A throughout the course of the study.

Visual Water Task

The grating acuity of all animals was assessed using the Visual Water Task, which has been described in detail previously (Prusky *et al.*, 2000a; Prusky et a *et al.*, 2000b; Prusky *et al.*, 2000c; Prusky *et al.*, 2002; McGill *et al.*, 2004). Briefly, the apparatus consisted of a trapezoidal-shaped pool with two computer monitors facing through a clear glass wall into the wide end of the pool and a midline divider extending into the pool from between the monitors, creating a Y-maze with a stem and two arms. Animals are trained to discriminate between different visual stimuli generated and projected on the screens using a computer program (Vista©; CerebralMechanics).

Training and testing followed the same procedure as described elsewhere (McGill *et al.*, 2004) with the following exceptions: animals in this study were tested once per month commencing at four months of age. ARPE19-transplanted and sham-treated

animals were tested up to seven months of age at the University of Lethbridge, at which point they were transported to the University of Utah for physiological and anatomical analyses (Sauve, *et al.,* 2003).

Statistical analysis

Repeated measures analyses of variance (ANOVA) were used to compare ARPE19 and sham-operated groups. The probability level at which the null hypothesis was rejected is represented by p; statistical significance was at $p<0.05$.

Results

Training

All rats readily learned to associate swimming to the platform with escape from the water. On average, about 100 trials (i.e. 4-5 days) were required for animals to reach 90% accuracy over 40 trials and there were no obvious group differences in this ability.

Testing

During testing, all animals maintained a high level of performance at spatial frequencies below threshold and made very few, if any, errors. As the spatial frequencies neared threshold values, the animals made an increasing number of errors until performance dropped below 70%. Although the grating thresholds generally decreased with age, there was no evidence of any accompanying cognitive deficits, motor deficits, or lack of general ability to perform the task. Figure 9 displays frequency-of-seeing curves for a representative animal from each group at four and seven months of age. Note that although the thresholds differ between animals, the shapes of the functions are remarkably similar.

Sham controls

Eighteen animals were used as sham operated controls. These animals were tested from four to seven months of age, over which time the group's average acuity dropped from 0.445 c/d to 0.265 c/d. An analysis of variance confirmed that the visual acuity of the sham animals decreased significantly with age ($F_{(3,45)}$=33.935, P=0.000).

ARPE19

The grating acuity of the twelve dystrophic RCS rats with ARPE19 cell transplants was tested from four to seven months of age. Transplanted animals exhibited an average visual acuity of 0.69 c/d at four months, which fell to 0.38 c/d by seven months. The best animals performed at 0.72 c/d at four months and the best at five months gave a figure of 0.55 c/d. An analysis of variance was used to compare ARPE19 and sham operated control groups. There was a significant effect of age on ARPE19 transplantation ($F_{(3,33)}$=78.652, P=0.000) and a significant interaction of age and ARPE19 treatment ($F_{(3,78)}$=7.147, P=0.000). Posthoc comparisons showed that ARPE19-transplanted animals had a higher acuity than sham controls at all ages. Figure 10 shows the average grating acuity of the ARPE19-transplanted group compared with the sham transplanted animals across all ages tested.

Summary

The results of this study show that the sub-retinal transplantation of human-derived RPE cell line, ARPE19, into the RCS rat retina limits the loss of visual acuity for up to at least seven months of age. At all points tested, the visual (sine-wave grating) acuity of the ARPE19 transplanted group was significantly better than that of the sham-operated group, with an average acuity over twice that of unoperated controls at four months of age. All animals, both transplanted and sham-operated, performed

competently in the task even as their vision degenerated, indicating that only threshold values, and not general behavioral proficiency, changed over time. Similarly, no peripheral conditions of the treatment appeared to affect the ability of animals to distinguish salient visual stimuli, signifying that the effects of cell transplantation can be directly evaluated.

This builds on previous anatomical and physiological work showing that various types of RPE cells, either freshly harvested or immortalized cell lines as used here, preserve not only photoreceptors but also visual responsiveness (Lund *et al.,* 2001; Coffey *et al.,* 2002). A range of visual behaviors have been shown to be preserved by this treatment, including parameters of the ERG response (Sauve *et al.,* 2003) and pupillary light reflex (Whiteley *et al.*, 1996) as well as relative retinal sensitivity in the area of graft preservation (Sauve *et al.*, 2002), single unit physiological responses in the visual cortex (Girman *et al.*, 2003) and head tracking to moving drums (Coffey *et al.*, 2002). Some of these effects, such as improvement in ERG and head tracking, are not generally sustained for long periods, even though measurements of other indicators such as cortical physiology show continued functional rescue. Previous ARPE19 transplant studies in rats used a terrestrial two-choice test to measure visual function, which was only able to record visual thresholds up to 0.35 c/d, even in normal pigmented rats. Although this method was able to discriminate performance in grafted vs unoperated dystrophic RCS rats, it failed to discriminate between grafted and non-dystrophic animals (Coffey *et al.*, 2000). The Visual Water Task, used here, is a higher resolution test which has made it possible to identify small differences in performance among animals and treatment groups. Taken together with the results of previous studies, our results show

that ARPE19 transplantation improves anatomical, physiological and functional measures of the visual system.

Although deterioration of spatial vision in these animals was significantly delayed, it was not stopped completely. Whether this reflected the inadequacy of cyclosporin as a complete immunosuppressant (Crafoord *et al.*, 2000; Del Priore *et al.*, 2003), or a physiological problem associated with other aspects of graft cell survival, or its ability to sustain photoreceptor rescue is unclear. Rejection or immune response was not measured in these animals and therefore it is possible that there was an immune reaction to the grafts. The issues surrounding immune-suppression will be addressed later.

Another measure of transplant success is the level of integration of the transplanted cells with the host retina. Ideally, the implanted cells should occupy an appropriate area on Bruch's membrane and phagocytose shed outer-segments replacing the function of the host RPE cells. Although the transplanted ARPE19 cells integrated themselves along the host RPE layer (Figure 11)(Lund *et al.*, 2001; unpublished data-Lund lab), and they survived in the host retina for a long period of time, there was no evidence that the transplanted cells phagocytosed photoreceptor outer-segments. This raises the question of how these cells function to preserve spatial vision in the RCS rat. In the normal retina, RPE cells phagocytose outer-segments as well as provide structural support and trophic support, such as bFGF (Malecaze *et al.*, 1993) to the photoreceptors. It has been shown that a number of trophic factors can support photoreceptor survival (Carwile *et al.*, 1998), suggesting that the transplanted ARPE19 cells provided the

required trophic support (Dunn *et* al., 1998) for photoreceptor survival which in return translated into improved visual function.

Chapter 4 - Mechanisms of Spatial Vision Deterioration Prevention

4.1 Effects of trophic factors on retinal degeneration

The mechanisms by which photoreceptor degeneration is delayed by subretinal cell transplantation are unclear; however previous studies have provided clues to what these mechanisms might be. In the ARPE19 cell transplantation study, there was no evidence that the transplanted cells assumed the physiological role of phagocytosing outer segments, however there still was functional improvement. The lack of evidence for phagocytosis suggests that there may have been a trophic role performed by the transplanted ARPE19 cells. A previous study (Faktorovich *et al.*, 1990) has shown that intra-vitreal injection of bFGF can preserve photoreceptors across the retina for up to at least two months. In addition, Carwile *et al.*, (1998) showed that CNTF, GDNF, and bFGF can all significantly delay the degeneration of photoreceptors.

The role of NTF's in preserving photoreceptors has been studied using a few different methods. These include injecting NTF's directly into either the vitreous or the subretinal space, vector mediated delivery, and cell transplantation. Direct injection of growth factors into the vitreous or subretinal space has been shown to preserve photoreceptors but it is not known exactly how long the benefits last (longer than two months) or whether multiple injections are needed. The use of viral vectors to deliver NTF's has also been found to preserve photoreceptors; however it is not known how long lasting these effects are.

4.2 Effect of Schwann cell transplantation on spatial vision in the RCS rat

Schwann cells have been shown to continually produce numerous growth factors (Neuberger *et al.*, 1993; Meyer *et al.*, 1992; Sendtner *et al.*, 1992) eliminating the need

for multiple injections. Transplantation of Schwann cell lines secreting BDNF and GDNF has been shown to preserve photoreceptors (Lawrence *et al.*, 2004). In addition, a previous study showed that when congenic Schwann cells are transplanted subretinally they can slow the accompanying loss of visual function as measured by head tracking to moving stripes, and visual receptive fields measured in the superior colliculus (Lawrence *et al.*, 2000); however, there has been no systematic description of how these transplants affect spatial vision. In addition, Schwann cells also have the advantage of being easily harvested from an individual and could be used for transplantation into that same person (syngeneic transplantation). This characteristic alone provides a method to overcome the major complication of immune-suppression, which is especially important when treating the elderly population. The objective of the present study is to evaluate whether the subretinal transplantation of human Schwann cells can limit the progressive loss of visual acuity in the RCS rat.

The rationale of this work is that as potential treatments for human retinal degenerative disease are developed, they are tested in animal models before clinical trials. Here, it was important to establish two important points: first, can human Schwann cells survive following transplantation, and second, can human Schwann cells preserve vision following transplantation.

Methods

Animals

Fifteen pigmented dystrophic (*rdy*$^{+}$, *p*$^{-}$) RCS rats were used in this study. The animals were housed and handled with the authorization and supervision of the Institutional Animal Care and Use Committee (IACUC) at the University of Utah, and

the University of Lethbridge animal care committee. Every procedure conformed to guidelines of the National Institutes of Health (NIH) and the Canadian Council on Animal Care (CCAC).

Animals were housed in isolated rooms with a 12 hour dark/light cycle, a constant temperature of 22°C, and food and water available ad libitum. They were maintained on the immunosuppressant agent cyclosporin A, administered in the drinking water (210mg/l; resulting blood concentration of around 300 μg/liter; Coffey *et al.*, 2002) from 2-3 days prior to transplantation until they were sacrificed after all behavioral testing.

Transplantation

The cell type transplanted was a human Schwann cell culture derived from organ donors (Casella *et al.*, 1996). A sham-injected group utilized fibroblasts as a control cell type (Casella *et al.*, 1996), because it is likely that the cultures were contaminated with a small number of fibroblasts. In addition, unoperated non-dystrophic (*rdy*+, *p*+) and dystrophic (*rdy*-, *p*+) rats provided reference material.

All cell transplantations were done in the Ray Lund laboratory and followed the procedure described in Lawrence *et* al., 2000. Briefly, nine rats aged PN21 received Schwann cell transplants and six rats aged PN21 received fibroblast transplants. All animals were anesthetized with 2,2,2 tribromoethanol (230mg/kg) and the eyes were further anesthetized locally with ophthaine. The pupil was dilated with tropicamide and the eye was proptosed slightly using a thread. Cell cultures were trypsinized, washed and delivered as a suspension in 2μl of Ham's F10 medium through a fine glass pipette (internal diameter 75-150μm) attached by tubing to a 10μl Hamilton syringe into the eye through a small scleral incision. The cell suspension contained about $2x10^4$ Schwann

cells. The cornea was punctured to reduce intraocular pressure and to limit the efflux of cells. Immediately after injection, the fundus was examined to check for retinal damage or signs of vascular distress. Any animals showing such problems were removed from the study. Donor cells were checked for viability at the beginning and end of a transplantation session using dye exclusion. A typical figure of greater than 95 % intact cells was obtained at both time points. All animals were treated identically, including receiving cyclosporin A throughout the course of the study.

Visual Water Task

The grating acuity of all animals was assessed using the Visual Water Task, which has been described in detail previously (Prusky *et al.*, 2000a; Prusky *et al.*, 2000b; Prusky *et al.*, 2000c; Prusky *et al.*, 2002; McGill *et al.*, 2004). Briefly, the apparatus consisted of a trapezoidal pool with two computer monitors facing through a clear glass wall into the wide end of the pool and a midline divider extending into the pool from between the monitors, creating a Y-maze with a stem and two arms. Animals are trained to discriminate between different visual stimuli generated and projected on the screens using a computer program (Vista©; CerebralMechanics).

Training and testing followed the same procedure as described elsewhere (McGill *et al.*, 2004) with the following exceptions: animals in this study were tested once per month commencing at four months of age. The Schwann cell and fibroblast cell transplanted animals were tested up to five months of age at the University of Lethbridge, at which point they were transported to the University of Utah for further study (Wang *et al.*, 2003). In order to collect correlative data at a stage when performance was optimal, post-surgical testing of the Schwann cell group was not extended to seven months of age.

Statistical Analysis

Repeated measures analyses of variance (ANOVA) were used to compare groups. The probability level at which the null hypothesis was rejected is represented by p; statistical significance was at $p<0.05$.

Results

Training

All rats readily learned to associate swimming to the platform with escape from the water. On average, about 100 trials (i.e. four-five days) were required for animals to reach 90% accuracy over 40 trials and there were no obvious group differences in this ability.

Testing

During testing, all animals maintained a high level of performance at spatial frequencies below threshold and made very few, if any, errors. As the spatial frequencies neared threshold values, the animals made an increasing number of errors until performance dropped below 70%. Although the grating thresholds generally decreased with age, there was no evidence of any accompanying cognitive deficits, motor deficits, or lack of general ability to perform the task. Figure 12 displays frequency-of-seeing curves for a representative animal from each group at four and five months of age. Note that although the thresholds differ between animals, the shapes of the functions are remarkably similar.

Fibroblast controls

Six fibroblast controls started the experiment; however, two animals died before the second test date and were thus removed from the study. The remaining four

fibroblast controls were tested at four and five months of age. The average acuity of 0.46 c/d at four months fell to 0.36 c/d by five months of age, however, this change was not significant ($F_{(1,3)}$= 3.968, P=0.140).

Schwann cells

The grating acuities of the dystrophic RCS rats with Schwann cell transplants were tested at four and five months of age. The average acuity of the group was 0.57 c/d at four months of age, which remained unchanged at five months of age (0.55 c/d; no significant effect of age $F_{(1,8)}$=1.561, P=0.247). The best performers gave figures of 0.72 c/d and showed no significant deterioration between the two time points. An analysis of variance was used to compare Schwann cell transplant group with sham operated and fibroblast controls. There was no significant difference between the fibroblast group and the sham group ($F_{(1,18)}$=0.017, P=0.898; Figure 13). There was a significant group effect of Schwann cells when compared with sham animals ($F_{(1,23)}$=10.738, P=0.003) and with fibroblasts ($F_{(1,11)}$=6.017, P=0.029), as well as a significant interaction of age and treatment ($F_{(1,11)}$=5.153, P=0.035). This indicated that the acuity of the Schwann cell group was significantly better than both control groups, even though the fibroblast group did not decrease significantly with age.

Summary

The results show that sub-retinal transplantation of human-derived Schwann cells into RCS rats can preserve the visual acuity in these animals for at least up to five months of age. In collaboration, Ray Lund's lab has shown that human-derived Schwann cells can survive in the RCS rat retina up to at least five months of age, and that following transplantation, the Schwann cells survive as undifferentiated groups of cells along the

RPE cell layer (Figure 14). The work done here also showed that Schwann cell transplanted animals had an average visual acuity twice that of unoperated controls at five months of age, and individual animals retained almost 90% of their original visual acuity at five months of age.

Earlier work on Schwann cell transplantation in RCS rats (Lawrence *et al.*, 2004; Lawrence *et al.*, 2000) showed that the cells survived in the subretinal space, rescued photoreceptors, sustained visual threshold responses recorded from the superior colliculus, slowed the deterioration of head tracking, and preserved the receptive field properties of cortical neurons. Here, the Schwann cells do not appear to have a phagocytotic role ingesting photoreceptor outer-segments, suggesting that any beneficial effects of the transplants are caused solely from the trophic factors produced by the cells. Schwann cells produce a range of neurotrophic factors, including CNTF, GDNF and BDNF (Carwile *et al.*, 1998; Neuberger & De Vries, 1993; Meyer *et al.*, 1992; Sendtner *et al.*, 1992), which are known to preserve photoreceptors when introduced into the eye. Because there is always the possibility of some contamination with fibroblasts in the isolation procedure, a fibroblast control was an important step to rule out the possibility that these cells might be responsible for at least some of the rescue. It should also be noted that the animals were not prescreened and some transplants may have failed. If this was the case, the mean visual acuity of the cell transplanted group is underestimated.

It still remains to be discovered exactly how the Schwann cells are acting to preserve vision in these animals. It is unclear whether the trophic support provided by the Schwann cells is directly affecting the photoreceptors themselves or rather increasing

the rate of phagocytosis in the defective RPE cells, either of which is likely to have a beneficial effect on preserving photoreceptors and consequently promote better vision.

The human-derived Schwann cells used here can survive following transplantation as well as delay the degeneration of visual acuity. This is a promising finding in that this is the first time that human derived Schwann cells have been transplanted into the RCS rat retina. These findings also provide support for using syngeneic Schwann cells transplants for treatment of human retinal degenerative disease.

Chapter 5-Discussion

5.1 Rat Model of Retinal Degeneration

The studies presented here support the use of rats, in particular the RCS rat, for use in studying retinal degenerative disease. Rats have fundamentally similar visual systems and visual capabilities to those of humans. The RCS rat also has a homologous mutation to patients with RP, and analogous photoreceptor loss and visual dysfunction to patients with AMD. The work done here adds to the vast amount of anatomical and physiological work that has been done on the RCS rat. Many previous studies in the RCS rat have determined the rates of structural deterioration and loss of photoreceptors, and of degeneration of physiological responses such as ERGs and single-unit recordings; however, until recently (McGill *et al.*, 2004) there have been no systematic psychophysical measurements of the RCS rat's visual capabilities.

Here, the RCS rat's visual acuity was quantified using the same measurement as those used for determining human visual capabilities (visual acuity). It is important to have comparable measures of visual function because the goals for treatment of human retinal degenerative disease are to provide patients with improved visual function for a long period of time. Because there is no animal model of naturally occurring AMD, the closest analogous model would be one with a RPE cell dysfunction. The RCS rat serves this purpose as well as being a homologous model for the *MERTK* gene mutated form of RP. For the first time, the RCS rat provides researchers with a model of retinal degenerative disease with its visual capabilities characterized allowing for further investigation of possible treatment techniques for humans to be evaluated in this animal model.

Many patients with RP have rhodopsin mutations and there is a rat model for these types of mutations (Dresner *et al.*, 1998). In future studies, it will be important to establish how these mutations affect spatial vision in animals with rhodopsin mutations. Because photoreceptor degeneration occurs in both the RCS and P23H rat, it is conceivable that spatial vision may deteriorate in a similar fashion. If this is the case, then similar methods of preventing photoreceptor loss and spatial vision loss might be effective.

5.2 Cell-Based Therapy

The results of these studies show that cell-based therapies may be an effective technique in the treatment of human retinal degenerative disease. In addition, these studies show that two very different cell types, as those transplanted here can be used to preserve spatial vision in an animal model of human retinal degenerative disease. At all ages tested, ARPE19 and Schwann cell transplanted animals significantly outperformed controls with the best animals retaining up to 70% of normal vision.

In the present study, it is remarkable that at four months the animals with the highest acuity in both the ARPE19 and Schwann cell transplanted groups was better than 0.7 c/d. This is well above the average acuity of sham operated and fibroblast control animals, which degenerated over time with a pattern similar to that observed in un-operated dystrophic RCS rats (McGill *et al.*, 2004). These findings indicate that not all cell types will provide a benefit to visual function, most likely only those which perform physiological functions similar to that of cells found in the normal retina.

One concern is that the mechanism of preservation of photoreceptors using the ARPE19 and Schwann cells remains unclear. It is possible that the activation of *Mertk*

by its ligand (Gas6) in photoreceptor outersegments could retard degeneration by a non-phagocytotic role such as activating synthesis/release of rescue-promoting substances. If this is the case, then both ARPE19 and Schwann cells must express *Mertk*, and fibroblasts must not. However, at present, there appears to be no evidence that any of the cell types used in this experiment express functional *Mertk*. On the other hand, ARPE19 cells do express other genes typical of RPE cells such as RPE65 and CRALBP (Dunn *et al.*, 1996). In addition, the ligand (Gas6) acts as a growth factor on Schwann cells increasing both their size and number (Li *et al.*, 1996), and the effects of Gas6 on ARPE19 cells is largely unknown. Although the experiments done in this thesis show a significant benefit to the visual acuity of treated animals, much work is needed to determine the exact mechanisms these cells are using.

Another concern is that the rescue of photoreceptors and vision may be limited to the local area of the transplants and not across the retina or the entire visual field. Anatomical studies that followed the behavioural work with the Schwann cell transplanted animals showed that the photoreceptor preservation ranged from 25% to 50% of the retina (Wang *et al.*, 2003a). Although the preservation is not universal across the entire retina, this proportion is enough to encompass the human macula and fovea. For human patients with AMD and cone-based RP, preserving the photoreceptors in this area (macula and fovea), should provide the most benefit to visual function. However, human patients with rod-based RP may require a different treatment method, providing a more widespread effect on the retina and peripheral vision.

For possible application clinically, the ARPE19 cell line may have an advantage in being homologous to the defective host RPE and therefore potentially capable of

replacing a number of physiological functions. It does have the drawbacks in not being syngeneic to the recipient and therefore requiring some level of immunosuppression, and in the safety concerns that attend all cell lines. Schwann cells, however, do appear to survive without pathological manifestations in the subretinal space and they do have the advantage that they could be harvested from a peripheral nerve of a patient and introduced into the subretinal space of that same patient. In demonstrating in this study that human Schwann cells have benefit to vision not very different from that of ARPE19 cells (Figure 15) in an animal model of retinal degenerative disease, it is clear that with the potential of performing autologous transplantation, the path from laboratory investigation to clinical trials is significantly simplified with the Schwann cell type.

The results from the studies done in this thesis are directly applicable to RP patients with a mutation in their *MERTK* gene, and that subretinal cell transplantation may delay their corresponding loss of vision. The results in this study are also applicable to the many other forms of blinding diseases that have secondary photoreceptor cell death, such as AMD. It is possible that independent of the primary cause of the disease, the treatment methods used here may prevent the loss of photoreceptors, and more importantly central vision.

5.3 Final Comments and Future Directions

These experiments are exciting in that they show that cell-based transplantation is a possible therapy for limiting the loss of spatial vision resulting from retinal degenerative disease. There are, however, a number of research questions that need to be resolved before formal clinical trials can be undertaken: 1) What is the mechanism by which transplanted cells preserve function? 2) How long can transplanted cells live and

preserve visual function? 3) How long do these treatments affect the visual performance when performed in a state of reduced visual function? 4) Can an autologous (self to self) transplantation technique be developed to avoid the use of immune suppressants that are currently required to prevent the transplanted cells from being attacked by the recipient's immune system.

First, the mechanisms by which photoreceptor degeneration is delayed by subretinal cell transplantation are unclear. Studies have shown that both RPE cells and Schwann cells produce a number of neurotrophic factors (NTF's), and some of these factors have been shown to preserve photoreceptors (Faktorovich *et al.*, 1990). Here, it was shown that subretinal transplantation of ARPE19 and Schwann cells can preserve visual function supporting the hypothesis that NTF's are the necessary component to photoreceptor preservation and survival. A number of methods are available for delivering NTF's subretinally. One such method is using viral vectors, and using this method the NTF GDNF has been shown to slow the rate of degeneration in mutant Tg rhodopsin rats for at least two months (McGee Sanftner *et al.,* 2001). However it remains to be seen whether vector delivered NTF's can preserve visual function, and if so, how long the benefits can last. Answering these questions will address the issues of which method of delivering NTF's is better, single vector delivery, multiple vector deliveries, or a single transplantation of cells.

Secondly, successful subretinal cell transplantation requires that the grafted cells survive and function for extended periods of time following transplantation. Recent experiments have shown that both RPE cells and Schwann cells, when transplanted early (p21) into the RCS rat, can survive up to at least six months of age (Sauve *et al.,* 2002;

Lawrence *et al.*, 2000). In addition, the studies in this thesis have shown that these cell types can preserve vision until at least 7 months of age for the ARPE19 cells and 5 months for the Schwann cells; however, it is not known how much longer the beneficial effects on vision can last.

Thirdly, the results of these studies correlate well with the disease histories of children with blinding diseases because the retinal deterioration found in the RCS rat begins at a very early age. However, the majority of blinding diseases affect the elderly population in which photoreceptor degeneration does not begin until a person's senior years. Also, visual dysfunction is usually not detected until a significant portion of the retina has deteriorated. Therefore, it is important to understand how the transplantation methods used in young animals would affect visual performance in aged animals (which are in a state of reduced visual function) before clinical trials are considered.

Fourth, immune suppression can be avoided with the use of an autologous (self to self) transplantation technique. This procedure involves performing a small biopsy on a peripheral nerve, harvesting and culturing Schwann cells, and transplanting those cells into the subretinal space of the animal that the cells came from. There are three questions to be addressed with this type of study 1) Can the transplanted cells survive without the aid of immune-suppression? 2) Do these grafts preserve vision? 3) Provided they do survive and preserve visual function, how long do the benefits last? All of these questions need to be addressed using an animal model before clinical application.

In summary, the results of these studies should be applicable to clinical therapies for both RP and AMD. It is essential to know whether an animal's central vision is preserved with a treatment such as transplantation, before any clinical application is

attempted. It is also important to evaluate visual performance under conditions of reduced input efficacy, such as the presence of a background, before attempting experimentally to enhance information capture. As a potential therapy, autologous Schwann cell transplantation promises an added benefit of not requiring immune-suppression for the transplanted cells to survive and function, which would be vital when treating elderly patients. As well, delivery of various growth factors via vectors may also be a method of circumventing immune-suppression while preserving visual function. Addressing these questions will contribute both to the understanding of normal visual processing and to the potential of a therapy that might serve to improve visual outcome in a large group of patients with progressive retinal disease.

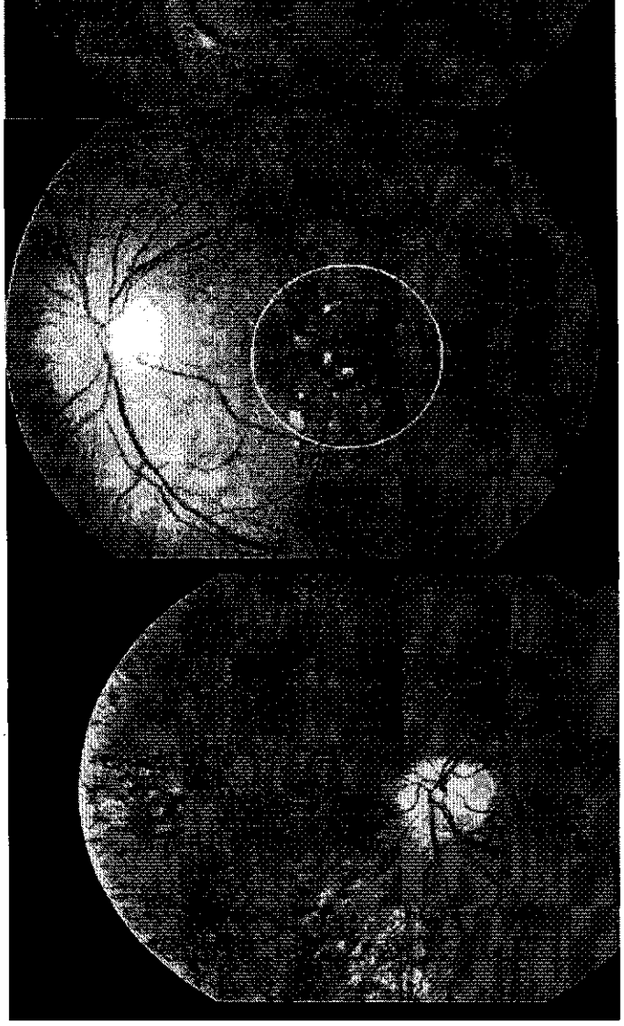

Figure 1. Morphology of a normal retina, an AMD retina and a RP retina. Images were taken from The Retina Center (Permission obtained from Edward Goldman, M.D. at www.focusonretina.com) and Retina Australia (Permission obtained from David McKay at www.eyemac.com.au)

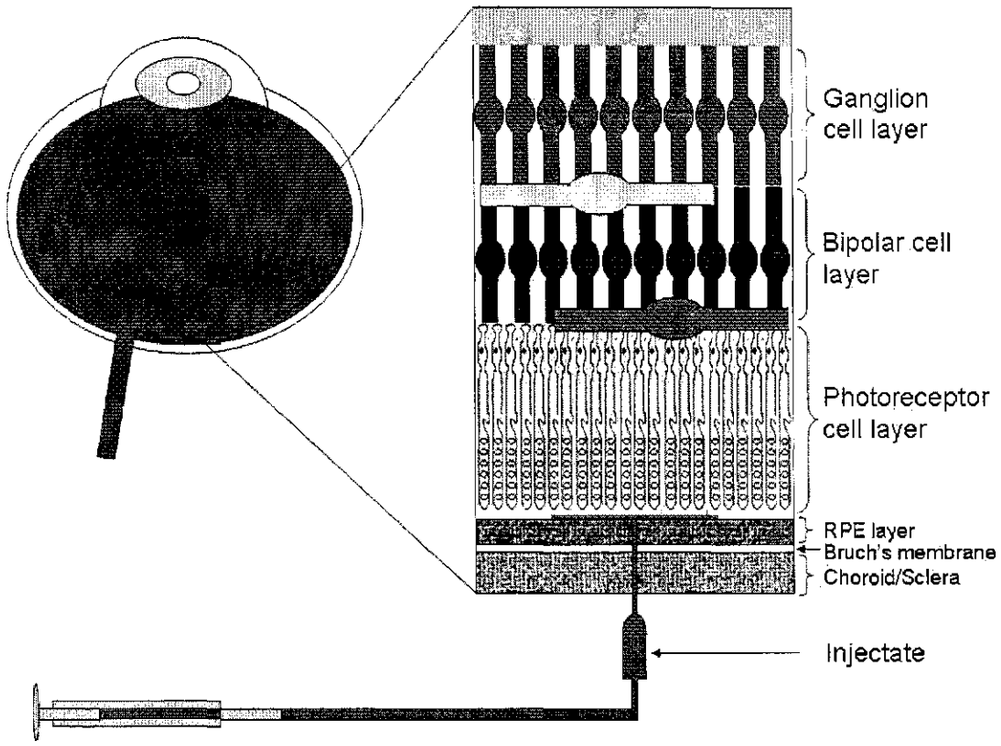

Figure 2. The Figure shows the anatomical components of the retina and the subretinal transplantation procedure.

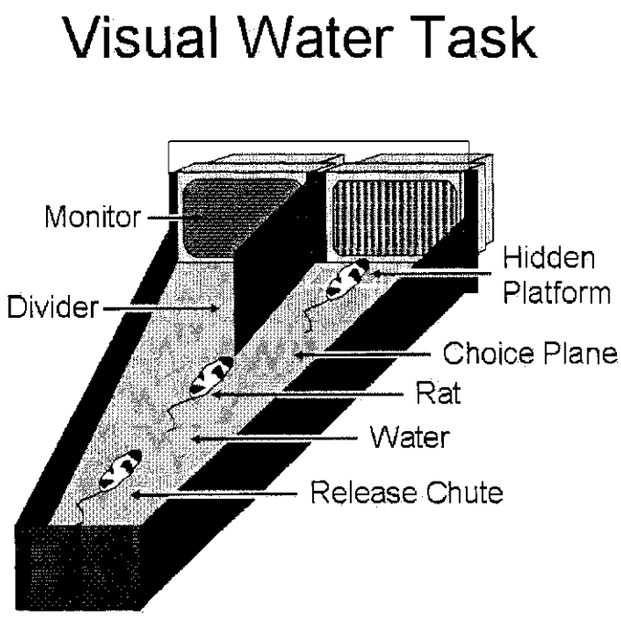

Figure 3. The Visual Water Task.

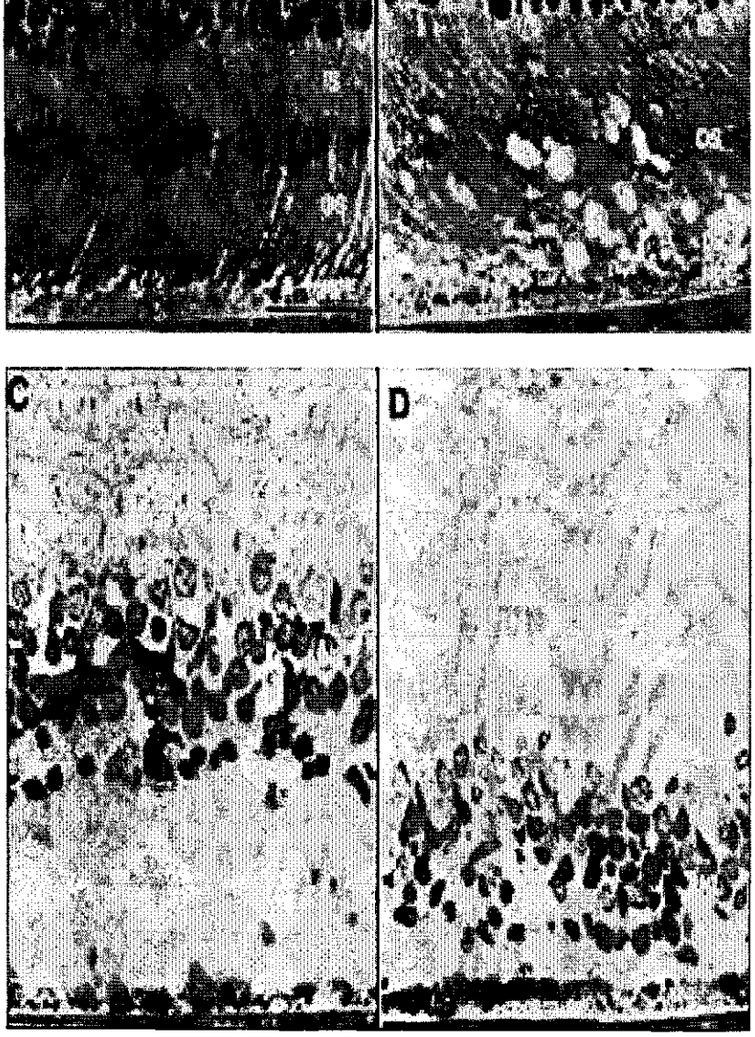

Figure 4. The figure shows photoreceptor degeneration in the RCS rat over the course of six months. Micrograph A was taken from an animal three weeks of age, B from an animal six weeks, C at three months and D and six months. Figure is adapted (with permission) from Dr. Ray Lund's lab (Sauve *et al.*, 2002).

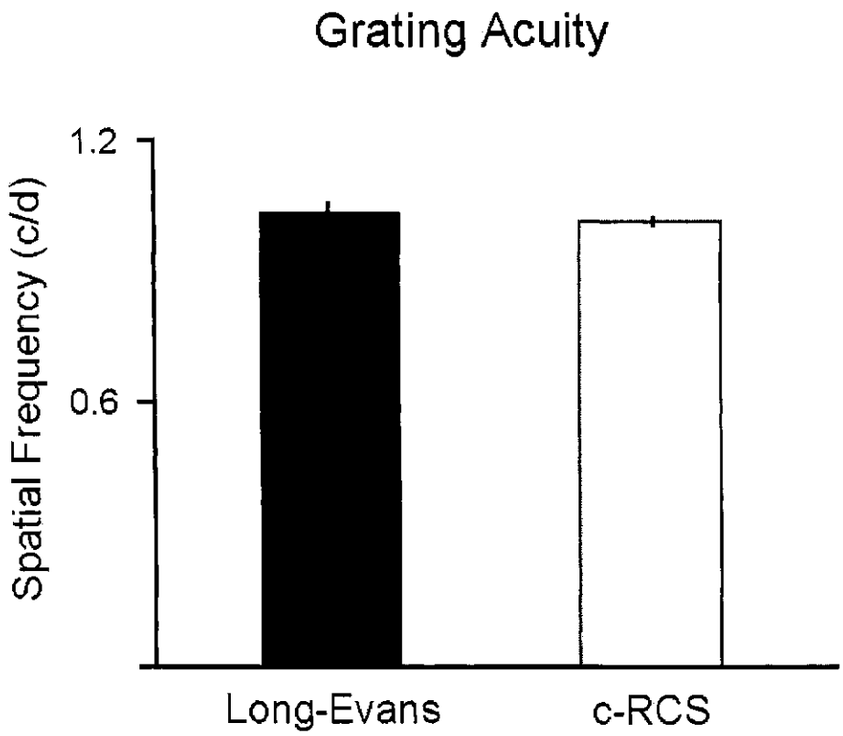

Figure 5. Grating acuity of adult Long-Evans and non-dystrophic RCS rats. Both strains have acuities of approximately 1.0 c/d. There were no significant differences between the groups. See text for details.

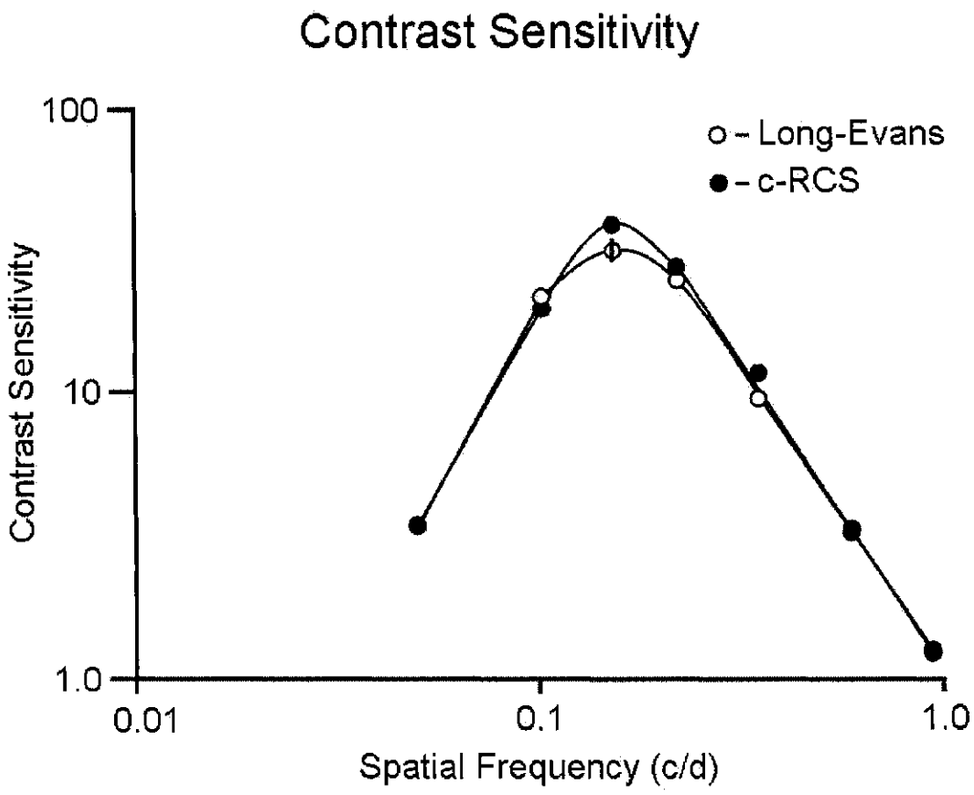

Figure 6. Contrast sensitivity of Long-Evans and non-dystrophic (control (c)) RCS rats measured in the Visual Water Task. Each strain displayed a characteristic inverted "U" shaped function that peaked near 0.2 c/d. There were no significant strain differences in the contrast sensitivity functions. SE for the peak is shown which may be smaller than the data point marker. See text for details.

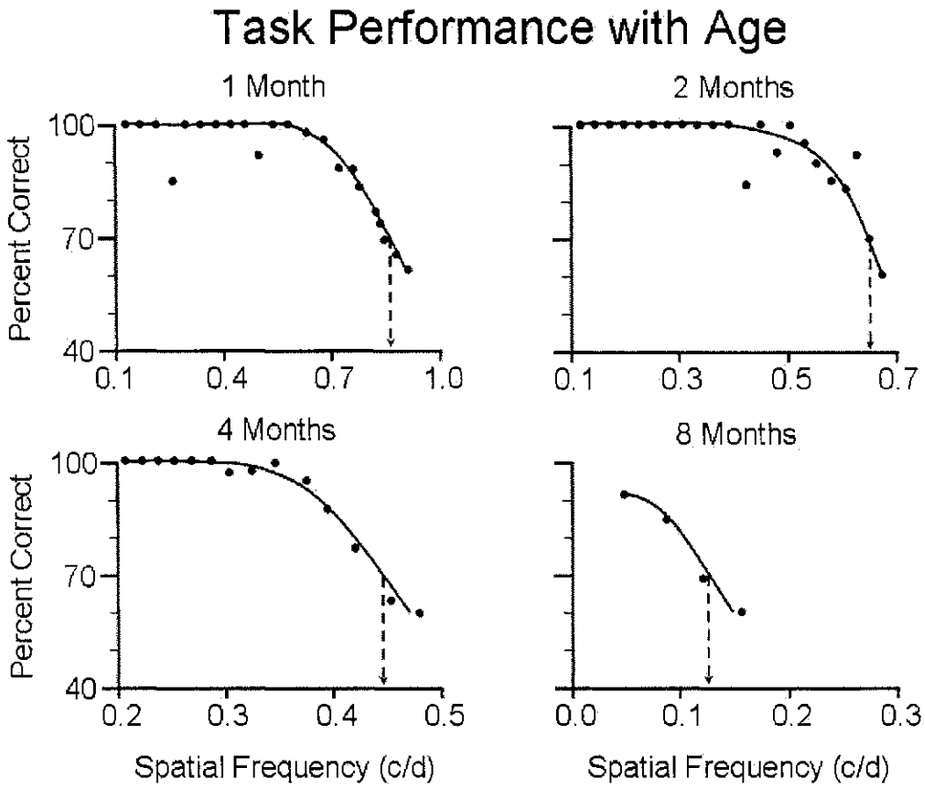

Figure 7. Frequency-of-seeing curves used to calculate the acuity of an individual dystrophic RCS rat at 1 month, 2 months, 4 months and 8 months of age (arrows indicate the grating threshold). A high level of performance was maintained at spatial frequencies below threshold as the animal aged and its acuity decreased. See text for details.

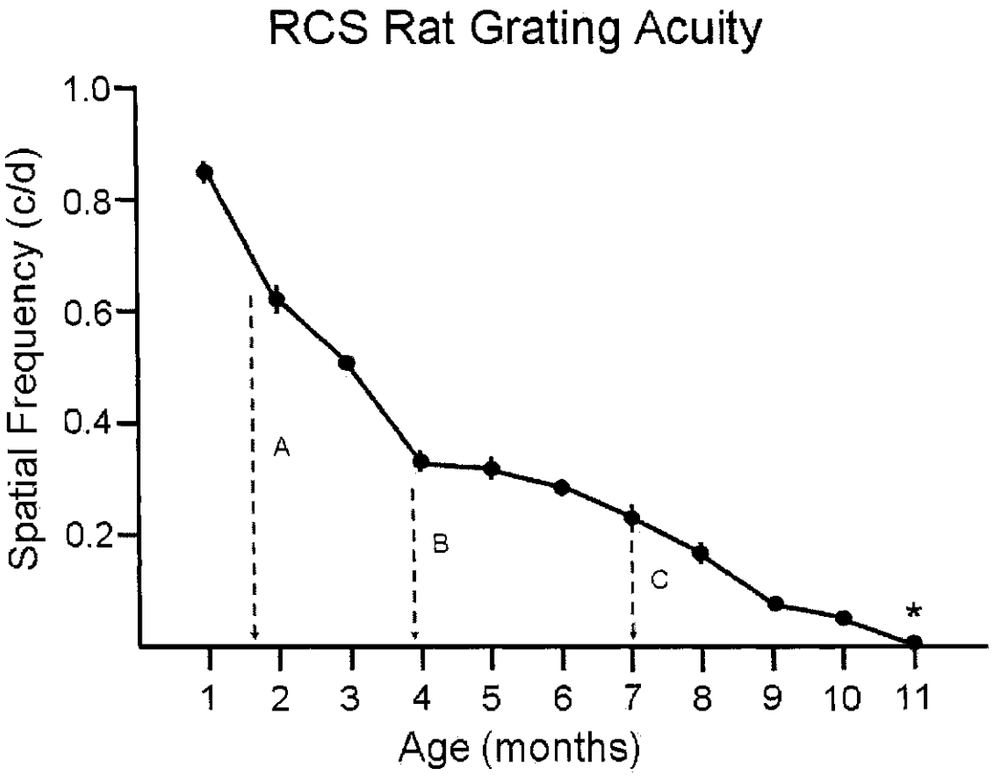

Figure 8. Mean grating acuity of six RCS rat as a function of age. There was a rapid decrease in the grating acuity until 4 months of age. A slower decline in acuity then occurred until blindness at 11 months. The asterisk indicates an inability to determine a black computer screen from a white one. A indicates the age at which RCS rats lose the retinal a-wave (Sauve Y, et al. *IOVS* 2003; ARVO abstract 485), B indicates the age when the b-wave is lost (Sauve Y, et al. *IOVS* 2003; ARVO abstract 485), and C indicates the age when there is a loss of responses in superior colliculus to small spots (Girman S, et al. *IOVS* 2003; ARVO abstract 482). Any non visible SE's are smaller than the data point marker.

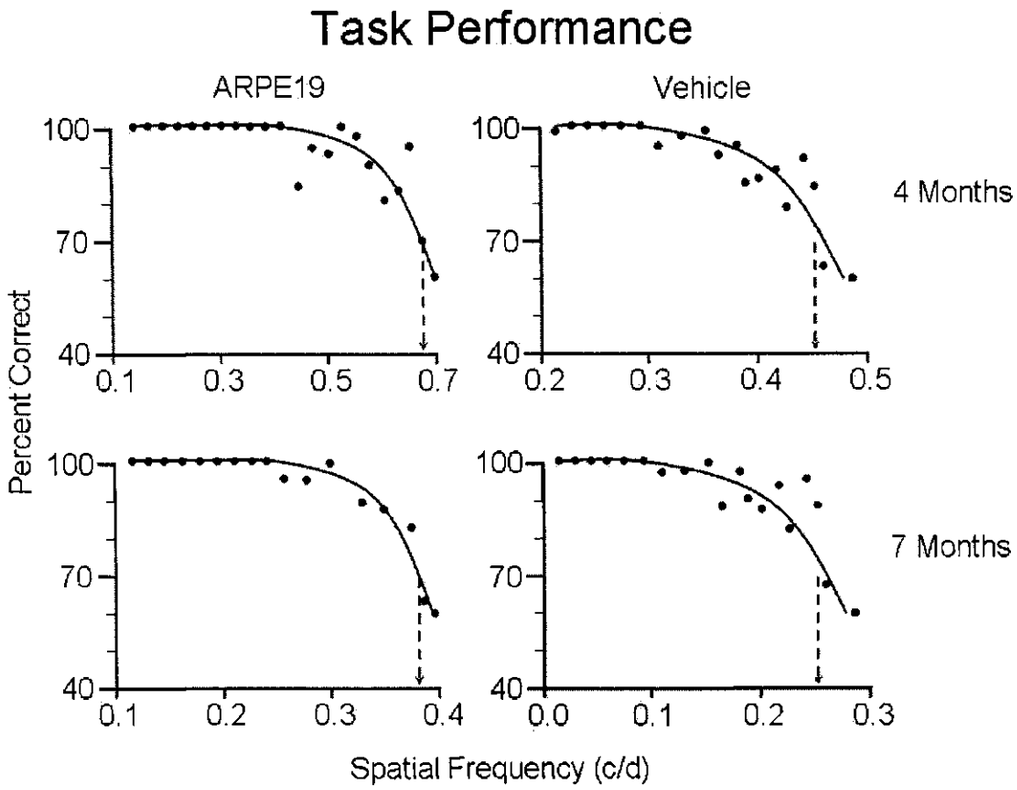

Figure 9. Task performance of individual ARPE19 and vehicle transplanted animals at both four and seven months of age.

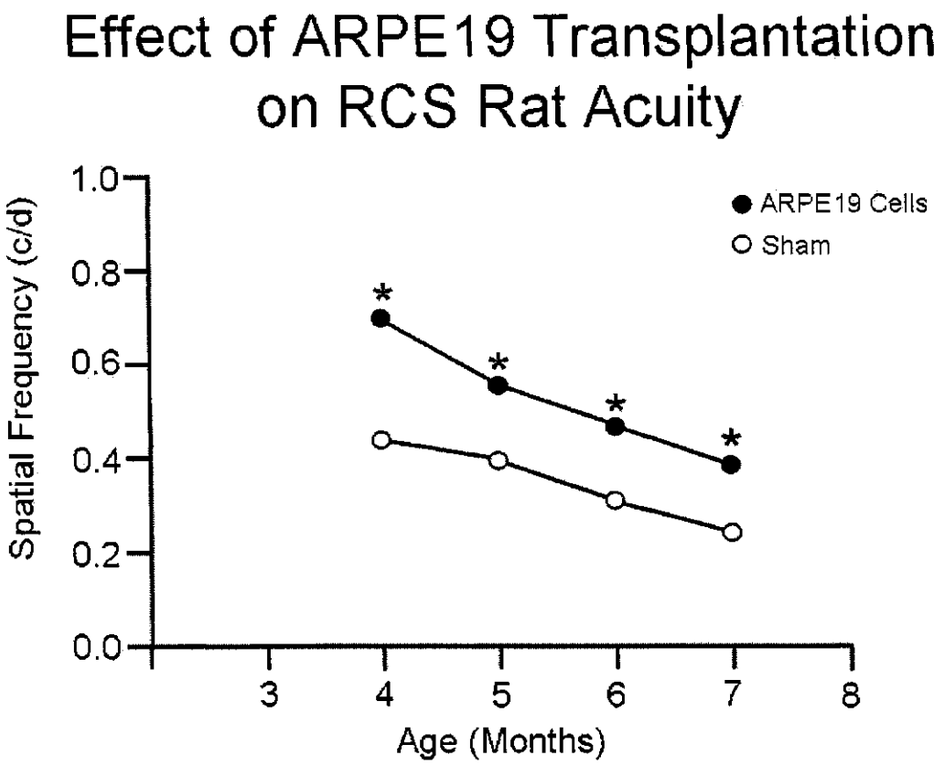

Figure 10. Effect of sub-retinal ARPE19 transplantation on the mean grating acuity of dystrophic RCS rats compared with sham surgery (vehicle injections), measured in the Visual Water Task. The ARPE 19 group performed significantly better than the sham group ($F_{(3,78)}$=7.147, P=0.000). See text for details.

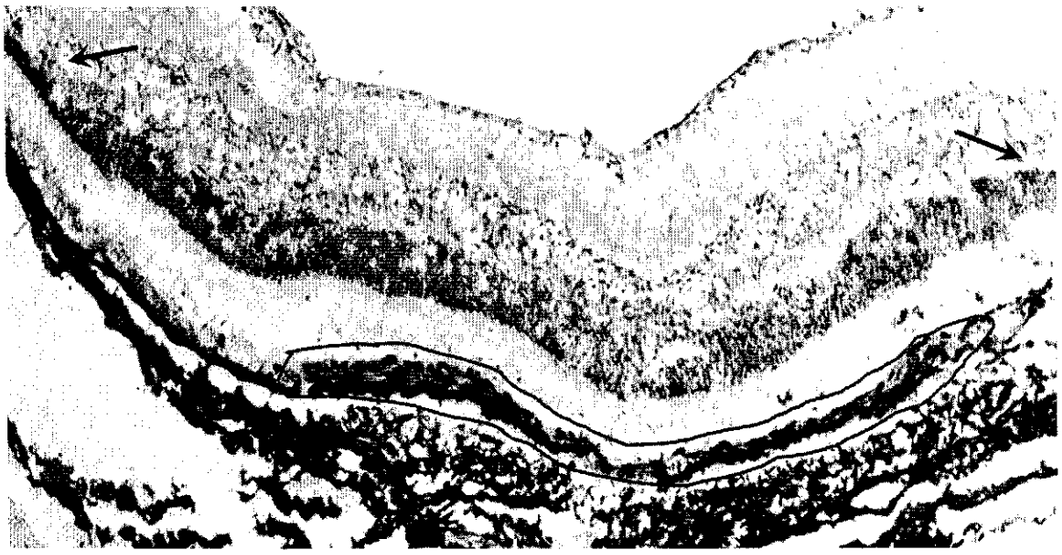

Figure 11. The figure displays ARPE19 cell transplantation into a RCS rat retina. The highlighted area shows the site of ARPE19 cell transplantation at six months of age. The ARPE19 cells appear as a purple cell layer on a dark background, being the host RPE cell layer. The figure is orientated so the inner retina is at the top and the choroid is at the bottom. The arrows indicate areas where photoreceptor survival is impaired which is outside the graft area. (Unpublished data, permission obtained from Dr. Ray Lund)

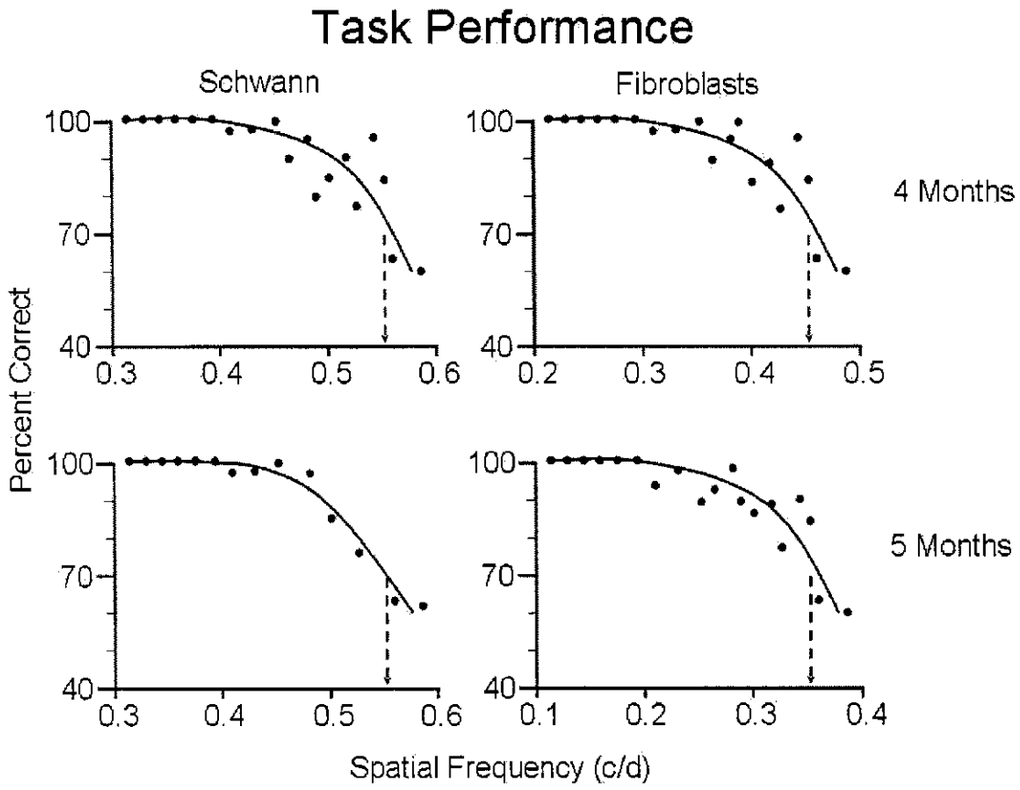

Figure 12. Task performance of individual Schwann cell and fibroblast cell transplanted animals at both four and five months of age.

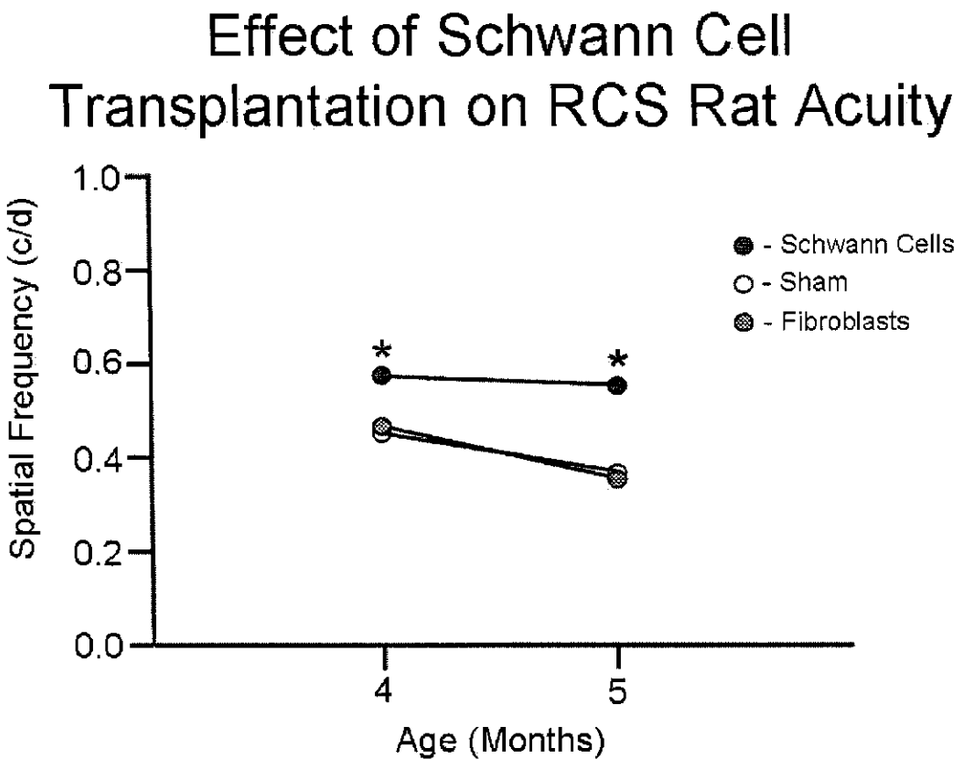

Figure 13. Effect of sub-retinal Schwann cell transplantation on the grating acuity of dystrophic RCS rats compared with sham animals (vehicle injections) and fibroblast controls; measured in the Visual Water Task. The Schwann cell group performed significantly better than both control groups ($F_{(1,11)}$=5.153, P=0.035). See text for details.

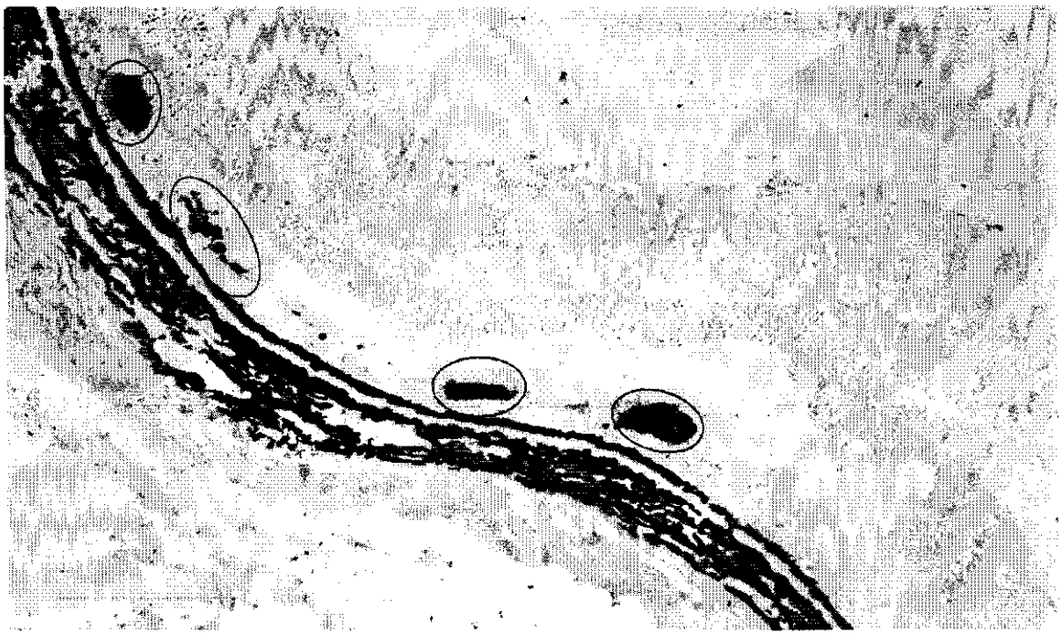

Figure 14. Human derived Schwann cells transplanted into a RCS rat retina. The figure is from an animal six months of age and is orientated so that the inner retina is at the top. The area highlighted shows the Schwann cells as undifferentiated groups of cells between the host RPE cell layer and the photoreceptor outer-segments. The figure also shows a distinguished photoreceptor cell body layer. (Unpublished data, permission obtained from Dr. Ray Lund)

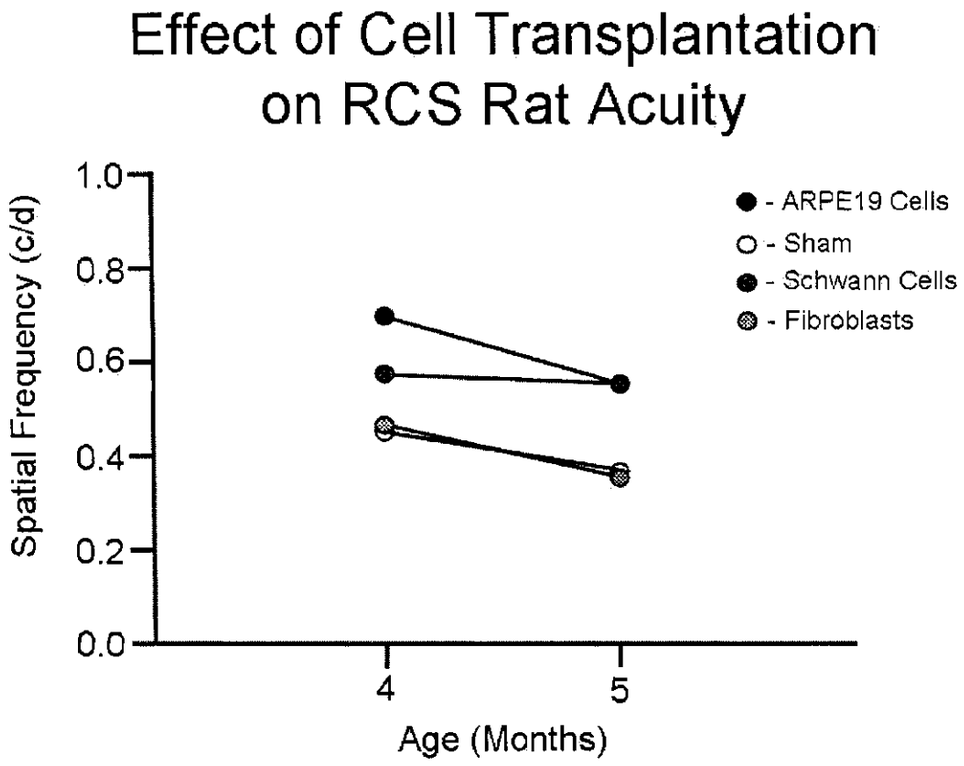

Figure 15. A comparison of the average visual acuity of all groups at four and five months of age. At four months, both ARPE19 and Schwann cell transplantation resulted in a significant benefit to vision when compared to sham and fibroblast groups. The ARPE19 group was also significantly better than the Schwann cell transplanted group. At five months, there was no difference between the ARPE19 cell transplanted group and the Schwann cell transplanted group, however, both ARPE19 and Schwann cell transplanted groups were better than control groups. See text for details.

References

Adler R, Curcio C, Hicks D, Price D, Wong F (1999) Cell death in age-related macular degeneration. Mol Vis 5:31.

Akimoto M, Miyatake S, Kogishi J, Hangai M, Okazaki K, Takahashi J, Saiki M, Iwaki M, Honda Y (1999) Adenovirally expressed basic fibroblast growth factor rescues photoreceptor cells in RCS rats. Invest Ophthalmol Vis Sci 40:273-279.

Algvere P, Berglin L, Gouras P, Sheng Y (1994) Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. Graefes Arch Clin Exp Ophthalmol 232:707-716.

Algvere P, Berglin L, Gouras P, Sheng Y, Kopp E (1997) Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. Graefes Arch Clin Exp Ophthalmol 235:149-158.

Algvere P, Gouras P, Dafgard Kopp E (1999) Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. Eur J Ophthalmol 9:217-230.

Allikmets R (1999) Molecular genetics of age-related macular degeneration: current status. Eur J Ophthalmol 9:255-265.

AREDSRG. (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol 119:1417-1436.

Behbehani M, Bowyer D, Ruffolo J, Kranias G (1984) Preservation of retinal function in the RCS rat by laser treatment. Retina 4:257-263.

Berson E, Rosner B, Sandberg M, Hayes K, Nicholson B, Weigel-DiFranco C, Willett W (1993) A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. Arch Ophthalmol 111:761-772.

Birch D, Jacobs G (1979) Spatial contrast sensitivity in albino and pigmented rats. Vision Res 19:933-937.

Bird A, Bressler N, Bressler S, Chisholm I, Coscas G, Davis M, De Jong P, Klaver C, Klein B, Klein R (1995) An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. Surv Ophthalmol 39:367-374.

Bourne M, Campbell D, Tansley K (1938) Hereditary degeneration of the rat retina. Br J Ophthalmology 22:613-623.

Bowden W, Douglas R, Prusky G (2002) Horizontal Bias in Rat Visual Acuity. Society for Neuroscience (abstract) online.

Bressler N, Group. ToA-RMDwPTTS (2001) Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2. Arch Ophthalmol 119:198-207.

Bundey S, Crews S (1984) A study of retinitis pigmentosa in the City of Birmingham. I Prevalence. J Med Genet 21:417-420.

Bunker C, Berson E, Bromley W, Hayes R, Roderick T (1984) Prevalence of retinitis pigmentosa in Maine. Am J Ophthalmol 97:357-365.

Carwile M, Culbert R, Sturdivant R, Kraft T (1998) Rod outer segment maintenance is enhanced in the presence of bFGF, CNTF and GDNF. Exp Eye Res 66:791-805.

Casella G, Bunge R, Wood P (1996) Improved method for harvesting human Schwann cells from mature peripheral nerve and expansion in vitro. Glia 17:327-338.
Chader G (2002) Animal models in research on retinal degenerations: past progress and future hope. Vision Res 42:393-399.
Coffey P, Girman S, Wang S, Hetherington L, Keegan D, Adamson P, Greenwood J, Lund R (2002) Long-term preservation of cortically dependent visual function in RCS rats by transplantation. Nat Neurosci 5:53-56.
Coffey P, Whiteley S, Lund R (2000) Preservation and restoration of vision following transplantation. Prog Brain Res 127:489-499.
Crafoord S, Algvere P, Kopp E, Seregard S (2000) Cyclosporine treatment of RPE allografts in the rabbit subretinal space. Acta Ophthalmol Scand 78:122-129.
Curcio C, Medeiros N, Millican C (1996) Photoreceptor loss in age-related macular degeneration. Invest Ophthalmol Vis Sci 37:1236-1249.
Curcio C, Millican C (1999) Basal linear deposit and large drusen are specific for early age-related maculopathy. Arch Ophthalmol 117:329-339.
Das T, del Cerro M, Jalali S, Rao V, Gullapalli V, Little C, Loreto D, Sharma S, Sreedharan A, del Cerro C, Rao G (1999) The transplantation of human fetal neuroretinal cells in advanced retinitis pigmentosa patients: results of a long-term safety study. Exp Neurol 157:58-68.
D'Cruz P, Yasumura D, Weir J, M. M, Abderrahim H, LaVail M, Vollrath D (2000) Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. Hum Mol Genet 9:645-651.
Dean P (1981) Visual pathways and acuity hooded rats. Behav Brain Res 3:239-271.
Dean P, Pope S (1981) Visual discrimination learning in rats with lesions of superior colliculus: door-push and approach errors in modified jumping stand. Q J Exp Psychol B 33:141-157.
Del Priore L, Ishida O, Johnson E, Sheng Y, Jacoby D, Geng L, Tezel T, Kaplan H (2003) Triple immune suppression increases short-term survival of porcine fetal retinal pigment epithelium xenografts. Invest Ophthalmol Vis Sci 44:4044-4053.
Dentchev T, Milam A, Lee V, Trojanowski J, Dunaief J (2003) Amyloid-beta is found in drusen from some age-related macular degeneration retinas, but not in drusen from normal retinas. Mol Vis 14:184-190.
DiLoreto DJ, del Cerro C, Cox C, del Cerro M (1998) Changes in visually guided behavior of Royal College of Surgeons rats as a function of age: a histologic, morphometric, and functional study. Invest Ophthalmol Vis Sci 39:1058-1063.
Dowling J, Sidman R (1962) Inherited retinal dystrophy in the rat. J Cell Biol 14:73-109.
Drenser K, Timmers A, Hauswirth W, Lewin A (1998) Ribozyme-targeted destruction of RNA associated with autosomal-dominant retinitis pigmentosa. Invest Ophthalmol Vis Sci 39:681-689.
Dunaief J, Kwun R, Bhardwaj N, Lopez R, Gouras P, Goff S (1995) Retroviral gene transfer into retinal pigment epithelial cells followed by transplantation into rat retina. Hum Gene Ther 6:1225-1229.
Dunn K, Aotaki-Keen A, Putkey F, Hjelmeland L (1996) ARPE-19, a human retinal pigment epithelial cell line with differentiated properties. Exp Eye Res 62:155-169.

Dunn K, Marmorstein A, Bonilha V, Rodriguez-Boulan E, Giordano F, Hjelmeland L (1998) Use of the ARPE-19 cell line as a model of RPE polarity: basolateral secretion of FGF5. Invest Ophthalmol Vis Sci 39:2744-2749.
Eckmiller R (1997) Learning retina implants with epiretinal contacts. Ophthalmic Res 29:281-289.
Edwards R, Szamier R (1977) Defective phagocytosis of isolated rod outer segments by RCS rat retinal pigment epithelium in culture. Science 197:1001-1003.
Eisner A, Klein M, Zilis J, Watkins M (1992) Visual function and the subsequent development of exudative age-related macular degeneration. Invest Ophthalmol Vis Sci 33:3091-3102.
Engel H, Dawson W, Ulshafer R, Hines M, Kessler M (1988) Degenerative changes in maculas of rhesus monkeys. Ophthalmologica 196:143-150.
Faktorovich E, Steinberg R, Yasumura D, Matthes M, LaVail M (1990) Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. Nature 347:83-86.
Ferris Fr, Fine S, Hyman L (1984) Age-related macular degeneration and blindness due to neovascular maculopathy. Arch Ophthalmol 102:1640-1642.
Gal A, Li Y, Thompson D, Weir J, Orth U, Jacobson S, Apfelstedt-Sylla E, Vollrath D (2000) Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. Nat Genet 26:270-271.
Gartner S, Henkind P (1982) Pathology of retinitis pigmentosa. Ophthalmology 89:1425-1432.
Gaynes B (2003) AREDS misses on safety. Arch Ophthalmol 121:416-417.
Girman S, Lu B, Lund R (2003) Light Adaptation Study in RCS Rats, Untreated and with Subretinal Graft of Human RPE Cells. Invest Ophthalmol Vis Sci Abstract.
Girman S, Sauve Y, R. L (1999) Receptive field properties of single neurons in rat primary visual cortex. J Neurophysiol 82:301-311.
Girman S, Wang S, Lund R (2003) Cortical visual functions can be preserved by subretinal RPE cell grafting in RCS rats. Vision Research 43:1817-1827.
Gooley J, Lu J, Fischer D, Saper C (2003) A broad role for melanopsin in nonvisual photoreception. J Neurosci 23:7093-7106.
Gorin M, Breitner J, De Jong P, Hageman G, Klaver C, Kuehn M, JM. S (1999) The genetics of age-related macular degeneration. Mol Vis 3:29.
Gouras P, Algvere P (1996) Retinal cell transplantation in the macula: new techniques. Vision Res 36:4121-4125.
Gu S, Thompson D, Srikumari C, Lorenz B, Finckh U, Nicoletti A, Murthy K, Rathmann M, Kumaramanickavel G, Denton M, Gal A (1997) Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. Nat Genet 17:194-197.
Haimovici R, Gantz D, Rumelt S, Freddo T, Small D (2001) The lipid composition of drusen, Bruch's membrane, and sclera by hot stage polarizing light microscopy. Invest Ophthalmol Vis Sci 42:1592-1599.
Hetherington L, Benn M, Coffey P, Lund R (2000) Sensory capacity of the royal college of surgeons rat. Invest Ophthalmol Vis Sci 41:3979-3983.
Husain D, Kramer M, Kenny A, Michaud N, Flotte T, Gragoudas E, Miller J (1999) Effects of photodynamic therapy using verteporfin on experimental choroidal

neovascularization and normal retina and choroid up to 7 weeks after treatment. Invest Ophthalmol Vis Sci 40:2322-2331.
Imaki H, Moretz R, Wisniewski H, Neuringer M, Sturman J (1987) Retinal degeneration in 3-month-old rhesus monkey infants fed a taurine-free human infant formula. J Neurosci Res 18:602-614.
Jacobs G, Fenwick J, Williams G (2001) Cone-based vision of rats for ultraviolet and visible lights. J Exp Biol 204:2439-2446.
Johnson P, Lewis G, Talaga K, Brown M, Kappel P, Fisher S, Anderson D, Johnson L (2003) Drusen-associated degeneration in the retina. Invest Ophthalmol Vis Sci 44:4481-4488.
Jones B, Watt C, Frederick J, Baehr W, Chen C, Levine E, Milam A, Lavail M, Marc R (2003) Retinal remodeling triggered by photoreceptor degenerations. J Comp Neurol 464:1-16.
Kalloniatis M, Fletcher E (2004) Retinitis Pigmentosa: understanding the clinical presentation mechanisms and treatment options. Clin Exp Optom 87:65-80.
Kaplan H, Tezel T, Berger A, Wolf M, Del Priore L (1997) Human photoreceptor transplantation in retinitis pigmentosa. A safety study. Arch Ophthalmol 115:1168-1172.
Kaplan J, Bonneau D, Frezal J, Munnich A, Dufie rJ (1990) Clinical and genetic heterogeneity in retinitis pigmentosa. Hum Genet 85:635-642.
Keller J, Strasburger H, Cerutti D, Sabel B (2000) Assessing spatial vision - automated measurement of the contrast-sensitivity function in the hooded rat. J Neurosci Methods 97:103-110.
Klaver C, Allikmets R (2003) Genetics of macular dystrophies and implications for age-related macular degeneration. Dev Ophthalmol 37:155-169.
Klein R, Klein B, Linton K (1992) Prevalence of age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology 99:933-943.
Klein R, Klein B, Tomany S, Meuer S, Huang G (2002) Ten-year incidence and progression of age-related maculopathy: The Beaver Dam eye study. Ophthalmology 109:1767-1779.
Kwan A, Wang S, Lund R (1999) Photoreceptor layer reconstruction in a rodent model of retinal degeneration. Exp Neurol 159:21-33.
Lashley K (1930) The mechanism of vision: I. A method for rapid analysis of pattern vision in the rat. J Gen Psych 37:453-460.
LaVail M (1981) Photoreceptor characteristics in congenic strains of RCS rats. Invest Ophthalmol Vis Sci 20:671-675.
Lavail M, Li L, Turner J, Yasumura D (1992) Retinal pigment epithelial cell transplantation in RCS rats: normal metabolism in rescued photoreceptors. Exp Eye Res 55:555-562.
LaVail M, Sidman R, Gerhardt C (1975) Congenic strains of RCS rats with inherited retinal dystrophy. J Hered 66:242-244.
Lawrence J, Keegan D, Muir E, Coffey P, Rogers J, Wilby M, Fawcett J, Lund R (2004) Transplantation of Schwann cell line clones secreting GDNF or BDNF into the retinas of dystrophic Royal College of Surgeons rats. Invest Ophthalmol Vis Sci 45:267-274.

Lawrence J, Sauve Y, Keegan D, Coffey P, Hetherington L, Girman S, Whiteley S, Kwan A, Pheby T, Lund R (2000) Schwann cell grafting into the retina of the dystrophic RCS rat limits functional deterioration. Royal College of Surgeons. Invest Ophthalmol Vis Sci 41:518-528.

Lehrman S (1999) Virus treatment questioned after gene therapy death. Nature 401:517-518.

Li L, Turner J (1988) Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. Exp Eye Res 47:911-917.

Li R, Chen J, Hammonds G, Phillips H, Armanini M, Wood P, Bunge R, Godowski P, Sliwkowski M, Mather J (1996) Identification of Gas6 as a growth factor for human Schwann cells. J Neurosci 16:2012-2019.

Li Z, Wong F, Chang J, Possin D, Hao Y, Petters R, Milam A (1998) Rhodopsin transgenic pigs as a model for human retinitis pigmentosa. Invest Ophthalmol Vis Sci 39:808-819.

Liang F, Dejneka N, Cohen D, Krasnoperova N, J. L, Maguire A, Dudus L, Fisher K, Bennett J (2001) AAV-mediated delivery of ciliary neurotrophic factor prolongs photoreceptor survival in the rhodopsin knockout mouse. Mol Ther 3:241-248.

Liang J, Williams D, Miller D (1997) Supernormal vision and high-resolution retinal imaging through adaptive optics. J Opt Soc Am A 14:2884-2892.

Lopez R, Gouras P, Brittis M, Kjeldbye H (1987) Transplantation of cultured rabbit retinal epithelium to rabbit retina using a closed-eye method. Invest Ophthalmol Vis Sci 28:1131-1137.

Lucas R, Hattar S, Takao M, Berson D, Foster R, Yau K (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. Science 299:245-247.

Lue C (1994) Rod cell activity in retinal degenerative rats. J Formos Med Assoc 93:605-610.

Lund R, Adamson P, Sauve Y, Keegan D, Girman S, Wang S, Winton H, Kanuga N, Kwan A, Beauchene L, Zerbib A, Hetherington L, Couraud P, Coffey P, Greenwood J (2001) Subretinal transplantation of genetically modified human cell lines attenuates loss of visual function in dystrophic rats. Proc Natl Acad Sci 98:9942-9947.

Malecaze F, Mascarelli F, Bugra K, Fuhrmann G, Courtois Y, Hicks D (1993) Fibroblast growth factor receptor deficiency in dystrophic retinal pigmented epithelium. J Cell Physiology 154:631-642.

Marc R, Jones B, Watt C, Strettoi E (2003) Neural remodeling in retinal degeneration. Prog Retin Eye Res 22:607-655.

Marlhens F, Bareil C, Griffoin J, Zrenner E, Amalric P, Eliaou C, Liu S, Harris E, Redmond T, Arnaud B, Claustres M, Hamel C (1997) Mutations in RPE65 cause Leber's congenital amaurosis. Nat Genet 17:139-141.

Marshall E (1999) Gene therapy death prompts review of adenovirus vector. Science 286:2244-2245.

Maw M, Kennedy B, Knight A, Bridges R, Roth K, Mani E, Mukkadan J, Nancarrow D, Crabb J, Denton M (1997) Mutation of the gene encoding cellular retinaldehyde-binding protein in autosomal recessive retinitis pigmentosa. 17:198-200.

McGee Sanftner L, Abel H, Hauswirth W, Flannery J (2001) Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa. Mol Ther 4:622-629.

McGill T, Douglas R, Lund R, Prusky G (2004) Quantification of spatial vision in the Royal College of Surgeons rat. IOVS 45:932-936.

Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H (1992) Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. J Cell Biol 119:45-54.

Milam A, Li Z, Fariss R (1998) Histopathology of the human retina in retinitis pigmentosa. Progress in Retinal and Eye Research 17:175-205.

Miller H, Miller B, Ryan S (1986) The role of retinal pigment epithelium in the involution of subretinal neovascularization. Invest Ophthalmol Vis Sci 27:1644-1652.

Miller J, Schmidt-Erfurth U, Sickenberg M, Pournaras C, Laqua H, Barbazetto I, Zografos L, Piguet B, Donati G, Lane A, Birngruber R, van den Berg H, Strong A, Manjuris U, Gray T, Fsadni M, Bressler N, Gragoudas E (1999) Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration: results of a single treatment in a phase 1 and 2 study. Arch Ophthalmol 117:1161-1173.

Mitchell P, Smith W, Attebo K, Wang J (1995) Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. Ophthalmology 102:1450-1460.

Mohan K, Shukla D, Namperumalsamy P, Kim R (2003) Management of age-related macular degeneration. J Indian Med Assoc 101:471-474, 476.

Muir D, Mitchell D (1975) Behavioral deficits in cats following early selected visual exposure to contours of a single orientation. Brain Res 85:459-477.

Mullen R, LaVail M (1976) Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. Science 192:799-801.

Narfstrom K, Nilsson S (1987) Hereditary rod-cone degeneration in a strain of Abyssinian cats. Prog Clin Biol Res 247:349-368.

Neuberger T, De Vries G (1993) Distribution of fibroblast growth factor in cultured dorsal root ganglion neurons and Schwann cells. II. Redistribution after neural injury. J Neurocytol 22:449-460.

Neve A, Prusky G, Douglas R (2002) Perception of Motion Coherence in Rats. SFN Program No. 353.17.

Petrukhin K, Koisti M, Bakall B, Li W, Xie G, Marknell T, Sandgren O, Forsman K, Holmgren G, Andreasson S, Vujic M, Bergen A, McGarty-Dugan V, Figueroa D, Austin C, Metzker M, Caskey C, C. W (1998) Identification of the gene responsible for Best macular dystrophy. Nat Genet 19:241-247.

Peyman G, Blinder K, Paris C, Alturki W, Nelson NJ, Desai U (1991) A technique for retinal pigment epithelium transplantation for age-related macular degeneration secondary to extensive subfoveal scarring. Ophthalmic Surg 22:102-108.

Prusky G, Douglas R (2003) Developmental plasticity of mouse visual acuity. Eur J Neurosci 17:167-173.

Prusky G, Harker K, Douglas R, Whishaw I (2002) Variation in visual acuity within pigmented, and between pigmented and albino rat strains. Behav Brain Res 136:339-348.

Prusky G, Reidel C, Douglas R (2000) Environmental enrichment from birth enhances visual acuity but not place learning in mice. Behav Brain Res 114:11-15.

Prusky G, West P, Douglas R (2000) Behavioral assessment of visual acuity in mice and rats. Vision Res 40:2201-2209.

Prusky G, West P, Douglas R (2000) Experience-dependent plasticity of visual acuity in rats. Eur J Neurosci 12:3781-3786.

Radtke N, Aramant R, Seiler M, Petry H (1999) Preliminary report: indications of improved visual function after retinal sheet transplantation in retinitis pigmentosa patients. Am J Ophthalmol 128:384-387.

Rasmussen H, Chu K, Campochiaro P, Gehlbach P, Haller J, Handa J, Nguyen Q, Sung J (2001) Clinical protocol. An open-label, phase I, single administration, dose-escalation study of ADGVPEDF.11D (ADPEDF) in neovascular age-related macular degeneration (AMD). Hum Gene Ther 12:2029-2032.

Rivolta C, Sharon D, DeAngelis M, Dryja T (2002) Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. Hum Mol Genet 11:1219-1227.

Sarks S (1982) Drusen patterns predisposing to geographic atrophy of the retinal pigment epithelium. Aust J Ophthalmol 10:91-97.

Sauve Y, Girman S, Wang S, Keegan D, Lund R (2002) Preservation of visual responsiveness in the superior colliculus of RCS rats after retinal pigment epithelium cell transplantation. Neuroscience 114:389-401.

Sauve Y, Girman S, Wang S, Lawrence J, Lund R (2001) Progressive visual sensitivity loss in the Royal College of Surgeons rat: perimetric study in the superior colliculus. Neuroscience 103:51-63.

Sauve Y, Klassen H, Whiteley S, Lund R (1998) Visual field loss in RCS rats and the effect of RPE cell transplantation. Exp Neurol 152:243-250.

Sauve Y, Lu B, Wang S, Lund R (2003) Correlation of Full Field Corneal Electroretinogram (ERG) with Retinal Thresholds Recorded from the CNS in Rodent Retinal Degeneration and Rescue. Invest Ophthalmol Vis Sci Abstract.

Schmidt-Erfurth U, Miller J, Sickenberg M, Laqua H, Barbazetto I, Gragoudas E, Zografos L, Piguet B, Pournaras C, Donati G, Lane A, Birngruber R, van den Berg H, Strong H, Manjuris U, Gray T, Fsadni M, Bressler N (1999) Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration: results of retreatments in a phase 1 and 2 study. Arch Ophthalmol 117:1177-1187.

Seddon J, Ajani U, Sperduto R, Hiller R, Blair N, Burton T, Farber M, Gragoudas E, Haller J, Miller D (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA 272:1413-1420.

Seddon J, Cote J, Rosner B (2003) Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. Arch Ophthalmol 121:1728-1737.

Seigel D (2002) AREDS investigators distort findings. Arch Ophthalmol 120:100-101.

Sendtner M, Stockli K, Thoenen H (1992) Synthesis and localization of ciliary neurotrophic factor in the sciatic nerve of the adult rat after lesion and during regeneration. J Cell Biol 118:139-148.
Sibulesky L, Hayes K, Pronczuk A, Weigel-DiFranco C, Rosner B, Berson E (1999) Safety of <7500 RE (<25000 IU) vitamin A daily in adults with retinitis pigmentosa. Am J Clin Nutr 69:656-663.
Sidman R, Green M (1965) Retinal Degeneration in the Mouse: Location of the RD Locus in Linkage Group XVII. J Hered 56:23-29.
Sieving P, Yashar B, Ayyagari R (1999) Juvenile retinoschisis: a model for diagnostic testing of x-linked ophthalmic disease. Am J Ophthalmol 129:833.
Simpson D (1986) Exudative age-related maculopathy. J Am Optom Assoc 57:113-115.
Smith A, Schlichtenbrede F, Tschernutter M, Bainbridge J, Thrasher A, Ali R (2003) AAV-Mediated gene transfer slows photoreceptor loss in the RCS rat model of retinitis pigmentosa. Mol Ther 8:188-195.
Smith S (1999) The role of antioxidants in AMD: ongoing research. J Ophthalmic Nurs Technol 18:68-70.
Sommer A, Tielsch J, Katz J, Quigley H, Gottsch J, Javitt J, Martone J, Royall R, Witt K, Ezrine S (1991) Racial differences in the cause-specific prevalence of blindness in east Baltimore. N Engl J Med 325:1412-1417.
Suner I, Espinosa-Heidmann D, Marin-Castano M, Hernandez E, S. P-S, SW. C (2004) Nicotine increases size and severity of experimental choroidal neovascularization. Invest Ophthalmol Vis Sci 45:311-317.
Sunness J, Massof R, Johnson M, Bressler N, Bressler S, Fine S (1989) Diminished foveal sensitivity may predict the development of advanced age-related macular degeneration. Ophthalmology 96:375-381.
Ulshafer R, Allen C, Rubin M (1990) Distributions of elements in the human retinal pigment epithelium. Arch Ophthalmol 108:113-117.
Ulshafer R, Engel H, Dawson W, Allen C, Kessler M (1987) Macular degeneration in a community of rhesus monkeys. Ultrastructural observations. Retina 7:198-203.
van den Bergh H, Ballini J, Sickenberg M (2004) On the selectivity of photodynamic therapy of choroidal neovascularization associated with age-related macular degeneration. J Fr Ophtalmol 27:75-78.
Veske A, Nilsson S, Narfstrom K, Gal A (1999) Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. Genomics 57:57-61.
Villegas-Perez M, Vidal-Sanz M, Lund R (1996) Mechanism of retinal ganglion cell loss in inherited retinal dystrophy. Neuroreport 7:1995-1999.
Virdi P, Hayreh S (1982) Ocular neovascularization with retinal vascular occlusion. I. Association with experimental retinal vein occlusion. Arch Ophthalmol 100:331-341.
Vollrath D, Feng W, Duncan J, Yasumura D, D'Cruz P, Chappelow A, Matthes M, Kay M, LaVail M (2001) Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. Proc Natl Acad Sci U S A 98:12584-12589.
Wang S, Lu B, Lawrence J, Wood P, Kolb H, Lund R (2003) Human Schwann Cells Grafted into the Retina of Dystrophic RCS Rats Delay Anatomical Deterioration. SFN Abstract Program No. 453.2.

Wang S, Sauvé Y, Lu B, Wood P, Lawrence JM, Lund R (2003) Human Schwann Cell Grafting into the Retina of the Dystrophic RCS Rat Limits Anatomical and Functional Deterioration. IOVS Online Abstract.

Weisz J, Humayun M, De Juan EJ, Del Cerro M, Sunness J, Dagnelie G, Soylu M, Rizzo L, Nussenblatt R (1999) Allogenic fetal retinal pigment epithelial cell transplant in a patient with geographic atrophy. Retina 19:540-545.

Whiteley S, Litchfield T, Coffey P, Lund R (1996) Improvement of the pupillary light reflex of Royal College of Surgeons rats following RPE cell grafts. Exp Neurol 140:100-104.

Yamamoto S, Du J, Gouras P, Kjeldbye H (1993) Retinal pigment epithelial transplants and retinal function in RCS rats. Invest Ophthalmol Vis Sci 34:3068-3075.

Yang Y (2004) Preserving vision with verteporfin photodynamic therapy. Hosp Med 65:39-43.

Yates J, Moore A (2000) Genetic susceptibility to age related macular degeneration. J Med Genet 37:83-87.