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Prediction and Shaping of Visual Cortex Activity for Retinal Prostheses

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the requirements of the degree of

Doctor of Philosophy

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Abstract

Retinal prostheses are a promising treatment for blindness caused by photoreceptor degeneration. Electrodes implanted in the retina deliver electrical stimuli in the form of current pulses that activate surviving neurons to restore a sense of vision. Clinical trials for such devices have shown that the visual percepts evoked are informative, and can improve the day-to-day life of recipients. However, the spatial resolution of retinal prostheses is a limiting factor, with those who have the highest reported acuity measures still classified as legally blind. Simultaneous stimulation of multiple electrodes is a possible strategy to improve device resolution without increasing the number of physical electrodes. However, electrode interactions that occur during simultaneous stimulation are not well understood. This thesis investigates the characteristics of cortical responses to simultaneous stimulation of multiple electrodes.

We formulated a quantitative model to characterise the responses of visual cortex neurons to multi-electrode stimulation of the retina to understand how simultaneous stimulation can improve resolution. Activity was recorded in the visual cortex of normally-sighted, anaesthetised cats in response to temporally sparse, spatially white stimulation with 21 or 42 electrodes in the suprachoroidal space of the retina. These data were used to constrain the parameters of a linear-nonlinear model using a spike-triggered covariance technique. The recovered model accurately predicted cortical responses to arbitrary patterns of stimulation, and demonstrated that interactions between electrodes are predominantly linear. The linear filters of the model, which can be considered as weighting matrices for the effect of the stimulating electrodes on each cortical site, showed that cortical responses were topographically organised.

Photoreceptor degeneration results in a number of changes in the surviving cells of the retina that can negatively impact stimulation strategies. Therefore, in the second study, we investigated the effect of multi-electrode stimulation on the degenerate retina. Characteristics of cortical responses to simultaneous stimulation of multiple electrodes

were evaluated in unilaterally, chronically blind anaesthetised cats, bilaterally implanted with suprachoroidal retinal prostheses. Significant differences were found between responses to stimulation of the normally sighted and blind eyes, which may help to explain the varied perceptual observations in clinical trials with simultaneous stimulation.

The success of the linear-nonlinear model in predicting responses to arbitrary patterns of stimulation indicated that it may provide a basis for optimising stimulation strategies to shape cortical activity. Therefore, we investigated the possibility of inverting the model to generate stimuli aimed at reliably altering the spatial characteristics of cortical responses. An *in vivo* preparation with a normally sighted, anaesthetised cat showed that the response characteristics derived by the model could be exploited to steer current and evoke predictable cortical activity.

Overall, these results demonstrate that cortical responses to simultaneous stimulation of both the normal and degenerate retina are repeatable, and can be predicted by a simple linear-nonlinear model. Furthermore, the interactions between electrodes are predominantly linear, and can be harnessed to shape cortical activity through inversion of the model. The method shows promise for improving the efficacy of retinal prostheses and patient outcomes.

Declaration

I hereby declare that this thesis comprises of my original work towards the degree of Doctor of Philosophy at the University of Melbourne. All work included in this thesis, except where acknowledged in the Declaration of Authorship, is my original work. All other work has been duly acknowledged.

This thesis is fewer than the maximum word limit of 100,000 words exclusive of tables, figures, bibliographies and appendices.

Signed: Kerry Halupka

A handwritten signature in black ink, appearing to read 'Kerry Halupka', written in a cursive style.

13th April, 2017

Declaration of Authorship

I hereby declare that this thesis and the work presented in it are original and generated by me as the result of my own investigations. Except where acknowledged below, I was responsible for the data collection, data analysis, software programming, and generation of images and graphical data.

Due to the multidisciplinary aspect of this work, the following thesis would not have been possible if it were not for the contributions detailed below.

- Joel Villalobos and Chris Williams designed the stimulating arrays.
- Owen Burns and Vanessa Maxim fabricated the stimulating arrays.
- Penelope Allen and Chi Luu surgically implanted the stimulating arrays.
- Carla Abbott, Alice Brandli, Alexia Saunders, Michelle McPhedran, Alison Neil, Dimitra Stathopoulos, Stephanie Epp, and Ceara McGowan assisted with electrophysiological experiments and animal handling during surgery.
- Evgeni Sergeev developed the stimulator software.
- James Fallon developed the software used to perform artefact removal from electrophysiological data.
- Thomas Spencer, Faith Lamont, Ali Almasi, Felix Aplin, Rosemary Cicione, Patrick Thien, Emma Johnson and Ronald Leung assisted with data collection during the long overnight shifts.
- Carla Abbott, Chi Luu, and Alice Brandli performed intraocular injection of ATP and clinical assessments of retinal structure and function (full-field flash electroretinogram, optical coherence tomography and funduscopy) in Chapter 3.

The following chapters include published works that have resulted from the research presented in this thesis.

- Chapter 3 is a slightly modified version of the published article:

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Wong, Y.T., Burkitt, A.N., and Meffin, H. Prediction of cortical responses to simultaneous electrical stimulation of the retina. *Journal of Neural Engineering*, 14(1):016006, 2016.

- Chapter 4 is a modified version of the published article:

Halupka, K.J., Abbott, C.J., Wong, Y.T., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Sergeev, E.N., Luu, C.D., Brandli, A., Allen, P.J., Meffin, H., and Shivdasani, M.N. Neural responses to multi-electrode stimulation of healthy and degenerate retina. *Investigative Ophthalmology & Visual Science*, 58(9):3770-3784, 2017.

- While not included in this thesis, the following article was also published during my candidature:

Wong, Y.T., Halupka, K.J., Kamenev T., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Meffin, H., and Shivdasani, M.N. Spectral distribution of local field potential responses to electrical stimulation of the retina. *Journal of Neural Engineering*, 13(3):036003, 2016.

- A number of conference abstracts have also been published:

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Ibbotson, M.R., Meffin, H. Prediction of cortical responses to simultaneous retinal electrical stimulation using a linear-nonlinear model, *Society for Neuroscience* (Chicago, Illinois USA), October 17-21, 2015.

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Meffin, H. Predicting cortical responses to simultaneous electrical stimulation of the retina using a linear-nonlinear model, *Joint Symposium on Cognitive Neuroengineering and Computational Neuroscience* (Adelaide, Australia), February 5-6, 2015.

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Meffin, H. Predicting cortical spiking responses to simultaneous electrical stimulation of the retina using a linear-nonlinear model, *Students of Brain Research* (Melbourne, Australia), October 30, 2014.

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Meffin, H. A linear-nonlinear model predicts cortical spiking responses to simultaneous electrical stimulation of the retina, *The Eye and the Chip* (Dearborn, Michigan, USA), September 28-30, 2014.

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Meffin, H. Prediction of cortical responses to simultaneous electrical stimulation of the retina using a linear-nonlinear model, *St Vincents Research Week* (Melbourne, Australia), August 18-22, 2014.

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Meffin, H. A linear-nonlinear model accurately predicts cortical responses to simultaneous electrical stimulation with a retinal implant, *Twenty-Third Annual Computational Neuroscience Meeting* (Québec City, Canada), July 26-31, 2014.

Halupka, K.J., Shivdasani, M.N., Cloherty, S.L., Grayden, D.B., Burkitt, A.N., Meffin, H. Modelling cortical responses to simultaneous electrical stimulation of the retina, *NeuroEng* (Adelaide, Australia), February 2-3, 2014.

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Abbreviations

AMD		Age-related Macular Degeneration
ATP		Adenosine triphosphate
EEP		Electrically evoked potential
EP		Evoked potential
ERF		Electrical receptive field
ERG		Electroretinogram
FDR		False discovery rate
FMP		Focused multipolar stimulation
INL		Inner nuclear layer
LFP		Local field potential
LGN		Lateral geniculate nucleus
LN		Linear nonlinear (model)
LNL		Linear nonlinear linear (model)
MUA		Multi-unit activity
OCT		Optical coherence tomography
ONL		Outer nuclear layer
PSTH		Peri-stimulus time histogram
RGC		Retinal ganglion cells

RP	Retinitis Pigmentosa
RPE	Retinal pigment epithelium
SD	Standard deviation
SE	Standard error
SUA	Single-unit activity
V1	Primary visual cortex
VEP	Visually evoked potential

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Chapter 1

Introduction

1.1 Abstract

Blindness affects over 40 million people worldwide (Resnikoff et al., 2004) and costs approximately ten billion dollars annually in Australia alone (Taylor et al., 2006). With such statistics, it is no surprise that technologies that could restore a sense of vision to individuals who are experiencing blindness are widely studied. Electrical stimulation of various positions along the visual pathway has been investigated in detail over the last several decades, and overwhelming evidence has emerged showing that such stimulation elicits sensations of light, termed phosphenes (Tukahara and Abe, 1951; Gebhard, 1952; Riggs et al., 1957; Brindley and Lewin, 1968). However, the field has exploded in the last two decades with the advancement of materials technology and fabrication techniques. Now, electrode arrays are able to be implanted within the retina (Humayun et al., 1996), around the optic nerve (Veraart et al., 1998), or on the surface of the visual cortex (Dobelle, 2000). Currently, there are multiple research groups worldwide investigating visual prostheses for the treatment of blindness. The majority of this research is concentrated around retinal implants; however, optic nerve implants and cortical devices continue to be studied, although both are yet to be commercialized (Dagnelie, 2012).

1.2 The Visual System

In healthy visual systems, perception begins when light enters through the front of the eye and is focused by the lens onto the photoreceptors located in the outer retina. Photore-

ceptors synapse onto bipolar cells, which in turn synapse onto ganglion cells. A significant amount of visual processing occurs through complicated patterns of communication between cells in the retina. The axons of the ganglion cells meet at the optic disc to form the optic nerve, which extends toward the brain (Figure 1.1). At the optic chiasm, projections from the nasal retina of both eyes cross over to synapse at the lateral geniculate nucleus on the contralateral side, while projections from the temporal retina synapse on the ipsilateral side. The lateral geniculate nucleus acts as a relay station to the visual cortex. While this route via the lateral geniculate nucleus is not the only pathway between the retina and cortex, it is the dominant pathway, and of most interest for conscious vision (Merigan and Maunsell, 1993). The visual cortex further processes the arriving information.

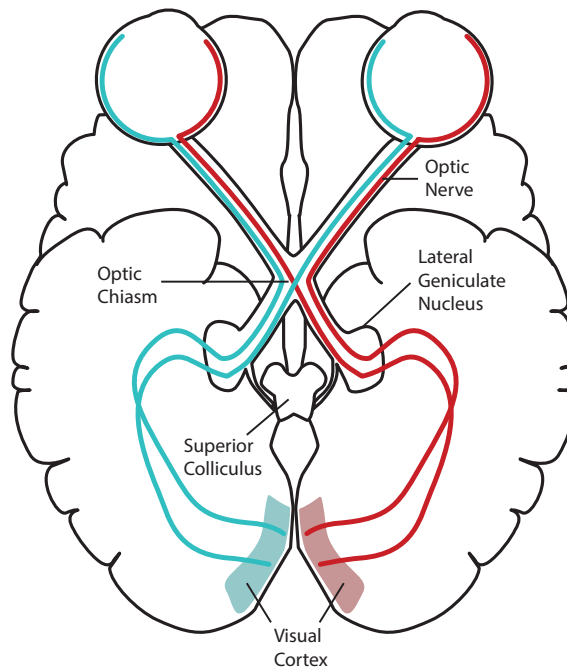


Figure 1.1: Anatomy of the visual pathway. Red and blue lines depict the flow of information from the eye through to the optic chiasm, lateral geniculate nucleus and then the visual cortex.

1.2.1 The Eye

The first step in the visual pathway is the eye (depicted in Figure 1.2A), a spherical structure whose form is maintained by the outermost layers: the cornea and the sclera (Davson, 2012). Light enters the eye through the cornea, which, together with the lens, refracts the light to focus it on the retina. The amount of light that is able to enter the

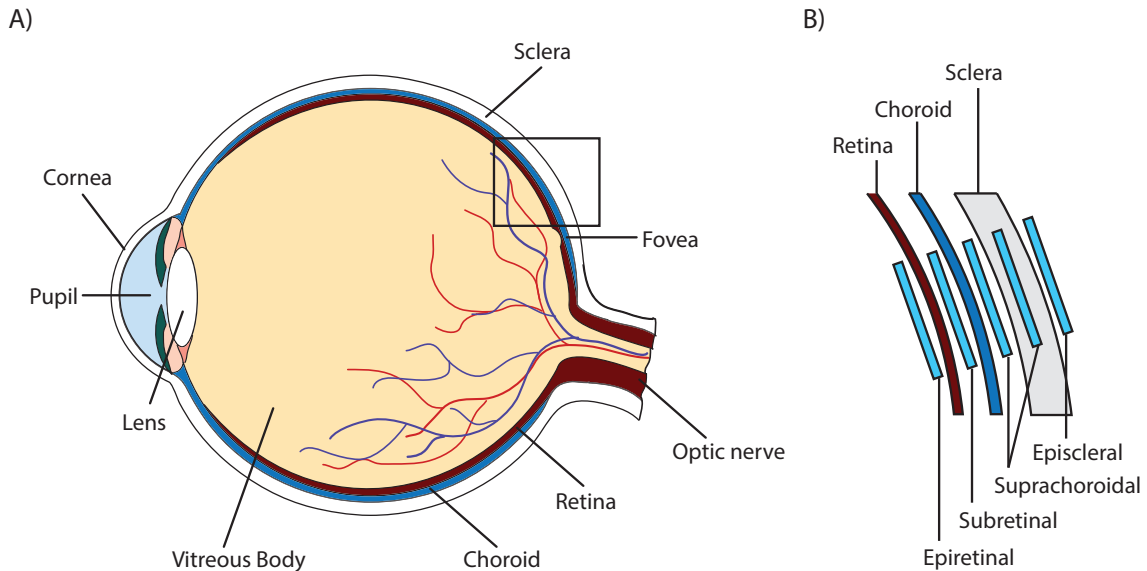


Figure 1.2: A schematic cross-section of the human eye (A), with an enlargement of a portion at the back of the eye showing epiretinal, subretinal, suprachoroidal and episcleral positions for retinal prostheses.

eye is regulated by the pupil, which constricts in response to bright light and dilates in the dark. The sclera is a fibrous protective tissue layer, continuous with the cornea and forming the back five-sixths of the outermost coating of the eye. Between the sclera and the retina lies the choroid, a vascular layer that supplies the outer layers of the retina.

1.2.2 The Retina

The inner layer of the eyeball is the retina, a layered structure containing five major cell classes: photoreceptors, bipolar cells, horizontal cells, amacrine cells, and ganglion cells (Figure 1.3A). Each cell type contributes to the processing of information traversing the retina. The photoreceptor cells, consisting of rod and cone cells, are located adjacent to the pigment epithelium (Kolb, 2003). Rod cells function well in low light conditions and outnumber the cones at a ratio of 20:1, except in the fovea, which only contains cones. Cone cells are responsible for high acuity vision and colour vision with sufficient light. Photoreceptors synapse with bipolar cells and horizontal cells. Horizontal cells have a large receptive field, receiving inputs from many photoreceptors and providing

inhibitory feedback (Kolb, 2003). There are at least 10 types of bipolar cells, and each type is represented in the group of cells to which each cone synapses (Masland, 2012). Therefore, multiple bipolar cells will receive input from each cone, and it is believed that each transmits a different component of the visual signal (Masland, 2012). Bipolar cells synapse with retinal ganglion cells (RGCs), while amacrine cells interact with both bipolar cells and RGCs. Amacrine cells transmit information laterally throughout the retina, similar to horizontal cells. There are at least 12 different types of RGCs in the mammalian retina. These types differ both functionally and morphologically (Sanes and Masland, 2015), such that different RGCs encode different aspects of the visual scene, and have a mosaic structure within the retina such that cells of a particular type are evenly spaced with respect to other cells of the same type (Rockhill et al., 2000). This organisation gives rise to a uniform sampling of visual space, with each RGC encoding local features within a small patch of the visual scene, termed a *receptive field* (Hartline, 1938). Receptive fields are organised in centre-surround fashion, with ON-centre receptive fields detecting light areas on dark backgrounds, and OFF-centre receptive fields detecting dark areas on light backgrounds (Kuffler, 1953). RGCs with ON- and OFF-centre receptive fields form separate ON and OFF pathways from the retina to the brain. These pathways work in parallel and begin in the inner retina, where bipolar cells are considered either ON or OFF, and synapse onto ON and OFF retinal ganglion cells, respectively (Kolb, 2003). Horizontal and amacrine cells provide inhibitory surround feedback, resulting in centre-surround organisation of bipolar cells.

1.2.3 The Optic Nerve and Lateral Geniculate Nucleus

The optic nerve is composed of approximately 1.2 million retinal ganglion cell axons (Prasad et al., 2011). At the optic chiasm, fibres of nasal ganglion cells cross to join axons from temporal ganglion cells of the contralateral eye. In this way, the axons from both eyes that view the same portion of the visual field are brought together (Figure 1.1). From here, the optic nerves extend to the lateral geniculate nucleus (LGN), where most of the cells synapse. The LGN is organised in a retinotopic fashion (Schneider et al., 2004), with six layers, and axons from the ipsilateral and contralateral eyes synapse in different layers (layers 2, 3 and 5, and 1, 4, and 6, respectively) (Chacko, 1948). Additionally, the centre-surround receptive field properties found in the retina are maintained in the cells of the LGN (Derrington and Lennie, 1984). The LGN is an important relay station that controls the information flow to the visual cortex based on modulating inputs from the thalamic reticular nucleus and layer 6 of the visual cortex (Guillery and Sherman, 2002).

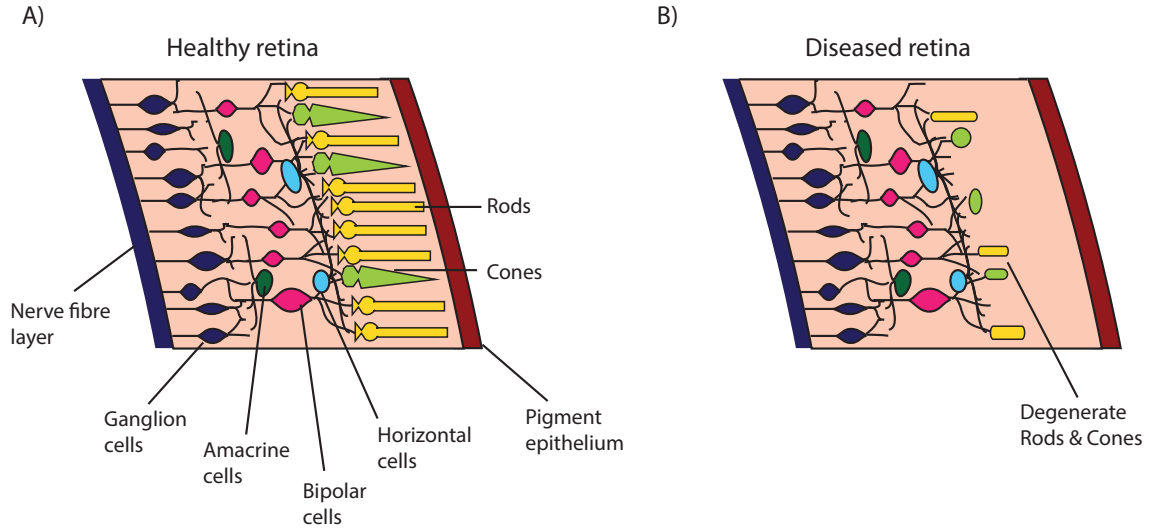


Figure 1.3: A) Schematic representation of the healthy retina, showing different cell types and their connective pathways. (B) Diseases such as Retinitis Pigmentosa and Age-related Macular Degeneration cause degeneration of the photoreceptor cells.

1.2.4 The Primary Visual Cortex

The primary visual cortex (V1) is located in the occipital lobe, at the back of the brain, along with four other distinct visual areas (V2-V5). V1 is the principle visual area responsible for perception of vision, while areas V2-V5 deal with the processing of colour, depth, form and movement information (Nicholls et al., 2001). V1 is composed of six horizontal layers; however, in primates, these layers can be further subdivided. For instance, layer 4 is further divided into layers labelled 4A, 4B, 4C α , and 4C β , where the main LGN afferent inputs terminate in layer 4C (Burkhalter and Bernardo, 1989). The topographical organisation of the LGN is maintained in the visual cortex, where there is a large magnification factor (approximately 9.9 mm/degree at 1 degree eccentricity (Horton and Hoyt, 1991)) when compared to the retina. Additionally, cells of the visual cortex also exhibit receptive fields (Hubel and Wiesel, 1959). Cells are classified as either simple or complex depending on their receptive field characteristics. The receptive fields of simple cells are composed of elongated ON and OFF subregions, arranged to detect lines or edges at specific orientations. Complex cells also respond to specifically oriented lines and edges, but responses to such stimuli tend to be spatially invariant within the receptive field, due to a lack of excitatory and inhibitory zones (Hubel and Wiesel, 1962). While receptive fields of simple and complex cells were originally mapped by analysing their responses to various visual stimuli, including dots, lines and edges, quantitative methods of receptive field mapping

have helped to refine our understanding (discussed further in Section 1.6). As well as simple and complex categories, cells in V1 are grouped according to other factors such as eye dominance and their preference for orientation, spatial and temporal frequency, colour, and direction (Hubel and Wiesel, 1965; LeVay et al., 1975; De Valois et al., 2000).

From V1, two streams project to other visual cortices; these streams are the ventral and dorsal streams, otherwise referred to as the “what” and “where” pathways (Goodale and Milner, 1992). The ventral stream travels from V1, through V2 and V4, to the inferior temporal cortex, and plays a major role in the identification of objects. The dorsal stream projects from V1 to V2, then V5 and finally through the the posterior parietal cortex. It is associated with motion, object location and control of eye movements.

1.3 Diseases of the Outer Retina

Blindness can be caused by degeneration or damage of any point along the visual pathway, from the cornea through to the visual cortex. Of particular importance to this study is blindness due to the loss of photoreceptors in the outer retina, since retinal prostheses require the presence of viable cells in the inner retina (Weiland and Humayun, 2014). A schematic of the retina affected by outer retinal degeneration is shown in Figure 1.3B. Age-related macular degeneration (AMD) and Retinitis Pigmentosa (RP) are the two most common outer retinal degenerative diseases, for which retinal implants show promise (Weiland and Humayun, 2008).

1.3.1 Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is the name given to a group of hereditary retinal diseases that result in photoreceptor loss and eventual blindness. The incidence of RP has been estimated at 1 in 4000 people (Boughman et al., 1980), and the onset of symptoms varies widely from childhood through to adulthood (Hartong et al., 2006). The symptoms generally exhibited include night blindness (indicating a loss of rod photoreceptors) and a loss of mid-peripheral visual field (due to loss of cone photoreceptors). A decline in visual acuity often isn’t noticed until the late stages of the disease, since patients can lose 90% of the cones in the fovea before visual acuity is reduced (Geller and Sieving, 1993). The disease tends not to have a direct effect on bipolar, horizontal, amacrine or ganglion cells; however, as the disease progresses, these cells sometimes degenerate (Santos et al., 1997). With the absence of input from photoreceptor cells, inner retinal cells may also begin

to migrate into other layers of the retina and form new connections, termed remodelling (Jones et al., 2016).

1.3.2 Age-related Macular Degeneration

Age-related macular degeneration is the cause of almost half of all cases of blindness in Australia (Taylor et al., 2005). AMD is caused by a build up of drusen, which is a material secreted from malfunctioning retinal pigment epithelium (RPE) cells and deposited between the RPE and the choroid (Bressler et al., 1988). A primary characteristic of this disease is that the deposits occur on the macula, a region in the centre of the retina (within which is the fovea). The accumulation of the deposits causes the RPE to atrophy, and the photoreceptor cells, which are dependent on those sections of RPE, are subsequently lost (Young, 1987). This causes a loss of vision in the centre of the visual field, the worst possible location since this is the area with the highest visual acuity. Similar to RP, the inner retina and ganglion cells are relatively well preserved with AMD (Kim et al., 2002).

1.3.3 Impact on the Inner Retina

While the primary impact of RP and AMD is the loss of photoreceptor cells, studies suggest that the inner retina undergoes a series of changes, termed remodeling, as the outer retina degenerates (Jones et al., 2012). Retinal remodeling encompasses many specific changes in the inner retinal neurons, including the retraction of bipolar and horizontal cell dendrites with deafferentation. This is followed by migration of cells into other areas of the retina, and the generation of new synaptic connections (Marc et al., 2003). By the late stages of remodeling, the structure of the inner retina has undergone large-scale reorganisation. Given the aforementioned reliance of visual function on the complex information pathways in the retina, even if the lost input from photoreceptor cells were to be replaced, spatial processing may be too corrupt to regain function. Therefore, it is crucial to the success of interventional strategies such as visual prostheses that they be validated in retinæ that have undergone remodeling.

1.4 Restoration of Vision with Visual Prostheses

Given the impact that vision loss has, not only on an individual basis but also on society, it is not surprising that strategies to restore vision have been heavily investigated. Treat-

ments for vision loss include visual prostheses, optogenetic therapies (Barrett et al., 2014), and stem-cell treatments (Uy et al., 2013). This review will focus on visual prostheses, which have been investigated for almost one hundred years, beginning with the work of Foerster (1929). Foerster (1929) reported that upon electrical stimulation of the occipital lobe, a subject perceived small, motionless spots of light in his visual field (termed phosphenes). Brindley and Lewin’s highly influential work in 1968 followed on from this, showing that stimulation of the primary visual cortex through a grid of surface electrodes in a blind human also elicited repeatable phosphenes (Brindley and Lewin, 1968). Tassicker (1956) showed that a curved plate of photosensitive material located in the suprachoroidal space resulted in an area of uniform light in the previously dark visual field of a subject with retinal degeneration. The 1990’s heralded the start of acute experiments involving temporary implantation of stimulating arrays in the eyes of blind test subjects (Humayun et al., 1996, 1999). These studies showed that potentially useful, retinotopically organised percepts could be elicited from electrical stimulation of the degenerate retina, and that multiple percepts could be combined to form simple patterns. This discovery prompted increased interest in devices that could restore sight to blind people through electrical stimulation, otherwise known as visual prostheses. In order to do so, the prosthesis must detect light from the environment and transduce it into an electrical stimulus. One approach to this consists of an external camera mounted on a set of glasses that captures images of the visual environment. The captured video is processed by an external processing unit worn by the user, and converted into a series of electrical stimulation commands which are sent to the implant.

However, the road to restoring vision by replacing the lost input from photoreceptors, or bypassing the retina completely, is beset with a number of challenges. Along with the ability of any device to restore functional vision, other aspects that must be taken into account in the design of a visual prosthesis include: safety and invasiveness of surgical implantation, toleration of the device in the body, amount of electrical stimulation required for successful device operation (since the tissue and electrodes can be damaged by excessive current levels (Cohen et al., 2011)), and long-term reliability of the device. Many types of visual prostheses are currently being investigated throughout the world, each of which has advantages and disadvantages. The three main types of visual prostheses are named according to their location in the visual pathway: higher visual pathway (LGN and visual cortex), optic nerve and retina.

1.4.1 LGN and Visual Cortex Prostheses

Stimulation of higher areas of the visual pathway has the potential to treat a wider range of vision loss. Presently, the LGN and visual cortex are being investigated as potential sites for electrical stimulation.

The LGN is well characterised and retinotopically organised, and can be accessed using surgical techniques developed extensively for deep brain stimulation (Pezaris and Eskandar, 2009). However, since the LGN is located behind the optic chiasm, bilateral LGN implantation would be required in order to access both halves of the visual field. While studies into LGN prostheses have not yet reached the stage of clinical implantation, initial acute animal experiments in alert monkeys show promising results (Pezaris and Reid, 2007; Panetsos et al., 2009), with chronic implantations to follow (Kyada et al., 2017).

Following on from Brindley and Lewin’s successful use of cortical stimulation to elicit phosphenes (Brindley and Lewin, 1968), Dobelle (2000) have described the results of a long term trial, where several volunteers were chronically implanted with cortical stimulating arrays, which received information from a digital video camera system. At the time of publishing, Dobelle (2000) stated that two of the volunteers in the study had retained their implants for more than 20 years without infection or other issues. Despite this, visual cortex prostheses are still limited by the inherent invasiveness of surgical implantation, and have a higher risk of complications than other types of prostheses. Also, prostheses implanted at this late stage in the visual pathway cannot take advantage of the processing that occurs in the retina and LGN. However, if these risks are mitigated and further processing of the digital signals can replace the bypassed processing stages, visual cortex prostheses have several possible advantages over other prostheses. These advantages include the much lower current required to produce a percept (Schmidt et al., 1996) and their applicability to a wider range of patients, such as those suffering loss or damage to the eye through disease or trauma.

1.4.2 Optic Nerve Prostheses

Optic nerve prostheses consist of either a spiral or penetrating cuff of electrodes that encircles the optic nerve. Stimulation at this site has been shown to produce phosphenes that are distributed in a $85^\circ \times 50^\circ$ section of the visual field, and range in colour and size (Veraart et al., 1998). Percepts can be moved reproducibly by changing stimulation parameters such as current amplitude, pulse duration, pulse frequency and number of

pulses in the stimulus train (Delbeke et al., 2003). Percepts can also be arranged such that pattern recognition and orientation discrimination is possible (Brelén et al., 2005). However, a major draw-back of optic nerve prostheses is the non-retinotopic nature of the optic nerve, which therefore requires a model to relate stimulation location to position of the evoked percept in the visual field (Delbeke et al., 2003). Additionally, it has so far not been possible to change the intensity of the elicited phosphenes directly, as is possible with retinal or cortical stimulation (Veraart et al., 2003). Also, specificity of activation is difficult due to the close proximity of nerve axons in a bundle (Veraart et al., 1998). Despite these drawbacks, optic nerve prostheses do have less complicated implantation surgeries than cortical prostheses, and have lower thresholds for eliciting percepts than retinal prostheses (Margalit et al., 2002).

1.4.3 Retinal Prostheses

Retinal prostheses electrically stimulate surviving cells in the retina to create a sense of vision. Electrodes implanted in the retina deliver this electrical stimulus in the form of current pulses, which affect the membrane potential of the surrounding cells. Four main types of retinal prostheses have been investigated by several different groups worldwide in both acute and chronic human trials. These types are named according to the location of the electrode array: epiretinal (Rachitskaya and Yuan, 2016; Klauke et al., 2011; Kesterü et al., 2012; Hadjinicolaou et al., 2012), subretinal (Stingl et al., 2015; Rizzo III, 2011), suprachoroidal (Fujikado et al., 2011; Sinclair et al., 2016) and extraocular (Chowdhury et al., 2008; Siu and Morley, 2008a; Gerding, 2007). The location of each type of prosthesis is shown in Figure 1.2B.

Epiretinal Prostheses

Epiretinal devices are placed in front of the retina, in close proximity to the retinal ganglion cells. Advantages of this approach include the close proximity of the stimulating electrodes to the retinal ganglion cells, resulting in low stimulation current thresholds for eliciting a response. This, in turn, facilitates smaller electrode sizes and thus higher spatial resolution (Shepherd et al., 2013). Additionally, the vitreous fluid in the eye can act as a heat sink to dissipate heat produced by the device. However, the epiretinal surface as a location for a prostheses presents its own complications. These include the difficulty of the surgery in comparison to other device locations (Ong and da Cruz, 2012) and the potential for mechanical damage to the retina due to the need for retinal tacks or other forms of affixing

the device to the retina (Guenther et al., 2012).

Second Sight Medical Products, Inc. (California, USA) has performed the most extensive retinal prosthesis clinical trials to date. The first clinical trial by this company began in 2002 with their first generation device, the Argus I, with an array located epiretinally consisting of 16 platinum electrodes, connected via a cable to electronics outside of the eye (Humayun et al., 2003). The electrode array is attached to the retina via a tack. The Argus I clinical trial resulted in a number of important findings: subjects were able to perceive light when the device was activated, with perceptual thresholds below the safe limit (de Balthasar et al., 2008; Mahadevappa et al., 2005); perceived phosphenes were round, oval or elongated in shape (de Balthasar et al., 2008; Nanduri et al., 2008); phosphene brightness depended on stimulation amplitude and frequency, with higher amplitudes and frequencies being related to brighter phosphenes (Horsager et al., 2009; de Balthasar et al., 2008; Nanduri et al., 2008); increased amplitude also caused phosphene size to increase (Horsager et al., 2009; Nanduri et al., 2012); synchronous stimulation of electrodes produced percepts at an acuity level matching the distance between electrodes (Caspi et al., 2009), but interactions occurring between electrodes stimulated synchronously or close in time influenced percept quality (Horsager et al., 2010, 2011).

Following the success of Argus I, the Argus II was developed with the intention of commercial use. The second generation implant has an increased number of electrodes (60), decreased electrode size (200 μm), and decreased centre-to-centre spacing (575 μm) (Humayun et al., 2012). The clinical trial for the device started in 2006, and in 2011, it received European Union approval followed by US Food and Drug Administration approval in 2013. The clinical trial involved 29 people with Retinitis Pigmentosa and one person with Choroideremia (Ho et al., 2015), and has thus far shown impressive results. Subjects have shown improved target localisation as measured by their ability to point to a white square on a black background at random locations, with 89% of subjects showing higher accuracy with the system on than with the system off (Ho et al., 2015). Just over half (56%) of the subjects showed improvement with respect to detecting the direction of motion of a white bar moving against a black background (Ho et al., 2015), and the best visual acuity obtained thus far based on a grating visual acuity test is 20/1262 (1.8logMAR) (Humayun et al., 2012). In terms of utility in day-to-day life, the Argus II has also been shown to improve the ability of subjects to read white letters on a black background, with some able to identify simple 2-4 letter words (da Cruz et al., 2013), and aid subjects in identifying common household objects (when the object to background contrast is high) (Luo et al., 2014). Subjects have also shown improvements in finding a door across a room

and following a white line on the ground (Humayun et al., 2012).

Other clinical trials of epiretinal prostheses have been reported by Intelligent Medical Implants (IMI Inc, Zug, Switzerland) and Epi-Ret. IMI, a German company, developed a 49-electrode array with varying electrode diameters (100, 200, and 360 μm). Information is transferred at a high rate to the intraocular part of the device using an infrared optical link, which is interrupted by the eyelid, thereby preventing sight if the eye is closed (Hornig et al., 2007). In an acute study with 20 patients, 19 subjects reported visual percepts when the device was stimulated, and thresholds were within safe limits of charge capacity (Keserü et al., 2012). A 9-month long study revealed that the implant was well tolerated by subjects; however, no further data is available beyond that time frame (Richard et al., 2007). Recently IMI Inc. were acquired by Pixium Vision (France), and have since begun a clinical trial of a device called the IRIS II, which is based on the IMI device, and has since been awarded CE mark approval. The IRIS II has 150 electrodes, and is designed to be explantable (Hornig et al., 2017). Separately, Epi-Ret developed the EPIRET3, a device that fits entirely within the eyeball (excluding the camera and image processor), rather than having intra- and extra-ocular electronics connected transcerally (Klauke et al., 2011). The array contains 25 electrodes of 100 μm diameter and protruding to a height of 25 μm , with 500 μm separation. The array was implanted for four weeks in six subjects with RP. Stimulation elicited percepts in all subjects, with thresholds below safety limits. In contrast to reports with the Argus II device, percept brightness was related more strongly to pulse duration than current amplitude (Klauke et al., 2011). Subjects were also able to discriminate between different stimulation patterns (Klauke et al., 2011).

Bionic Vision Australia are currently developing a diamond encapsulated epiretinal implant with 256 individually addressable electrodes (Ahnoood et al., 2016). A separate component containing a magnet is inserted between the sclera and choroid, such that the epiretinal component is magnetically secured to the retina without damaging the retina with a tack (Ahnoood et al., 2016). The diamond encapsulation of the device is particularly noteworthy, being well tolerated by the retina and providing long term implant stability.

Subretinal Prostheses

Subretinal stimulating electrode arrays are placed between the bipolar cell layer and the retinal pigment epithelium. The advantage of this placement is proximity to the inner nuclear layer, which also means that all remaining cell layers can contribute to the processing of the signal, provided remodeling is not too extensive. While the array location means

that the retina will hold it closer to the cells to be stimulated, there is a limited amount of space, so the size of the array and electronics is severely limited. There is also debate about whether the location could obstruct heat dissipation and nutrient transport to the retina, leading to atrophy of tissue (Sailer et al., 2007; Peachey and Chow, 1999). An interesting development in subretinally placed prostheses is the use of micro-photodiode arrays, which generate stimulation signals in response to light (Stingl et al., 2015; Chow et al., 2004; Palanker et al., 2005; Salzmann et al., 2006; Zrenner et al., 2008). A clinical trial of such a device, the Artificial Silicon Retina from Optobionics, was the first implant of this type to enter clinical trials. Unfortunately, however, investigators found that the incident light onto the device was not sufficient to drive meaningful activity (Palanker et al., 2005).

The Alpha IMS implant, developed by Retina Implant AG in Germany also uses a micro-photodiode array; however, their 1500 element array also contained an external power source and an amplifier, such that the current delivered to the retina via 50 μm^2 electrodes was sufficient to drive responses (Stingl et al., 2013). The entire array spans a 9 mm^2 area, covering a visual angle of approximately $11^\circ \times 11^\circ$. The device gained European regulatory approval for the treatment of late stage RP in 2013. Both an investigational device and a commercial device have been trialled in clinical studies. In the former study, 11 patients were implanted with the device in 2005; 7 with RP, 3 with cone-rod dystrophy and 1 with choroideremia (Zrenner et al., 2011). The investigational device also contained a 4×4 array of electrodes for direct stimulation independent from incident light, and a transdermal power cable, while the commercial device used a subdermal power module for wireless transmission (Stingl et al., 2013). Though the device trial was only for a few weeks, results of the study were encouraging: percepts from direct stimulation appeared as spatially confined, round dots with a white or yellow hue, and were retinotopically arranged (Zrenner et al., 2008); the size and brightness of percepts were dependent on stimulation strength (Zrenner et al., 2008); similar to the Argus II results, some subjects were able to locate high contrast objects, and one could identify common objects (Zrenner et al., 2011). The most successful subject in the trial was able to read large letters and combine them in to words, even without a training period (Zrenner et al., 2011). However, it was noted that simultaneous direct stimulation of multiple electrodes resulted in more complex percepts than the spatial composites of single electrode stimulation (Zrenner et al., 2008), which the authors suggested might limit spatial resolution. Later, (Wilke et al., 2011b) confirmed this finding when they compared the ability of subject to recognise simple spatial patterns with either sequentially or simultaneously activated electrodes. They observed that patterns involving simultaneous stimulation of electrodes could not be described or discriminated

correctly by some subjects. However, when electrodes in the patterns were stimulated sequentially with >100 ms interstimulus intervals, subject success rates improved (Wilke et al., 2011b). The subsequent clinical trial starting in 2009 saw 29 patients implanted with the commercial device (Stingl et al., 2015). In this trial the efficacy of the device was measured in terms of the improvement in activities of daily living, mobility, acuity and object recognition. Most subjects showed improvements in at least one area, with 13 participants reporting that the implant was useful in daily life. Four participants recorded visual acuity scores using the Landolt C-rings, with a best visual acuity of 20/546 (the same patient recorded 20/200 with a grating acuity measure) (Stingl et al., 2015).

Two other groups are developing subretinal implants, but are currently in the pre-clinical stages. A photovoltaic retinal prosthesis is being developed by Palanker et al. (Palanker et al., 2005; Mathieson et al., 2012), in which visual inputs are converted to near-infrared laser light that is projected onto silicon photodiodes implanted in the retina. Results of pre-clinical trials with this device are promising, with Lorach et al. (2015) finding that spatial resolution of the device in rats with retinal degeneration was similar to normally sighted rats. The Boston Retinal Implant Project has developed an array comprising 256 independently driven electrodes (Rizzo III, 2011), which is currently in pre-clinical trials. In contrast to the other subretinal devices, the array does not use micro photodiode-technology, and therefore requires an integrated circuit chip for independently addressing each channel (Kelly et al., 2013).

Suprachoroidal Prostheses

Suprachoroidal devices are placed either in the space between the sclera and choroid, an area referred to as the suprachoroidal space, or within the sclera itself (intra-scleral). Since the array is separated from the bipolar cell layer of the retina by the choroid, thresholds are increased relative to epiretinal or subretinal electrode positions. Animal studies have indicated that thresholds are approximately 15 times higher for suprachoroidal compared to subretinal stimulation (Yamauchi et al., 2005); however, in humans with Retinitis Pigmentosa, the difference was found to be much less severe at approximately 1.8 times higher (Shivdasani et al., 2014), possibly since the choroid undergoes shrinkage with degeneration (Ayton et al., 2013). Despite its distance from the retina, the array can still stimulate the target cells at safe charge levels from this position (Shivdasani et al., 2010). The advantages of the suprachoroidal position include the mechanical stability of the device, the relative simplicity of surgical implantation, and close contact to the choroid which acts as a heat sink for dissipating heat (Villalobos et al., 2013).

Fujikado et al. in Japan developed a suprachoroidal transcleral device, where the stimulating array is placed in a scleral pocket, with a return electrode located in the vitreous body (Nakauchi et al., 2005; Fujikado et al., 2011; Nishida et al., 2010). The device was trialled in two patients with RP over a four week period. The array consisted of 49 cylindrical electrodes with diameter and height of 500 μ m. While only 9 of the electrodes were functional, percepts could be obtained within safe charge levels from 6 electrodes in one patient, and 4 electrodes in the other. Despite the low number of functioning electrodes, both patients showed improvement in the ability to discriminate two bars with the system on versus off. A second generation implant has since been developed by the group, and 1-year outcomes of a clinical trial involving three patients with Retinitis Pigmentosa have been released (Fujikado et al., 2016). Results were encouraging, with 24 to 28 out of the 49 implanted electrodes able to evoke phosphenes with currents below 1 mA.

The Bionic Vision Australia consortium developed a suprachoroidal retinal prosthesis comprising 33 platinum electrodes with two different diameters (400 μ m and 600 μ m), and two larger return electrodes with 2 mm diameter. A clinical trial was conducted in 2012, in which three subjects with RP were implanted with the device, as well as a remote return electrode located under the skin behind the ear (Ayton et al., 2014). The outer ring of 13 electrodes was electrically coupled to form an alternate return configuration. Therefore, 20 out of the 33 electrodes could be individually addressed for stimulation. The clinical trial lasted for 2 years, within which time perceptual outcomes were encouraging. Phosphenes could be evoked in all three patients at safe charge levels, with thresholds depending significantly with distance between the electrodes and retina (Shivdasani et al., 2014). Phosphenes had varying complexity, but were retinotopically arranged for two of the subjects, and contained unique information (Sinclair et al., 2016). For one patient a visual acuity measure of 20/8397 (2.62logMAR) was achieved (Ayton et al., 2014). Additionally, performance in certain tasks was improved by pre-processing the image acquired image with a Nyquist bandlimited image filter, suggesting that targeted vision processing schemes could improve patient outcomes (Barnes et al., 2016).

Episcleral Prostheses

Devices where electrodes are placed on the external surface of the globe, and sutured to the outer scleral wall, are referred to as episcleral prostheses. Such devices stimulate the surviving retinal cells through the sclera. Due to the increased distance between stimulating electrodes and target cells compared to other retinal prosthesis locations, larger

electrodes and higher stimulating currents are required (Ranck, 1981), thereby limiting the resolution of vision that could be achieved. However, this device placement avoids damage to the retina that could be incited by attaching an array to it (as in the epiretinal approach) and also would not obstruct nutrient flow to the retina (as might occur with subretinal prostheses). Chowdhury et al. (2008) showed that a device with 21 platinum disk electrodes (700 μ m diameter, 950 μ m centre-to-centre spacing), sutured to the sclera of anaesthetised cats, could evoke cortical responses with biphasic pulses at amplitudes below the safe charge-density limits for chronic neural stimulation. Furthermore, Siu and Morley (2008a) showed that stimulation of the outer scleral wall with a 700 μ m diameter electrode elicited retinotopically organised cortical responses in rabbits with photoreceptor degeneration. This indicates that such a device could be used as a low-resolution aid for blind patients.

Gerding (2007) sought to mitigate the distance between electrodes and target cells by developing a transcleral implant, where the electrodes penetrate the eye via the sclera, with all other components are positioned extraocularly. To reduce damage on the retina, in particular the choroid, electrode arrays are initially sutured to the sclera with mild tension, and electrode tips in contact with the sclera. Over a period of days or weeks the electrodes slowly penetrate the sclera, choroid, and retinal pigment epithelium (Gerding, 2007).

1.4.3.1 Shortcomings of Clinical Retinal Prostheses

Retinal prostheses have clearly progressed a long way since their inception, with subjects able to use the useful percepts they evoke to aid in spatio-motor tasks (Barry et al., 2012; Kotecha et al., 2014; Stingl et al., 2015) and reading (da Cruz et al., 2013). However, spatial resolution of retinal prostheses is severely limited at present. Resolution is an important factor that influences performance in daily tasks, such as reading and face recognition (Bach et al., 2010). Additionally, perception of rapid movement is severely limited, with subjects requiring relatively slow image update speeds to avoid fading of percepts (Fornos et al., 2012; Stingl et al., 2013). Furthermore, and of particular interest to the work presented in this thesis, are the complex interactions that have been observed between multiple electrodes stimulated simultaneously in clinical studies (Humayun et al., 1999; Rizzo et al., 2003b; Auner et al., 2007; Horsager et al., 2010; Wilke et al., 2011b). Specifically, inconsistencies have been observed regarding percept predictability and reproducibility when electrodes in simple geometric patterns such as lines and squares are stimulated simultaneously (Humayun et al., 1999; Rizzo et al., 2003b; Horsager et al., 2010;

Wilke et al., 2011b; Zrenner et al., 2008). Some studies have shown that simultaneously driven electrodes evoke more complex percepts than the sequentially driven composites (Zrenner et al., 2008; Rizzo et al., 2003b; Wilke et al., 2011b), while others have reported that percepts were arranged in expected shapes albeit sometimes brighter (Horsager et al., 2010) or in continuous lines rather than individual phosphenes (Humayun et al., 1999). As well as increased complexity, electrode interactions between concurrently driven electrodes may detrimentally affect spatial resolution (Wilke et al., 2011b). These reasons contribute to sequential stimulation being the preferred method of stimulation. However, to accommodate for the growing numbers of electrodes on prostheses, the timing between sequentially stimulated electrodes will be compressed, possibly to the point of requiring concurrent stimulation. To this end, research has been undertaken in animal models to investigate methods of reducing interactions between electrodes (Cicione et al., 2012; Matteucci et al., 2013; Wong et al., 2009; Spencer et al., 2016). However, other studies have suggested that the interactions could possibly be harnessed to improve device resolution (Jepson et al., 2014b) or increase the range of percepts available with a retinal implant (Dumm et al., 2014), and thereby also addressing the shortcoming of spatial resolution. In light of the inconsistencies of clinical results, the crucial first step in either reducing or harnessing electrode interactions is to investigate the predictability and reproducibility of responses to simultaneous stimulation. This is a difficult task, particularly given the large numbers of electrodes of retinal prostheses, since it would take a great deal of time to individually evaluate the perceptual outcome of every possible electrode combination and stimulation paradigm.

Animal models, both *in vitro* and *in vivo*, are used to validate retinal prostheses prior to human testing. This is ethically appropriate and in some cases necessary from a safety perspective. Additionally, the use of animal models provides unique opportunities to investigate how retinal cells respond to electrical stimulation, and how those responses are transmitted to the cortex. While animal testing allows for a greater range of stimulation paradigms to be evaluated than what is generally available in clinical tests, some paradigms (particularly concurrent stimulation strategies) require particularly large numbers of situations to be trialled. In such cases, the use of computational models of neural systems can assist, thereby reducing the numbers of animals used for experimentation. The remainder of this literature review will discuss the recent pertinent studies in *in vitro* and *in vivo* research, particularly studies pertaining to stimulation strategies to improve patient outcomes. Additionally, we present an overview of quantitative models of visual system cells used to understand the system and aid in developing retinal prostheses.

1.5 Pre-Clinical Retinal Prosthesis Research

An important area of research for all types of visual prostheses is the characteristics of the elicited phosphenes and the parameters of stimulation that affect them. The analysis of these effects in human acute and chronic studies use perception as a measure of threshold, with patients providing feedback on the size and shape of percepts (Humayun et al., 2003; Rizzo et al., 2003b; Yanai et al., 2007; Gekeler et al., 2006; Zrenner et al., 2008). However, it is ethically appropriate to first assess the safety and efficacy of different parameters both *in vitro* and *in vivo* in animal models. Additionally, such studies mitigate the subjectivity of reports from human subjects.

1.5.1 *In Vitro* Studies

Many questions remain unanswered in retinal prosthesis research. This is unsurprising given the large number of variables involved and the complexity of the visual system. *In vitro* studies of electrical stimulation of the retina serve to elucidate these questions, and are generally aimed at either understanding how retinal cells respond to electrical stimulation or improving stimulation strategies. Strategies that more accurately emulate natural retinal firing patterns are likely to result in improved prosthetic vision and patient outcomes. In the following, several aspects of these studies are reviewed: spatial and temporal resolution and ability to selectively stimulate different cell types.

Spatial Resolution

Stimulation strategies generally investigate a particular target for activation, whether that is a retinal layer (inner retinal cells or RGCs) or a specific cell type (e.g., OFF brisk transient). For the former, targets of activation are considered in terms of their relation to RGCs. Direct activation refers to the activation of the ganglion cells directly with stimulation, as compared to indirect activation, where cells presynaptic to the RGCs are activated. Also considered is activation of passing axons (Jensen et al., 2003, 2005b), which project from the peripheral retina to the optic nerve, passing closer than the ganglion cell bodies to epiretinal electrode arrays. Activation of these passing axons may result in ellipsoid as opposed to circular percepts (Horsager et al., 2009; Nanduri et al., 2012). Given that activation of these axons is more likely from epiretinal stimulation, this can negatively impact the spatial resolution available to epiretinal arrays. However, short duration pulses from epiretinal arrays produce focal RGC activity while avoiding activation of axons of passage

(Sekirnjak et al., 2008; Jepson et al., 2013; Behrend et al., 2011; Jensen et al., 2005b), but only for stimulation amplitudes less than 40% above threshold, above which passing axons may also be activated (Weitz et al., 2015). Other strategies used to circumvent activation of passing axons include using bipolar stimulation perpendicular to the fibre (Grumet, 1999) or stimulation with cathodal pulses, which in the rabbit retina activates cell bodies at lower current amplitudes than axons (Jensen et al., 2003). More recently, Jepson et al. (2014b) showed that a model based on RGC responses to simultaneous stimulation of multiple epiretinal electrodes could be used to predict the optimal stimulation pattern to selectively activate a target cell while minimising the activity of a neighbouring cell. Such a method could be tailored to a number of situations, including the avoidance of axon stimulation, and may be useful in increasing the spatial resolution of retinal prostheses.

Indirect activation is generally characterised by prolonged multi-spike responses (Tsai et al., 2009; Jensen and Rizzo III, 2008) and, therefore, has low temporal resolution. However, indirect activation has a higher spatial resolution than direct activation, since the bipolar cells, which are the target of indirect stimulation, do not project laterally. Sim et al. (2014) used the concept of electrical receptive fields (ERFs) to directly compare the spatial resolution available to epiretinal and subretinal arrays when stimulating isolated mouse retinæ with an array of 3200 electrodes. Similar to visual receptive fields, ERFs were defined as the area of the stimulating array that successfully elicited a spiking response in an RGC. They found smaller ERFs during subretinal stimulation compared to epiretinal, but suggested that the difference may be due to the extended distance between subretinal stimulation electrodes and RGCs compared to epiretinal, leading to less charge reaching distant RGCs. Sim et al. (2014) also compared the size and shape of ERFs for cells that exhibited both immediate and delayed responses, finding that the ERFs for the two components differed in size and shape, but overlapped. This supports the idea that the two components of response in the same cell were derived from direct and indirect activation, respectively (Sim et al., 2014).

Given the increased resolution resulting from indirect stimulation of RGCs, it is not surprising that several studies have investigated methods of selectively activating presynaptic cells. Interestingly, these studies have found that while short duration pulses produce direct RGC activation, long duration pulses elicit robust indirect activation (Behrend et al., 2011; Jensen et al., 2005b; Weitz et al., 2015). The same effect is seen for low frequency (<25 Hz) sinusoidal stimulation (Freeman et al., 2010a; Weitz et al., 2015). Of particular note is the study by Weitz et al. (2015), where calcium imaging was performed on isolated wild type and degenerate rat retinas to map the patterns of activation induced

by different stimulation paradigms. They found that long amplitude stimulus pulses (25 ms) activated inner retinal cells while avoiding activation of RGC axons in both the healthy and degenerate retinas (Weitz et al., 2015). This resulted in more focal responses with the longer duration stimuli as compared to the ‘streaks’ of activity seen with shorter duration pulses (Weitz et al., 2015). While such a strategy may result in increased spatial resolution, it also required higher charge densities to reach threshold (Weitz et al., 2015), which may result in unsafe charge densities when used clinically (Petoe and Shivdasani, 2016).

Many studies involving network mediated stimulation in normally sighted animal models cannot completely rule out the contribution of photoreceptor activation towards responses. Similar to inner retinal cells, photoreceptors can be activated from stimulation with both pulses (Jensen et al., 2005a) and low frequency sinusoidal waveforms (Freeman et al., 2010a). Considering that photoreceptors would largely not be present in clinical trials of prostheses, this presents a problem since activation of photoreceptors could lower thresholds for RGC response by depolarizing them. Boinagrov et al. (2014) overcame this issue by using synaptic blockers while recording from patch-clamped RGCs, showing that it is possible to discriminate between direct and network-mediated responses by considering response latency. Short latency responses were due to direct activation of the RGCs, and occurred less than 5 ms after stimulation, while network mediated responses occurred between 3 and 70 ms (originating in the inner nuclear layer) or after 40 ms (originating in photoreceptors).

Temporal Resolution

The maximum spike rate of healthy retina in response to light can exceed 250 Hz (O’Brien et al., 2002). Therefore, emulating natural firing patterns in the retina will require high temporal precision and the ability to follow high rates of stimulation. Studies in isolated retina suggest that spike trains elicited through direct activation can achieve high temporal resolution; however, the precise range of frequencies varies between studies. Sekirnjak et al. (2006) stimulated at a range of frequencies up to 100 Hz for an extended period of time (5-20 ms), reporting that short-latency responses (presumably from direct activation of RGCs) could reliably be elicited up to approximately 50 Hz, above which responses declined. Ahuja et al. (2008) recorded from the RGCs in isolated salamander retina while stimulating at various frequencies, using presynaptic blockers to isolate direct activation of RGCs from indirect. They found that, with direct activation, RGCs could reliably respond to stimulation at rates of up to 500 Hz. Sekirnjak et al. (2006) and Ahuja et al. (2008) also found that long-latency responses decline at high frequencies. Sekirnjak et al.

(2006) found that long latency responses could follow the stimulation only up to 5 Hz and were suppressed at higher frequencies. The upper rate of response for presynaptic activation in the study by Ahuja et al. (2008) was 10 Hz. Similar conclusions have also been drawn for the degenerate retina. Ryu et al. (2009) showed that short-latency spikes could be reliably evoked by high frequency epiretinal stimulation of mice with retinal degeneration (specifically, *rd1* mice, which exhibit photoreceptor degeneration with preservation of other retinal cells). However, long-latency spikes were suppressed at rates above 10 Hz (Ryu et al., 2009). While this evidence suggests that desensitisation to high frequencies occurs mainly for indirect activation, Cai et al. (2011) found that for much higher rates of stimulation (2000 Hz) responses of rabbit ganglion cells decayed rapidly after onset of stimulation, and that this decay was still present when pharmacological blockers were applied to synaptic input. This result suggests that the mechanism causing desensitisation is mediated entirely within the ganglion cell (Cai et al., 2011).

Fried et al. (2006) also showed that short duration ($< 0.15\text{ms}$) pulses elicited only a single RGC spike per pulse at frequencies up to 250 Hz. Furthermore, they showed that temporal spiking patterns derived from responses to light stimuli could be precisely emulated using trains of these pulses. Wong et al. (2014) showed a similar result in patch-clamped cat RGCs, where they were able to evoke spiking with epiretinal stimulation that emulated the response to natural images, in both ON-and OFF-centre brisk transient cells. Jepson et al. (2014a) extended this concept by reproducing precise spatiotemporal responses of a group of ON parasol cells. Collectively, these studies show that it is possible to stimulate a target cell such that it spikes at rates up to those reported in normal vision, and with naturalistic temporal patterns. While this seems to indicate that firing patterns similar to those seen with normal visual responses may be achievable, the fading of percepts reported by retinal implant recipients in response to high rates of stimulation suggests otherwise (Fornos et al., 2012; Zrenner et al., 2011; Stingl et al., 2013; Stronks and Dagnelie, 2014), indicating that more study into this phenomenon is clearly required. Additionally, none of these strategies have attempted to selectively activate different types of cells, which is imperative to accurately recreating retinal activity.

Discrimination Between Cell Types

Electrical pulses delivered by stimulating electrodes, particularly those with larger diameters used clinically, activate a large area of the retina, without discriminating between cell types. The primate retina contains at least 17 morphologically distinct RGC types (Dacey, 2004), each conveying different information about a visual scene to the brain (Field and

Chichilnisky, 2007). Therefore, any stimulation strategy seeking to replicate normal vision would require the ability to differentially activate separate RGC types. Activation of separate RGC types has been investigated using a number of strategies in different animal models. By measuring thresholds of three different RGC types in rabbit retina, Fried et al. (2009) found that alpha cells had the lowest response thresholds. Therefore they postulated that alpha cells could be selectively activated using low amplitude pulses. However, no such differences between cell type thresholds were found in the primate retina (Jepson et al., 2013). Even if differences were present in primate retina, however, such a strategy has the inherent limitation that selective stimulation is only achievable for one cell type and could not distinguish between cells.

A better outcome based on amplitude selectivity was reported by Cai et al. (2013), who measured responses of rabbit OFF brisk transient and ON-OFF directionally selective cells to trains of pulses at 2000 pulses per second. They found that the two types responded optimally at different stimulus amplitudes, suggesting that the cells could be activated selectively by adjusting the stimulus amplitude. More recently, by examining the responses of rabbit RGCs to high frequency stimulation (2000 pulses per second), Twyford et al. (2014) showed that by altering the envelope used to modulate stimulation amplitude, ON and OFF brisk transient cells could be differentially activated. Considering that these cell types often display opposing activity patterns in response to light stimulation, it is thought that high frequency stimulation with envelope modulation could be used to evoke activity that is more natural.

1.5.2 *In Vivo* Studies

While the overarching aim of many *in vitro* studies is to reliably recreate natural patterns of firing activity in the retina with electrical stimulation, it is not clear whether recorded responses are equivalent to perception in the cortex (Weiland and Humayun, 2014), particularly considering that threshold levels for responses *in vitro* are lower than perceptual thresholds in clinical studies. However, a stimulus that leads to activation of the cortex is likely to lead to perception of vision. Activity measures have been recorded in the visual cortex of several animal models in response to electrical stimulation of the retina. These types of studies are useful for providing a more global view of prosthesis functionality.

In early studies, *in vivo* implantation served to confirm that activation of retinal cells with electrical stimulation is transmitted to higher visual centres, and that the device, implantation procedure, and stimulation do not cause permanent damage to the retina. More

recent studies have investigated characteristics of cortical responses related to device performance, including the currents required to elicit neural activity, the spread of responses spatially, the temporal resolution of responses, interactions between electrodes stimulated simultaneously, and stimulation strategies to improve device performance. A number of *in vivo* animal models have also been developed that exhibit similar retinal degenerations to those seen in humans with RP or AMD, to study the cortical responses to electrical stimulation of the degenerate retina.

Response Measures

Activation in the visual cortex can be measured by non-invasive and invasive means. By positioning electrodes on the scalp, the low frequency activity of populations of cells (evoked potentials) can be measured non-invasively. However, higher spatial resolution can be achieved with invasive methods such as subdural or penetrating electrodes, since signals are not attenuated by the skull. Subdural recording electrodes, placed on the surface of the brain, can measure the electrocorticogram (ECoG). Alternatively, electrodes that penetrate the brain can measure local field potentials (LFPs) or the spiking responses of cells, in the form of single or multi-unit activity (SUA or MUA, respectively). The investigations presented in this thesis involve *in vivo* animal experiments where cortical activity in response to electrical stimulation of the retina was recorded with penetrating electrode arrays. Multi-unit activity and evoked potential responses were recovered and used to evaluate the effect of multi-electrode stimulation.

Multi-unit activity typically reflects the spiking of multiple (tens to hundreds) cortical cells located within 150-300 μm from the tip of the recording electrode (Gray et al., 1995; Henze et al., 2000). However, LFP activity originates from cells 500-3000 μm from the electrode tip (Mitzdorf, 1987). It is thought that the early component of LFPs (<30 ms post stimulus) is representative of signals entering the visual cortex, while action potentials are indicative of neuronal output from the visual cortex (Mitzdorf, 1985; Fried and Jensen, 2011). Broadband LFP power has been shown to contain useful information about electrode configurations and stimulus parameters for stimulation of the retina from the epiretinal (Cottaris and Elfar, 2009) and suprachoroidal (Wong et al., 2016) spaces.

For application to human studies, less invasive measures of activity recording are preferred in order to reduce the number of invasive surgeries a patient is required to undergo. One such measure is the electrically evoked potential (EEP) recorded at the level of the visual cortex by positioning electrodes on the scalp (however, it can also be

recorded with electrodes placed on the exposed surface of the brain or with penetrating electrodes). The EEP contains both supra-threshold and sub-threshold components, and extends over wider radii than MUA (Fregnac et al., 1996). The EEP has been used to evaluate the effectiveness of visual prostheses (Sun et al., 2011; Nakauchi et al., 2005), and to develop a model to chronically monitor the implanted prostheses (Nayagam et al., 2014). Moreover, the EEP is similar to the visually evoked potentials (VEPs) elicited by flash stimulus of the eye (Nadig, 1999; Nakauchi et al., 2005; Schwahn et al., 2001), making it a useful signal for comparing responses to visual and electrical stimulation. It should be noted, however, that the latency of the EEP is significantly faster due to the electrical stimulation bypassing the retinal processing of visual signal inputs (Chowdhury et al., 2005; Nakauchi et al., 2005; Rizzo III et al., 2004).

Thresholds

The amount of charge that is required to reliably evoke cortical responses is referred to as the threshold. Low thresholds are desirable since excessive stimulation of the retina can result in tissue and electrode damage (Cohen et al., 2011), and higher current amplitudes result in a larger spread of current, thereby reducing spatial resolution. Thresholds for cortical activation have been investigated in a range of *in vivo* animal models, using various sizes of electrodes and device placements.

Hesse et al. (2000) stimulated with biphasic pulses using an array placed in the epiretinal space in adult cats. The minimum threshold for EP (evoked potential) responses was 14 nC per phase. This was comparable to the minimum threshold of 15 nC reported by Rizzo III et al. (2004) using epiretinal stimulation in rabbits while recording cortical EEPs. Similar thresholds have been reported for subretinal electrode arrays. Schanze et al. (2006) stimulated with 200 μ S per phase biphasic pulses in the subretinal space of minipigs, and reported a minimum threshold of 17 nC. When stimulating in the subretinal space of rabbits while recording cortical EPs, Yamauchi et al. (2005) and Gekeler et al. (2004) reported slightly lower thresholds of 9 nC and 5 nC, respectively. Yamauchi et al. (2005) also reported that thresholds required to elicit a cortical EP when stimulating from the suprachoroidal space were significantly higher (150 nC) than those required in the subretinal space. Shivdasani et al. (2010) reported similar thresholds of approximately 140 nC when stimulating from the suprachoroidal space in adult cats. Other suprachoroidal stimulation studies have shown quite variable thresholds, though consistently higher than epiretinal and subretinal thresholds. Nakauchi et al. (2005) reported thresholds of approximately 28 nC in rabbits, while those of Sakaguchi et al. (2004) in albino rabbits were 33

nC. Differences in thresholds within studies using the same electrode placement can be attributed to differences in recording techniques, species, or electrode design. Given the large number of variables involved, it is difficult to compare the results of the experiments but, in general, charge thresholds are lower for arrays placed in the epiretinal or subretinal locations compared to the suprachoroidal space. This is unsurprising, given the increased distance to the neural tissue.

While the majority of studies in *in vivo* animal models have been limited to healthy retinas, retinal prostheses are designed for restoring sight to humans with degenerate retinas. As such, it is important to consider the effects of electrical stimulation of degenerate retinas in animal models. Chen et al. (2006) compared cortical EP responses to epiretinal stimulation of normal and *rd1* mice, characterising both an early (<10 ms) and late response (>50 ms), which they attributed to direct and indirect activation, respectively. They found that the early responses in normal mice had lower thresholds than early responses in *rd1* mice; however, the thresholds for the late responses were similar between models. The authors posit that the difference between normal and *rd1* is degeneration of the RGCs, since the early (purportedly direct) responses were the most affected by degeneration. However, considering the thresholds of the late responses in the wild type mice were significantly greater than the early responses, the difference could also be due to damage of the retina, particularly the inner cells and photoreceptors, from surgery or stimulation.

A similar phenomenon was reported by Lorach et al. (2015), who compared cortical EP thresholds between rats with normal and degenerate retinas using a subretinal photovoltaic implant. They found that thresholds between the two types were not significantly different. However, in an *in vitro* study by the same group in the same animal models, thresholds of the rats with retinal degeneration were twice as high as the normal thresholds (Mathieson et al., 2012). Lorach et al. (2015) suggest that, in the *in vivo* normally sighted experiments, the retina was separated from the retinal pigment epithelium above the implant resulting in local degeneration of the photoreceptors, which may have contributed to lower thresholds in the *in vitro* experiments. This damage to the photoreceptors is an important drawback to the subretinal placement of arrays, which generally does not occur with suprachoroidal placement (Yamauchi et al., 2005). Considering that photoreceptor degeneration can be patchy and incomplete in diseases of the retina (Marc et al., 2007), it may be in the best interest of the patient to attempt to preserve remaining healthy photoreceptors.

Aplin et al. (2014) developed a feline model of ATP-induced photoreceptor degen-

eration, which they found to exhibit progressive retinal degeneration and remodeling over time, similar to other RP in humans (Aplin et al., 2016b). This animal model is of particular interest since the method by which blindness is induced (ATP-injection into the vitreous) can be applied unilaterally, thereby maintaining normal vision in the contralateral eye. Aplin et al. (2016a) subsequently compared cortical responses to suprachoroidal stimulation in both the normal and degenerate eyes of the model (via bilaterally implanted penetrating electrode arrays in the visual cortex), showing that MUA thresholds were significantly higher from stimulation of the degenerate eye compared to the normal eye.

Siu and Morley (2008a) used a rabbit model of outer retinal degeneration (developed by Humayun et al. (1995)) to investigate the feasibility of an episcleral retinal prosthesis. Photoreceptor degeneration was induced with sodium iodate, which resulted in destruction of photoreceptors in the central and peripheral retina while having little impact on the inner retinal cells. Siu and Morley (2008a) demonstrated that cortical EEPs could be elicited with safe levels of charge injection, and that thresholds for normal and degenerate eyes were similar in magnitude. This suggests that the photoreceptor layer may be bypassed during electrical stimulation from an episcleral location, and that inner retinal cells are the target of activation. This contrasts with the increased thresholds for the degenerate eye found by Aplin et al. (2016b). However, this difference is likely due to the reorganisation of the inner retina found in the cat model of ATP-induced retinal degeneration developed by Aplin et al. (2016b), which was not found in the study by Siu and Morley (2008a). This suggests that changes to the inner retina with remodelling secondary to photoreceptor loss are the cause of increased thresholds seen in animal models and possibly in human subjects, as the Siu and Morley model resembled more of an acute blindness (7 days post administration).

Furthermore, in rats with retinal degeneration, response thresholds have been found to increase with age (Chan et al., 2008) and are correlated with the degree of retinal ganglion cell loss (Chan et al., 2011). This general trend towards higher thresholds in animal models with degeneration, and link between degree of degeneration and efficacy of stimulation indicates that patients with severe retinal degeneration may require higher current levels to evoke visual percepts.

Spatial Characteristics of Responses

The human visual cortex encodes signals such that the spatial information of an image is preserved in the form of a visual field map (Holmes, 1918; Horton and Hoyt, 1991). Due

to the retinotopic organisation of the visual cortex, it is possible to relate the position of perception to the area of the visual cortex that is activated. Furthermore, the spread of cortical activation is expected to correlate with the size of the resultant percept. Through mapping activity in the visual cortex, studies have shown that retinal prostheses elicit focal responses. Wilms et al. (2003) stimulated the retina of anaesthetised cats visually and electrically while recording LFPs from the visual cortex. They estimated the spatial resolution of epiretinal stimulation as 1.28 mm in cortex, corresponding to 1.49° visual angle (at an eccentricity of 4-9°), with a best recorded resolution of 0.5°. Results from Cottaris and Elfar (2009) concur with this figure, finding that electrodes separated by as little as 150 μm (corresponding to 0.68°) could be discriminated when stimulating epiretinally. However, Wilms et al. (2003) reported that spatial resolution decreased with higher stimulation currents, while Cottaris and Elfar (2009) found the opposite. This difference can be attributed to differences in methodologies and devices between studies, including the wider range of phase widths used by Cottaris and Elfar (2009) (0.1-0.9 ms) compared to Wilms et al. (2003) (0.2 ms) or the different electrode types used. Cottaris and Elfar (2009) speculate that the exposed-tip cone electrodes used by Wilms et al. (2003) may have resulted in more diffuse electrical fields, with current spreading towards the vitreous, as opposed to their own disk electrodes blanketed in perfluorocarbon liquid, which would cause more focused electrical fields.

Eckhorn et al. (2006) compared the spatial resolutions of epiretinal and subretinal arrays. Thin-film electrode arrays were used, with 100 μm diameter electrodes used epiretinally and 100 μm^2 rectangular electrodes used subretinally. Spatial resolution was estimated by converting the retino-cortical point spread function into degrees of visual angle. They found that resolutions were comparable with 1.2° epiretinally and 0.9-1.3° subretinally (Eckhorn et al., 2006). They also estimated the best resolution possible with epiretinal placement, using 20 μm diameter cone fibre electrodes, which provided a resolution of 0.68°, identical to that shown by Cottaris and Elfar (2009). Elfar et al. (2009) showed that stimulating epiretinally in an adult cat resulted in two temporally separated responses in the cortex, which the authors likened to the direct and indirect activation of RGCs recorded *in vitro*. This comparison is strengthened by the spatial location of the responses, since the late response was spatially more localised and corresponded better to the actual location of the stimulating electrode, while the early response extended towards the area centralis. (Elfar et al., 2009). This suggests that the late component might be due to presynaptic activation of inner retinal cells, while the early response might originate from stimulation of RGCs and passing axons. If true, this result points towards subretinal stimulation having better spatial resolution than epiretinal stimulation, the latter of which

is limited by activation of passing axons.

For suprachoroidal-transretinal stimulation, spatial resolution has been estimated with superior colliculus responses in rats (Kanda et al., 2004). Responses to stimulation of electrodes separated by 700 μ m were discriminable, suggesting that a spatial resolution of 2.4° is realistic (Kanda et al., 2004). Later, Yan et al. (2015), using smaller electrodes (200 μ m diameter) with closer spacing (400 μ m centre-to-centre) while recording EPs in the rabbit visual cortex, estimated the spatial resolution with suprachoroidal-transretinal stimulation as 2°. Using a suprachoroidal array, Cicione et al. (2012) reported a spatial resolution of 4.5°, which is considerably higher than that reported by Yan et al. (2015) and Kanda et al. (2004). However, this may be due to differences between evaluation methods, since Cicione et al. (2012) defined cortical spread as the area of the response significantly greater than spontaneous activity, while Yan et al. (2015) estimated spread by the half-width at half maximum of Gaussians fitted to the responses. Considered together, these studies show that the spatial resolution of suprachoroidal prostheses is lower than that of epiretinal or subretinal prosthesis locations. This is not surprising given the increased distance to target cells and higher charge required to elicit a response. The suprachoroidal approach remains desirable, however, due to the previously described mechanical stability and simplicity of surgery. Therefore, several studies have investigated methods of improving the spatial resolution of suprachoroidal prostheses.

One approach to improving spatial resolution is limiting the spread of current in the retina by using one or more local return electrodes (Cicione et al., 2012; Matteucci et al., 2013; Wong et al., 2009; Spencer et al., 2016) as opposed to conventional monopolar stimulation, where the return electrode is located in the vitreous or on the sclera (Zhou et al., 2008; Kanda et al., 2004; Yamauchi et al., 2005). Alternate return configurations that have been reported include bipolar (Cicione et al., 2012), common ground (Cicione et al., 2012), hexapolar (Cicione et al., 2012; Wong et al., 2009; Matteucci et al., 2013; Spencer et al., 2016), focused multipolar (Spencer et al., 2016), and quasimonopolar (Matteucci et al., 2013). Bipolar stimulation utilises either an electrode adjacent to the stimulating electrode, or one that is further removed, as the return, and results in a similar amount of current spread as monopolar stimulation (Cicione et al., 2012), despite showing slight improvements when used in cochlear implants (Busby et al., 1994). In common ground stimulation, all remaining electrodes on an array are connected together to serve as a return while stimulating a single electrode. This configuration has been shown to reduce the spread of current in the retina, but interestingly did not improve cortical selectivity (Cicione et al., 2012). Hexapolar stimulation, in which the six electrodes surrounding a

stimulating electrode (when electrodes are arranged in a hexagonal pattern) are used as returns, has shown even further reduced current spreads than common ground stimulation, but mixed reports regarding cortical selectivity (Cicione et al., 2012; Wong et al., 2009; Matteucci et al., 2013; Spencer et al., 2016). This difference can most likely be attributed to the cortical spread being calculated at different points in the dynamic range of the response in the two studies. Hexapolar stimulation has a higher threshold and lower dynamic range than monopolar stimulation, likely due to a large amount of current shunting to the return electrodes rather than activating target cells (Joarder et al., 2011).

Focused multipolar stimulation (FMP) has only recently been investigated for use with retinal prostheses (Spencer et al., 2016), but has shown promise in cochlear implants (George et al., 2014). FMP involves simultaneous stimulation of multiple electrodes with weights calculated to nullify voltage changes in the area surrounding the stimulating electrode. FMP has shown similar improvements in retinal and cortical selectivity as hexapolar stimulation, with similarly elevated thresholds (Spencer et al., 2016). High thresholds are undesirable in retinal prostheses. Therefore, quasimonopolar stimulation attempts to reduce the amount of ineffective shunting of current by stimulating concurrently using a distant monopolar and hexapolar return configuration (Matteucci et al., 2013). This effectively produces a ‘best of both worlds’ outcome, with the reduced current spread (and increased cortical selectivity) of hexapolar stimulation, but thresholds closer to that of monopolar stimulation (Matteucci et al., 2013). Collectively, strategies like this that attempt to restrict the spread of current in the retina are referred to as ‘current focusing’ and show promise in improving the spatial resolution of retinal prostheses.

Understanding how the cortex responds to stimulation of single electrodes within the retina is clearly of high importance in the development of retinal prostheses, particularly given that most arrays used clinically employ sequential stimulation of single electrodes to elicit percepts. However, the numbers of electrodes on arrays will increase in the future, and 45-155 ms is required between sequential stimulus pulses for individual percepts to be perceived (Wilke et al., 2011c). Therefore, it is highly likely that simultaneous active stimulation of some form will need to be applied. Consequently, it is important to investigate the characteristics of responses to simultaneous stimulation, particularly since the cortical responses to such stimulation are not equivalent to the linear combination of the responses to stimulation of each electrode individually (Eger et al., 2005; Schanze et al., 2003; Yan et al., 2015; Shivdasani et al., 2012; Cicione et al., 2014; Dumm et al., 2014). This is due to the interaction between overlapping current fields from different electrodes in the retina. When electrodes nearby are stimulated with pulses of the same phase, this

interaction can have the effect of lowering cortical response thresholds (Shivdasani et al., 2012; Yan et al., 2015). However, the interactions can also be more complex. Schanze et al. (2003) recorded LFPs in the cat visual cortex while stimulating the retina electrically, and found that electrodes separated by 3.5° of visual angle resulted in inhibitory-like neuronal interactions while other distances caused excitatory-like interactions.

While it is possible to lessen channel interactions by using strategies such as hexapolar stimulation (Wilke et al., 2011a), it is also possible to exploit the interactions to excite different areas of the tissue, referred to as ‘current steering’. Dumm et al. (2014) showed that, when stimulating two nearby electrodes concurrently with varying ratios of current, cortical MUA responses could be generated intermediate between those elicited by physical electrodes. This strategy has the possibility of improving perceptual outcomes for retinal prosthesis users by increasing the number of available percepts beyond those achieved with single electrode stimulation alone.

1.6 Quantitative Models of Visual System Neurons

Many of the *in vitro* and *in vivo* studies discussed in the previous section relied on the qualitative characterisation and comparison of visual neuron responses to electrical stimulation. This is analogous to early approaches to characterising visual cortical neurons: by mapping the cells’ responses to various visual stimuli, including dots, lines and edges (Hubel and Wiesel, 1959, 1962). However, our understanding of visual receptive fields has been refined through the application of quantitative models, which provide a description of behavioral performance and insight into neural organisation. In the same way, recent studies have suggested that quantitative models can be applied to help understand the responses of visual system cells to electrical stimulation in order to improve stimulation strategies for retinal prostheses (Sekhar et al., 2016; Maturana et al., 2016; Freeman et al., 2010b; Jepson et al., 2014b). In this thesis, a linear-nonlinear (LN) model is used to characterise cortical responses to simultaneous electrical stimulation of the retina. Therefore this section is a review of the use of linear-nonlinear models in visual system research.

The LN model, illustrated in Figure 1.5, conceptualises receptive fields as spatio-temporal filters of stimuli followed by rectification, and originated in a study of RGCs by Rodieck and Stone (1965). LN models help to explain the responses of cells at various stages of the visual pathway to arbitrary visual stimuli (Wiener, 1966; Naka et al., 1988). A particular advantage of LN models is the ability to simulate large numbers of stimulus situations that have not necessarily been presented to the cell, meaning that the number

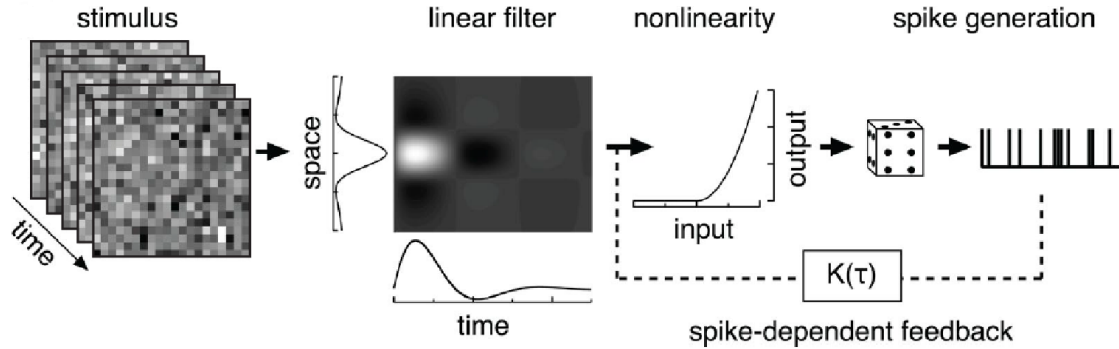


Figure 1.4: Linear-nonlinear model for ganglion cell responses. The input stimulus is passed through a spatiotemporal linear filter, followed by a time-independent nonlinearity. Adapted from Schwartz and Rieke 2011.

and duration of required experiments can be reduced. Additionally, LN models can be scaled up to encompass populations of cells. A LN model is often coupled with a Poisson spike generation process and sometimes includes a spike-dependent feedback term; both of these additions are shown in Figure 1.5. In a LN model, the spatiotemporal linear filter provides an estimate of the cells' spatial and temporal tuning properties, while the rectification step (commonly a static nonlinearity) accounts for nonlinear characteristics such as spike thresholds and saturation of responses (Chichilnisky, 2001; Schwartz and Rieke, 2011).

Classically, characterisation of visual system cells was achieved by presenting drifting and stationary bars, spots, sinusoidal gratings and other patterns while measuring spike counts following stimulus presentation. In some studies, such as that of Spekreijse (1969), multiple combinations of these stimuli were used in succession to identify cells. Later came the advent of characterising cells using white noise stimuli (Korenberg and Hunter, 1986; Sakai et al., 1988; Chichilnisky, 2001). While white noise has been used in identifying many different physiological systems, it has been most extensively applied to the visual system (Sakai, 1992). It is widely acknowledged that system characterisation through stimulation with white noise has several advantages over other techniques: it is more efficient since it covers a wide range of inputs due to being stochastic and interleaved; it doesn't lead to neuronal adaption from repeated, strong stimuli; and measurements may be taken from multiple cells during stimulation (Chichilnisky, 2001). Additionally, using white noise, the linear filter of the LN model can be readily obtained using the spike-triggered average of responses (Chichilnisky, 2001). However, if the purpose of the model is to predict responses of cells to non-white stimuli, such as natural images and patterns,

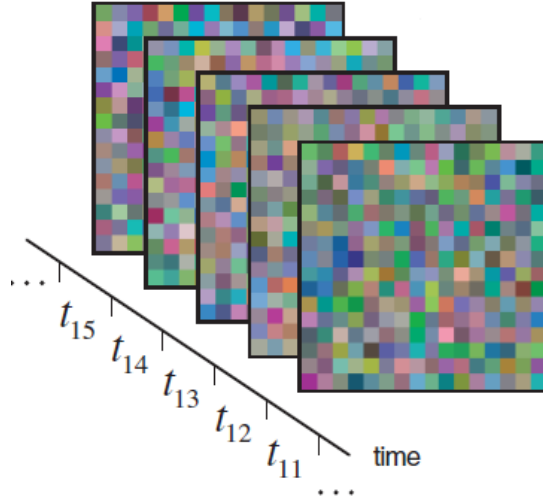


Figure 1.5: Example Gaussian white noise stimulus sequence, with spatial, temporal and chromatic modulation. Reproduced from Chichilnisky (2001).

the method may be inadequate (Gollisch, 2013). An example of a Gaussian white noise sequence is shown in Figure 1.5.

1.6.1 Adaptations to Classical Models

Several adaptations to the model have been proposed in recent studies, such as the addition of a spike-dependent negative feedback loop that makes the model dependent on recent spike history (Keat et al., 2001). The addition of the Poisson spike generation process adds a stochastic element to the model, mimicking the stochastic nature of spiking cells (Chichilnisky, 2001).

The addition of another linear filter following the nonlinear step is the fundamental difference between the Wiener model and the Korenberg model, which is referred to as a linear-nonlinear-linear (LNL) model (Korenberg and Hunter, 1986). Victor and Shapley (1979) compared the responses of a LNL model to observed second-order frequency responses of type Y RGCs in the cat retina. The two responses agreed well, and the authors proposed that the first linear filter could represent bipolar cells while the nonlinearity and second linear filter might be associated with amacrine cells (Victor and Shapley, 1979).

For both of these approaches, the spatial integration in the retina is approximated by a linear filter, thus assuming that the integration is a linear process. However, many

cells show nonlinear response properties, so the linear filter may appropriately estimate receptive field shapes but not account for nonlinearities within the receptive field (Gollisch, 2013; Schwartz and Rieke, 2011). Gollisch (2013) proposed that one reason so many studies show that LN models provide a good approximation of cell responses is that they are probed with white noise, which rarely has any of the spatial correlations of a natural scene. Some studies have included a nonlinear element prior to spatial integration, which has improved predictions for certain types of stimuli, but may also limit their use for general stimuli (Gollisch and Meister, 2008, 2010).

1.6.2 Application of Models to Retinal Prostheses

While classically LN models were used to describe the responses of cells to light, the model can also be used to explore the responses of cells to electrical stimulation. This was demonstrated by Guttman et al. (1974) to characterise the response of a squid axon to electrical stimulation and, more recently, by various groups to investigate the responses of retinal cells to electrical stimulation with retinal prostheses (Sekhar et al., 2016; Maturana et al., 2016; Freeman et al., 2010b).

Maturana et al. (2016) showed that a linear-nonlinear model could accurately predict RGC responses to simultaneous stimulation of multiple electrodes *in vitro*. The linear component of the model was computed using spike-triggered covariance analysis; the authors found that for the majority of cells, a single linear filter was sufficient to predict neural responses (Maturana et al., 2016). The nonlinearity was then estimated by projecting the spike-triggered stimuli onto the linear filter and fitting a sigmoid to the spiking probability using a least squares estimation. The linear filters found in this analysis characterise the cell's electrical receptive fields, while the sigmoidal nonlinearity describes the nonlinear aspects of a cell's firing rate, such as saturation at high charge levels. The authors suggest that the simplicity and success of the model lends itself to future use in real-time, closed-loop applications to improve clinical outcomes; however, they stipulate that some technical barriers remain before that goal can be accomplished (Maturana et al., 2016). These challenges include the requirement of a high density retinal prosthesis capable of both stimulation and recording on the same array.

Jepson et al. (2014b) used a piecewise linear model to predict the activation of a target cell in the retina *in vitro*. Fixed ratios of current at different amplitudes were delivered across two electrodes concurrently while responses were recorded. A line was then fit to the thresholds obtained with the set of stimulus patterns, with the goodness of

fit estimated by a nonlinearity index. For electrode pairs with particularly bad fits, lines were fit separately to different regions of the stimulus space. The authors showed that this simple model could accurately predict the activation of cells to stimulation of two electrodes, and demonstrated the generalisability of the model by successfully predicting responses to stimulation of three electrodes. Furthermore, they showed that patterns of stimulation to selectively activate a target cell could be predicted from a set of patterns (Jepson et al., 2014b); therefore, this work show promise in improving spatial resolution of prostheses, provided the responses recorded at such small scales *in vitro* translates to perception.

Cascade type models are most often used to further understand cell processing and are tested by predicting spiking responses such as in the studies by Maturana et al. (2016) and Jepson et al. (2014b). However, a LN model with Poisson spike generation has also been used as an “encoder” in a retinal prosthesis strategy (Nirenberg and Pandarinath, 2012). The encoder takes a natural image as an input and mimics the processing of the retina to deliver stimuli that more closely approximate the input that ganglion cells would normally receive (Nirenberg and Pandarinath, 2012). Interestingly, the stimulus set involved a combination of white noise and natural images, since they were seen to be complimentary (Nirenberg and Pandarinath, 2012). Also, the model was fitted using a machine learning approach, as opposed to a least squares estimation. With this approach, the authors found that the RGC firing patterns produced by the prosthesis quite closely approximated those produced with natural stimuli.

These studies demonstrate that the responses of retinal cells can be accurately predicted by a linear-nonlinear model and that a similar model can be used to predict optimal stimulation strategies for targeting specific cells. However, they have several limitations. The main limitation is that responses were only recorded from a small number of individual cells *in vitro*, since a particular aim was the generation of retinal activity similar to those seen in the healthy retina in response to light. However, it is not clear whether activity recorded in this way is akin to perception, whereas activity recorded in the visual cortex is more likely to be associated with perceivable occurrences in the visual field. Additionally, the study by Jepson et al. (2014b) used very small electrodes that are not used clinically; therefore, it is difficult to determine whether similar results would be seen with a human subject clinically. Furthermore, while Jepson et al. (2014b) showed that it was possible to predict the optimal response to activate a target cell while minimising the response on a nearby non-target cell, they did not record from surrounding neurons, meaning that any effects on the rest of the retina are unknown.

Chapter 2

Thesis Overview

2.1 Motivation

Retinal prostheses can restore a sense of vision to people blinded due to photoreceptor degeneration. Currently, most retinal prostheses employ sequential stimulation of individual electrodes to build up an image of the world. When stimulated simultaneously, interactions between adjacent electrodes may reduce spatial resolution of responses (Wilke et al., 2011a,b) and result in unpredictable, complex percepts that are not equivalent to sequentially driven composites (Zrenner et al., 2008; Rizzo et al., 2003b; Wilke et al., 2011b). However, with increasing numbers of electrodes being incorporated into retinal prostheses, more electrodes will be stimulated to build up an image. If update rates are to be kept constant or increased, simultaneous stimulation of multiple electrodes will be required in order to stimulate all required electrodes in each frame.

Some studies have shown that interactions between electrodes can be lessened by using nearby combinations of return electrodes (Cicione et al., 2012; Matteucci et al., 2013; Wong et al., 2009; Spencer et al., 2016). However, others suggest that actively stimulating multiple electrodes simultaneously may confer a larger range of percepts (Dumm et al., 2014) and improve device resolution (Jepson et al., 2011). Spatial resolution is currently a limiting factor of retinal prostheses, particularly for those far from target cells such as the suprachoroidal space, since larger currents are required, resulting in increased current spread. Therefore, if simultaneous stimulation can provide a means to improve device resolution while solving the foreseeable issue of excessive stimulation time for sequential stimulation, it clearly requires further research.

While the effect of electrode interactions has been variable based on the reports of subjects in clinical trials, differences may have been due to unreliable patient feedback. Therefore, the first aim of this thesis is to characterise cortical responses to simultaneous multi-electrode stimulation of the retina. By investigating responses *in vivo*, the effects of simultaneous stimulation can be quantitatively explored, thereby removing bias in patients interpretations.

The second goal is to characterise any differences between cortical responses to simultaneous stimulation in healthy versus degenerate retinæ. Given the changes that are known to occur in the retina following the loss of photoreceptors (Jones et al., 2012), it is possible that inconsistencies in clinical studies are related to varying levels of retinal degeneration. Therefore, understanding the characteristics of responses to multi-electrode stimulation of the degenerate retina is a crucial step towards eventual application in clinical studies.

The final goal is to explore the possibility of shaping neural activity by exploiting the characteristics of cortical responses to simultaneous stimulation. Some studies have showed promising results towards this goal (Jepson et al., 2011; Dumm et al., 2014), but have either used electrodes that are not clinically relevant while recording *in vitro* from only two cells (Jepson et al., 2011), or cannot be scaled up to include stimulation of many electrodes (Dumm et al., 2014). Successful application of neural activity shaping could result in improved outcomes for retinal prosthesis users.

2.2 Thesis Outline

Successful use of multi-electrode simultaneous stimulation requires an understanding of the characteristics of cortical responses to such stimuli. To achieve this, the work presented in this thesis makes use of a linear-nonlinear model fitted to the responses of cortical cells to electrical white noise stimulation of the retina in an *in vivo* cat model. Linear-nonlinear models have been frequently applied to investigate and predict responses of cells along the visual pathway to visual stimulation, and have recently been applied to understand the activity of RGCs in response to electrical stimulation. However, the responses of cortical cells are yet to be characterised, though they provide a closer analogue to human perception.

The work presented in this thesis was performed in both normally-sighted cats (Chapters 3 and 5) and cats with retinal degeneration (Chapter 4). The cat is well

established as a model for cortical visual neuroscience, and as such the synaptic physiology underlying the visual pathway in this species is well known (Orban, 2012; Ferster and Miller, 2000). Feline and human eyes are comparable with regard to size; the feline eye is only slightly smaller. The cat eye also possesses both a retinal and a choroidal circulation, and so is a better model of the human eye than is the rabbit eye (Bertschinger et al., 2008). Also, due to the well established cortical recording techniques and the above mentioned knowledge of the feline visual system, the cat model is widely used in measuring cortical activation from electrical retinal stimulation (Hesse et al., 2000; Dawson and Radtke, 1977; Shivdasani et al., 2010; Chowdhury et al., 2005; Walter et al., 2005; Schanze et al., 2002), and has been the most commonly used animal model in the development of vision prostheses so far (Fried and Jensen, 2011). Additionally, human perception is broadly consistent with models or predictions based on the response properties of cortical neurons recorded in cats and primates (Gilbert and Wiesel, 1990; van Wezel et al., 1997; von der Heydt and Peterhans, 1989; Knierim et al., 1992; Salzman et al., 1990). Therefore, it can also be assumed that the perception elicited by electrical stimulation of the retina in a human can be roughly approximated by the activation of cat visual cortex cells. For these reasons, the cat is an appropriate choice of animal model for these studies.

In Chapter 3, we show that a linear-nonlinear model accurately describes the cortical responses to simultaneous stimulation of a suprachoroidal prosthesis in a normally sighted cat. Of particular interest is information about responses conferred by the model, including electrical receptive fields of cortical sites. This study has been published (Halupka et al., 2016).

In Chapter 4, we explore the characteristics of cortical responses to multi-electrode stimulation of the degenerate retina, using the model developed in Chapter 3. A unilaterally blind feline model with bilaterally implanted stimulating and recording arrays is used, such that the responses to stimulation of healthy and degenerate retina can be compared in the same animal. This study was submitted to Investigative Ophthalmology and Visual Science (IOVS) on the 14th of December 2016, and is currently under review.

In Chapter 5, we present a proof of concept method of generating activity shaping stimulus patterns by partially inverting the linear-nonlinear model developed in Chapters 3 and 4.

In Chapter 6, we summarise the work described in this thesis, and discuss the key outcomes and future directions of this research.

Chapter 3

Prediction of Cortical Responses to Simultaneous Multi-Electrode Stimulation of the Retina

3.1 Abstract

Simultaneous electrical stimulation of multiple electrodes has shown promise in diversifying the responses that can be elicited by retinal prostheses compared to interleaved single electrode stimulation. However, the effects of interactions between electrodes are not well understood and clinical trials with simultaneous stimulation have produced inconsistent results. We investigated the effects of multiple electrode stimulation of the retina by developing a model of cortical responses to retinal stimulation. Electrical stimuli consisting of temporally sparse, biphasic current pulses with amplitudes sampled from a zero-centered Gaussian distribution were simultaneously delivered to the retina via a 42-channel electrode array implanted in the suprachoroidal space of anaesthetised cats. Visual cortex activity was recorded using penetrating microelectrode arrays. These data were used to identify a linear-nonlinear model of cortical responses to retinal stimulation. The ability of the model to generalise was tested by predicting responses to non-white patterned stimuli. The model accurately predicted two cortical activity measures: multi-unit neural responses and evoked potential responses to white noise stimuli. The model provides information about electrical receptive fields, including the relative effects of each stimulating electrode on every recording site. This demonstrates that a simple model accurately describes cortical responses to simultaneous stimulation of a suprachoroidal retinal pros-

thesis. Overall, our results show that cortical responses to simultaneous multi-electrode stimulation of the retina are repeatable and predictable, and that interactions between electrodes during simultaneous stimulation are predominantly linear. The model shows promise for determining optimal stimulation paradigms that exploit interactions between electrodes to shape neural activity, thereby improving outcomes for patients with retinal prostheses.

3.2 Introduction

Retinal prostheses produce phosphenes by electrically stimulating surviving retinal neurons in patients with severe photoreceptor degeneration. Clinical trials have shown that retinal prostheses elicit useful perceptions, resulting in improvements in spatio-motor tasks (Barry et al., 2012; Kotecha et al., 2014; Stingl et al., 2015) and reading (da Cruz et al., 2013). However, spatial resolution with present devices is severely limited with the highest reported visual acuity achieved of 20/546 (Stingl et al., 2013), while the majority of other reported acuities are much worse.

One strategy that shows promise for improving resolution of retinal prostheses is simultaneous stimulation of multiple electrodes. Interactions that occur when combinations of electrodes are stimulated simultaneously are capable of increasing the repertoire of visual percepts that can be elicited compared to conventional single-electrode stimulation. Simultaneous stimulation can activate groups of cells between electrodes (Dumm et al., 2014), reduce current spread by using one or more local return electrodes (Cicione et al., 2012; Habib et al., 2013; Matteucci et al., 2013), and elicit responses at the level of a single cell *in vitro* (Jepson et al., 2014b). Clinically, studies have mainly aimed to elucidate perceptual differences between sequential and simultaneous stimulation (Humayun et al., 1999; Rizzo et al., 2003b; Auner et al., 2007; Horsager et al., 2010; Wilke et al., 2011b). Although these studies were limited to stimulation of electrodes in simple geometric patterns such as lines and squares with identical biphasic pulses on all electrodes, inconsistencies were reported regarding percept predictability.

It is clear that simultaneous stimulation is a promising strategy for eliciting useful percepts. However, the crucial next step in either reducing or harnessing electrode interactions is to investigate the predictability and reproducibility of responses to simultaneous stimulation, and gain a better understanding of how electrode interactions affect neural responses. Here, we combine simultaneous stimulation of up to 42 suprachoroidal electrodes with multi-site recordings in the cat visual cortex to demonstrate that elec-

trode interactions at the level of the cortex are predominantly linear. Additionally, we show that a simple model comprised of a linear filter followed by a static nonlinearity (hereafter, linear-nonlinear model) can reliably predict neural spiking responses to several different simultaneous stimulation paradigms. We also demonstrate the ability of the same model to predict responses based on an alternative metric of cortical activity, namely, the power in the evoked responses. The predictive success of our model shows promise for efficiently determining optimal stimulation paradigms for shaping neural activity with a retinal prosthesis.

3.3 Methods

Experiments were performed in eight normally-sighted, adult cats. All procedures were approved by the Bionics Institute Animal Research Ethics Committee (Projects 12/255AB and 14/304AB).

3.3.1 Anesthesia and Surgery

Cats were anaesthetised with an initial dose of ketamine (intramuscular, 20 mg/kg) and xylazil (subcutaneous, 2 mg/kg). Anesthesia was maintained with an intravenous infusion of sodium pentobarbitone (60 mg/kg/hr) and Hartmanns solution (sodium lactate, 2.5 m L/kg/hour). Dexamethasone (intramuscular, 0.1 mg/kg) and Clavulox (subcutaneous, 10 mg/kg) injections were given daily to minimise brain swelling and infection. Physiological indicators were continuously monitored throughout the experiment.

Details of the surgical implantation of the suprachoroidal array have been described previously (Shivdasani et al., 2012). Briefly, a lateral incision through the sclera was performed to expose the choroid. The electrode array was then inserted into the suprachoroidal space via a pocket created between the sclera and choroid and sutured into place (Villalobos et al., 2012).

The stimulating and recording array placements are illustrated in Figure 3.1. The suprachoroidal array (Figure 3.1A) consisted of a flexible medical grade silicone substrate with 7 rows $\times$ 3 columns of platinum electrodes (used in six experiments), or 7 rows $\times$ 6 columns of electrodes (used in the remaining two experiments). Electrodes were 600 μ m in diameter arranged hexagonally with 1 mm center-to-center spacing (Villalobos et al., 2012).

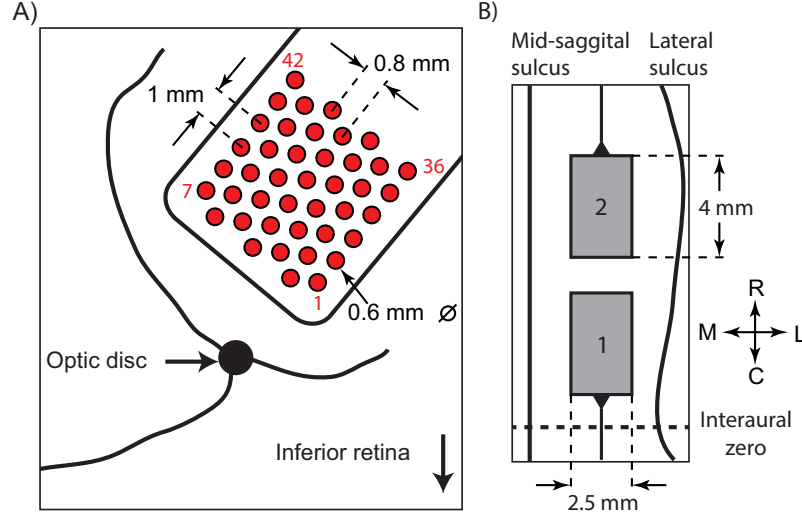


Figure 3.1: A) Schematic of stimulating array position and dimensions in relation to optic disc; electrode numbering for the four corner electrodes of the array are shown in red. B) Schematic of the recording array placements in the hemisphere of the visual cortex contralateral to the implanted eye. R: rostral, C: caudal, M: medial, L: lateral.

The visual cortex contralateral to the implanted eye was exposed and penetrating microelectrode arrays (either 6×10 or 6×6 channels, 1 mm length, 400 μm spacing, Blackrock Microsystems, USA) were implanted (Figure 3.1B). Evoked potentials (EPs) from the cortical surface in response to retinal stimulation were used to optimise the position of the implanted arrays (Cicione et al., 2012; Dumm et al., 2014). Neural signals were sampled at 30 kHz using the Cerebus Neural Processing System (Blackrock Microsystems, Salt Lake City, UT).

3.3.2 White Noise Stimuli

Temporally sparse (1 Hz) electrical pulses delivered simultaneously across the stimulating electrodes were used to characterise the system while responses were recorded in the visual cortex. The stimulus amplitude for each electrode was sampled from a zero-centered Gaussian distribution (hereafter, spatially white stimuli), where a positive amplitude reflected an anodic-first pulse, and a negative amplitude reflected a cathodic-first pulse. To balance the effect of each electrode on cortical activation, the standard deviation of the Gaussian distribution for each electrode was chosen to be the activation threshold for that electrode, as described below.

Thresholds were acquired by stimulating each electrode individually with cathodic-

first biphasic current pulses using 1 ms phase width, 25 μ s interphase gap, at 1 Hz presentation rate (the same pulse waveform was used for all electrical stimulation paradigms), and 0-750 μ A stimulus amplitude, in 50 μ A steps. A spike-rate (or power) versus stimulus amplitude input-output function for each stimulating electrode-recording channel combination was generated to which a sigmoid curve was fitted. For each sigmoid, the stimulus amplitude at 50% of the maximum response was defined as the threshold for that recording channel (Shivdasani et al., 2012). The threshold for each stimulating electrode was chosen to be the lowest threshold over all recording channels for which a sigmoid could be fit.

For white noise stimulation, all electrodes across the array were stimulated simultaneously with charge-balanced biphasic waveforms using a mix of anodic-first and cathodic-first polarities with current amplitudes sampled from their respective Gaussian distributions. There were 3600 white-noise stimulation patterns generated for each experiment; these were presented eight times, with the average of the responses on each recording channel used for analysis.

To investigate variability in cortical responses to repeated white noise stimuli, 60 repetitions of 30 different white noise patterns were presented in a randomised order in three of the experiments. The mean and standard deviation of the responses to each of these patterns was calculated.

3.3.3 Oriented Pattern Stimuli

Stationary, oriented electrical stimulus patterns were presented to test the prediction ability of the model with non-white stimuli. These patterns were electrical representations of sinusoidal gratings at six possible orientations (0, 30, 60, 90, 120, and 150 degrees), five spatial frequencies (ranging over approximately 1-3 cycles per array length), and scaled by five stimulating currents (i.e., amplitudes of stimulating currents on each electrode were scaled by 175, 235, 295, 355, or 415). Patterns consisted of three main types, as illustrated in Figure 3.2: (i) mixed-phase first stimuli (Figure 3.2A), where some electrodes on the array were stimulated with anodic-phase first biphasic pulses while others were stimulated with cathodic-phase first biphasic pulses; (ii) anodic-phase first stimuli (Figure 3.2B), where all electrodes on the array were stimulated with anodic-phase first biphasic pulses; (iii) cathodic-phase first stimuli (Figure 3.2C), where all electrodes on the array were stimulated with cathodic-phase first biphasic pulses. During each experiment, 30 different pattern stimuli were presented in a randomised order, with 10 repetitions of

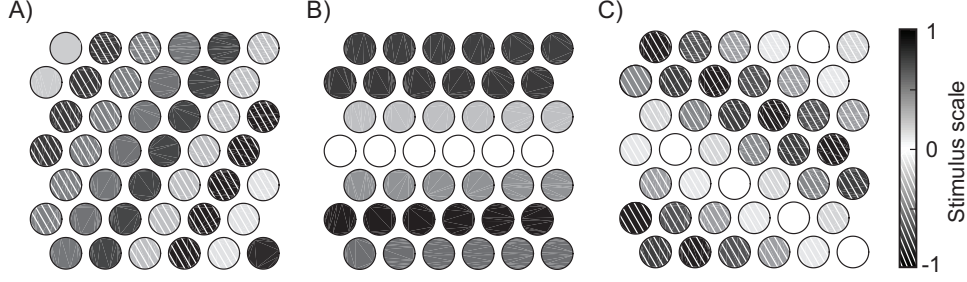


Figure 3.2: A) Example electrical stimulation patterns used as oriented electrical stimuli. The weighting of each electrode is represented by a gray scale, with black being the strongest weighting on the array (and thus the biggest current amplitude). White represents zero current amplitude. Electrodes with cathodic-phase first stimuli are hatched in white. A) Mixed-phase stimulus. B) Anodic-phase first only stimuli. C) Cathodic-phase first only stimuli.

each pattern stimulus.

3.3.4 Data Pre-Processing

Two measures were used to estimate the cortical activity that occurred within approximately 20 ms of the stimulus pulse: power in the evoked potential (EP power) in the 400-1400 Hz frequency band and multi-unit activity (MUA). To estimate the power in the evoked responses, offline multi-taper spectral analysis (Thomson, 1982; Mitra and Pesaran, 1999) was used to filter the raw data x_t , where x_t is the 30 kHz signal of length D recorded on a single electrode (the signal on each electrode was filtered independently). Multitaper spectral analysis involved multiplication of the data, x_t , by K orthogonal tapers, $w_t(k)$, and applying a fast Fourier transform to the tapered waveforms,

$$\tilde{x}_k(f) = \sum_{t=1}^D w_t(k) x_t \exp(-2\pi i f_0 t), \quad (3.1)$$

with a centre frequency f_0 of 900 Hz. The individual tapered spectral estimates, $\tilde{x}_k(f)$, were then averaged over the tapers to reduce variance and bias, and produce the direct multitaper spectral estimate $S_{MT}(T)$,

$$S_{MT}(f) = \frac{1}{K} \sum_{k=1}^K |\tilde{x}_k(f)|^2. \quad (3.2)$$

For the present study the taper functions were chosen to be discrete prolate spheroidal sequences (DPSSs) (Slepian and Pollak, 1961) for their optimal spectral concentration properties. DPSSs are parameterised by their length in time, L , and their bandwidth parameter, W . These two parameters were chosen iteratively by visual inspection, with $L = 4$ ms and $W = 500$ Hz, and the first two sequences were used, therefore $K = 2$. After filtering, the EP signal was downsampled to 3.0 kHz, before the spectrum for time-steps pre- and post-stimulation were estimated (MATLAB, Version 8.4, Mathworks, Inc. USA) using a frequency window of 500 Hz, moved in the time domain in 1 ms steps. The log power was then calculated from 3 ms after the end of the stimulus pulse (to exclude the stimulus artifact) until 20 ms after the pulse with the power in the 400-1400 Hz band in the 500 ms prior to each stimulus subtracted to exclude spontaneous activity.

For the MUA analyses, stimulus artefacts were first removed by computing a straight line between two sample points either side of each artefact, and replacing the stimulus artefact with values interpolated along this line (Heffer and Fallon, 2008; Cicione et al., 2012; Shivdasani et al., 2012). Following band-pass filtering using a third-order Butterworth filter (300-5000 Hz), multiunit spikes were detected as threshold crossings using a threshold of $4 \times$ root-mean-square amplitude that was calculated within a 60 s moving time window. Typically, MUA occurred 3-20 ms post-stimulus; however, in some instances, another burst of spikes followed at approximately 30 ms post-stimulus. Only the early component of spiking was analysed as this is considered to be a result of direct stimulation of retinal ganglion cells (RGCs) and indirect activation of the inner retina (Boinagrov et al., 2014). The spontaneous spiking rate on each channel in the 500 ms prior to each stimulus was subtracted to account for variation in the stimulus-independent activity. For both measures, responses on each channel were normalised by the maximum response recorded on the array to any presented stimulus.

3.3.5 Linear-Nonlinear (LN) Model

The Linear-Nonlinear (LN) model consisted of two parallel spatial linear filters that provided estimates of the spatial tuning properties of each cortical recording channel, followed by two parallel static nonlinearities that accounted for nonlinear characteristics of neurons such as response thresholds and saturation.

This model is similar to well-established linear-nonlinear models developed to describe light responses in the retina (Chichilnisky, 2001; Pillow et al., 2005; Schwartz and Rieke, 2011) and RGC responses to simultaneous stimulation of the retina (Maturana et al.,

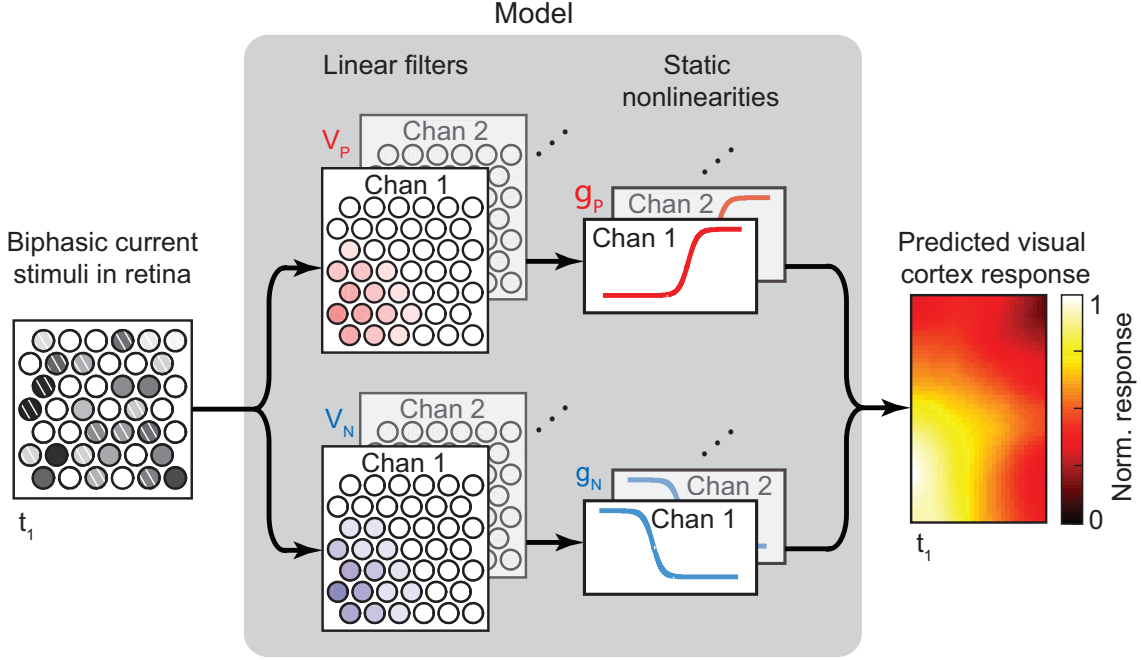


Figure 3.3: The linear non-linear cascade model of the pathway illustrated between the retina and visual cortex. Shown here is the input signal, arranged in the form of multiple electrode stimulation of the suprachoroidal array. The input is split by the model into a “positive” and a “negative” path, each representing the cortical response to net anodic first and net cathodic first stimuli respectively. After the linear and the nonlinear filters act on either side, the outputs are summed. The summed output is the predicted cortical response to a particular input stimulus. A total of 3600 white noise patterns were presented to the retina, here t_1 refers to a single stimulus and its resulting predicted response.

2016). However, several key adaptations have been made to the established model to accommodate the different stimulation method (i.e., electrical vs. light stimulation). First, as opposite phase pulses can produce strong, but differing responses (Jensen and Rizzo, 2006), the effects of both anodic-first and cathodic-first biphasic pulses were included separately in the model. Specifically, two different linear filters (V_P for net anodic-first (positive) and V_N for net cathodic-first (negative) stimuli) and their respective static nonlinearities (g_P and g_N) acted on the input (S) separately (Figure 3.3).

Our model was also adjusted to accommodate prediction of response amplitudes rather than spike probability. Additionally, we recorded cortical responses at multiple sites (up to 120) concurrently during stimulation and, as such, a LN model was fitted independently for each channel; thus, the index for each channel is omitted from the equations below for clarity.

An estimate of the activity (either spike count or EP power) at each time $t \in \{1 \dots T\}$, where T is the number of stimuli that resulted in a response, at a given site in the cortex is given by

$$\mathbf{R}_{\text{est}} = g_P \left(\mathbf{S}^{\text{tr}} \mathbf{V}_P \right) + g_N \left(\mathbf{S}^{\text{tr}} \mathbf{V}_N \right), \quad (3.3)$$

where tr is the matrix transpose. Each column of the stimulus matrix $\mathbf{S}$ contains the stimulation amplitude applied at time t with a row for each stimulating electrode $j \in \{1 \dots M\}$. The stimulus vector at time t is thus given by

$$\mathbf{s}_t = \begin{pmatrix} s_{1,t} \\ \vdots \\ s_{M,t} \end{pmatrix}. \quad (3.4)$$

The normalised stimulus vector is given by

$$\mathbf{s}_t = \begin{pmatrix} s_{1,t}/\sigma_1 \\ \vdots \\ s_{M,t}/\sigma_M \end{pmatrix}, \quad (3.5)$$

where σ_j ($j \in \{1 \dots M\}$), is the activation threshold of each stimulating electrode.

The combined effect of all stimulating electrodes on a response in a given cortical channel could be either net anodic-first or net cathodic-first. Responses of retinal cells may differ depending on pulse polarity (Jensen and Rizzo, 2006), therefore, the effects of both anodic-first and cathodic-first biphasic pulses were included separately in the model. Spike-triggered covariance analysis was used to identify the responses that were due to either net anodic-first stimulation or net-cathodic first stimulation. Since more than one spike was sometimes recorded per stimulus, the spike triggered covariance is given by

$$\mathbf{C}_{\text{STC}} = \frac{1}{N} \sum_{t=1}^T r_{i,t} \mathbf{s}_t \mathbf{s}_t^{\text{tr}}, \quad (3.6)$$

where $r_{i,t}$ is the response on recording channel $i \in \{1 \dots C\}$ at time t , and N is the sum of responses on that channel,

$$N = \sum_{t=1}^T r_{i,t}. \quad (3.7)$$

The first principal component of $\mathbf{C}_{\text{STC}}$ (denoted $\mathbf{u}_{\text{STC}}$) is the direction in the stimulus space in which the variance of the stimuli that evoked a response differs maximally from

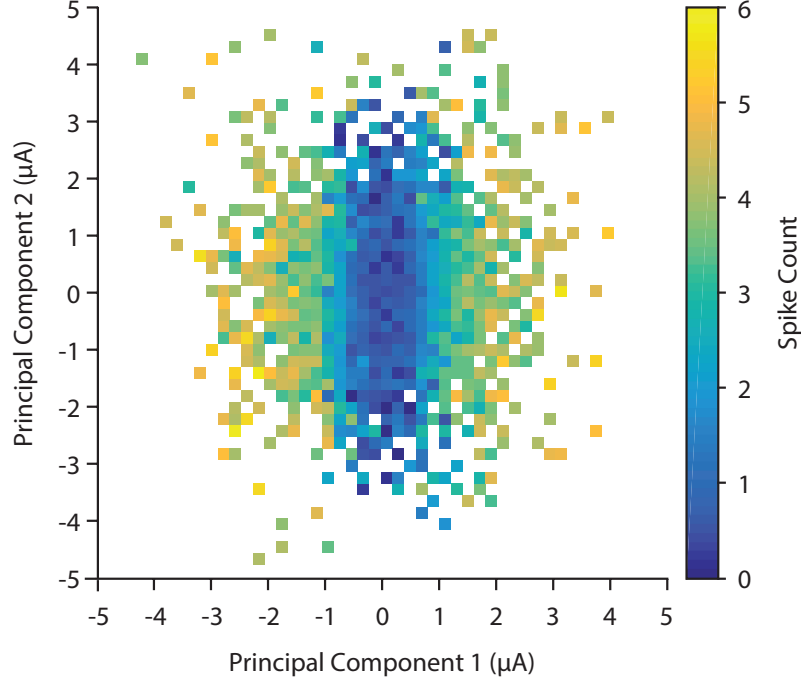


Figure 3.4: Projection of the stimuli onto the first (x-axis) and second (y-axis) principal components of the spike-triggered covariance C_{STC} for one recording channel. Projection onto the first component divided the stimulus space into positive and negative regions. Colours represent the average spike count evoked by stimuli within each bin (bin width = $0.2 \mu\text{A}$).

those stimuli that did not evoke a response. Projecting all stimuli onto $\mathbf{u}_{\text{STC}}$ served to divide the stimulus space into positive and negative regions. Responses due to a net anodic-first stimulation $\mathbf{R}_\text{P}$ were those where the projections of the stimulus onto the principal component of $\mathbf{C}_{\text{STC}}$ were positive. Stimuli for which this held true were denoted $\mathbf{s}_\text{P}$, such that

$$\mathbf{s}_\text{P} \cdot \mathbf{u}_{\text{STC}} > 0. \quad (3.8)$$

Responses to a net cathodic-first stimulation $\mathbf{R}_\text{N}$ were those for which the projections onto the principal component were negative,

$$\mathbf{s}_\text{N} \cdot \mathbf{u}_{\text{STC}} < 0, \quad (3.9)$$

where $\mathbf{s}_\text{N}$ were the corresponding cathodic-first stimuli. Figure 3.4 shows an example of the stimulus projected onto the first two principal components of $\mathbf{C}_{\text{STC}}$, coloured based on the response each evoked at one cortical recording channel.

The linear filter estimates $\widetilde{\mathbf{V}}_{\text{P,est}}$ and $\widetilde{\mathbf{V}}_{\text{N,est}}$ were then calculated as the spike triggered average (Chichilnisky, 2001) of response subsets $\mathbf{r}_{\text{P}}$ and $\mathbf{r}_{\text{N}}$ and their respective stimuli ($\mathbf{s}_{\text{P}}$ and $\mathbf{s}_{\text{N}}$),

$$\widetilde{\mathbf{V}}_{\text{P,est}} = \frac{\mathbf{r}_{\text{P}}^{\text{T}} \mathbf{s}_{\text{P}}}{N_{\text{P}}} \quad (3.10)$$

and

$$\widetilde{\mathbf{V}}_{\text{N,est}} = \frac{\mathbf{r}_{\text{N}}^{\text{tr}} \mathbf{s}_{\text{N}}}{N_{\text{N}}}, \quad (3.11)$$

where N_{P} and N_{N} are the sums of response subsets $\mathbf{r}_{\text{P}}$ and $\mathbf{r}_{\text{N}}$, respectively, such that $N_{\text{P}} + N_{\text{N}} = N$. The filters were normalised by dividing by their L-2 norm,

$$\mathbf{V}_{\text{N,est}} = \frac{\widetilde{\mathbf{V}}_{\text{N,est}}}{|\widetilde{\mathbf{V}}_{\text{N,est}}|} \quad (3.12)$$

$$\mathbf{V}_{\text{N,est}} = \frac{\widetilde{\mathbf{V}}_{\text{N,est}}}{|\widetilde{\mathbf{V}}_{\text{N,est}}|} \quad (3.13)$$

The normalised vectors $\mathbf{V}_{\text{P,est}}$ and $\mathbf{V}_{\text{N,est}}$ were considered to be weightings of the effects of the stimulating electrodes on a given cortical site (Chichilnisky, 2001).

The static nonlinearities g_{P} and g_{N} were approximated by sigmoids and parameterised by their saturation amplitudes (y_{P} and y_{N}), thresholds (a_{P} and a_{N}), which are 50% of the saturation level, and the gain of the sigmoids (b_{P} and b_{N}),

$$g_{\text{P}}(r) = \frac{y_{\text{P}}}{1 + e^{(-b_{\text{P}}(x_{\text{P}} - a_{\text{P}}))}} \quad (3.14)$$

$$g_{\text{N}}(r) = y_{\text{N}} - \frac{y_{\text{N}}}{1 + e^{(-b_{\text{N}}(x_{\text{N}} - a_{\text{N}}))}}. \quad (3.15)$$

We used the distributions of the outputs of the linear filters $x_{\text{P}} = \mathbf{s}_{\text{P}} \mathbf{V}_{\text{P,est}}$ and $x_{\text{N}} = \mathbf{s}_{\text{N}} \mathbf{V}_{\text{N,est}}$ (hereafter referred to as generator signals) and corresponding responses $\mathbf{r}_{\text{P}}$ and $\mathbf{r}_{\text{N}}$ to provide initial estimates for the parameters of non-linear functions g_{P} and g_{N} , respectively. The threshold values a_{P} and a_{N} were estimated to be the mean values of their respective generator signals, while y_{P} and y_{N} were estimated as the maximum average responses recorded on any channel (averaged over repeated trials). We used a Levenberg-Marquardt non-linear least squares algorithm (the `lsqnonlin` function in MATLAB Version 8.4, Mathworks, Inc. USA) to find the optimal non-linear parameters (a_{P} , a_{N} , b_{P} , b_{N} , y_{P} , y_{N}) and confirm that the optimal linear filters were $\mathbf{V}_{\text{P,est}}$ and $\mathbf{V}_{\text{N,est}}$. The outcome of

this process was a set of linear filters and static nonlinearities, each corresponding to a different recording channel (shown for one recording channel in Figure 3.6).

To test which electrodes significantly affected the response of each recording channel, we randomly time-shifted the response vector 1000 times and repeated the above analysis (Equations 3.3-3.13) for each new response subset. This generated a distribution for $\mathbf{V}_{P,est}$ and $\mathbf{V}_{N,est}$ to which the true vectors could be compared. Electrodes from the true $\mathbf{V}_{P,est}$ and $\mathbf{V}_{N,est}$ that were larger than the root-mean-square of the randomly-generated distribution were considered significant.

The spatial extent in the retina over which a cortical site is influenced is given by

$$D_P = \frac{\sum_{j=1}^M v_j^P d_j}{v_j^P} \quad (3.16)$$

$$D_N = \frac{\sum_{j=1}^M v_j^N d_j}{v_j^N}, \quad (3.17)$$

where d_j is the distance of each electrode j to the center of mass of the significant electrodes and v_j^P and v_j^N are the weights given by $\mathbf{V}_{P,est}$ and $\mathbf{V}_{N,est}$, respectively.

3.3.6 Statistical Analysis

The linear filters $\mathbf{V}_{P,est}$ and $\mathbf{V}_{N,est}$ were often visibly similar, therefore, a sign test was used to determine whether the magnitudes of $\mathbf{V}_{P,est}$ and $\mathbf{V}_{N,est}$ on an electrode by electrode basis for each channel were significantly different. False Discovery Rate correction (Benjamini and Hochberg, 1995) was used to control for multiple comparisons to a level of $p < 0.05$. We used the same analysis to compare the sizes of $\mathbf{V}_{P,est}$ and $\mathbf{V}_{N,est}$ between the two model types (MUA model and EP power model).

To demonstrate the ability of the model to predict cortical responses to spatially white electrical retinal stimulation, we performed a 6-fold cross validation analysis on each of the eight experiments for models fitted to both MUA and evoked response power. For this, the model was fitted using 5/6 of the white noise data and used to predict the remaining 1/6 of the data. To ensure that recording sites were driven by electrical stimulation of the retina, only recording sites that exhibited a monotonic increase of activity with current in response to single electrode stimulation of at least one stimulating electrode were included in model fitting and analysis. Cross-correlation analyses were performed to compare the predicted and recorded responses. The coefficient of determination and

slope of the line of best fit (where the predicted response was the independent variable) were calculated for each of the cross-validation sets independently. Significant differences between the prediction abilities of the model fitted to MUA versus that fitted to evoked response power were tested using a Wilcoxon Rank sum test ($p < 0.05$).

In order to rule out any correlations due to chance, we estimated the number of responses to white noise patterns in the trial set that could be accurately predicted by the model. A bootstrap test was performed (10,000 samples) comparing the absolute residual error of prediction for each white noise pattern in the trial set against a distribution composed of the absolute residual error of recorded responses versus time-shifted predictions, over all fitted channels. False Discovery Rate correction (Benjamini and Hochberg, 1995) was used to control for multiple comparisons to a level of $p < 0.05$.

We then tested the ability of a model trained with responses to white noise stimuli to predict responses to the oriented pattern stimuli. Models used in these predictions were fitted to all of the responses to white noise stimuli (rather than just 5/6 of the data). Here, the coefficient of determination and slope of the line of best fit was calculated for each of the five stimulation amplitudes separately for each of the eight experiments. Further bootstrap analyses, similar to those performed with the white noise data, were also performed on the pattern stimuli data to rule out any correlations that may have occurred due to chance. The same analysis was performed to compare the predicted and measured responses to single electrode stimulation using the data obtained to calculate single electrode thresholds. In addition to comparing the absolute residual error to the bootstrapped distribution, the coefficient of determination between the predicted and recorded responses to above-threshold single electrode stimulation was also compared in the same manner.

3.4 Results

Across eight experiments, visual cortex responses to multi-electrode stimulation of the retina were recorded and analysed in the form of multi-unit activity (MUA) and evoked potential (EP) power on a total of 696 recording channels.

3.4.1 Cortical Responses to Multi-Electrode Suprachoroidal Stimulation

Figures 3.5A and 3.5B show raw recording traces from two recording sites following a stimulus pulse, indicating evoked responses and the presence of spiking activity, respec-

tively (to a white noise stimulus). Evoked responses typically consisted of two prominent peaks occurring a few milliseconds apart (Figure 3.5A), sometimes followed by a third peak, resulting in a significant increase in power from the baseline response (Figure 3.5E). Both response measures exhibited a characteristic, monotonically increasing sigmoidal relationship with current amplitude (Figure 3.5C,D) (Cicione et al., 2012; Shivdasani et al., 2012; Dumm et al., 2014). However, significantly more channels exhibited this effect for EP power than MUA (MUA vs. EP power: $66.6\% \pm 6.1$ vs. $92.4\% \pm 3.2$, $p = 0.0029$; Rank sum test; $N=8$).

3.4.2 Prediction of Cortical Responses

Models for the response on each channel were fitted independently and parametrised by two linear filters and two static nonlinearities (Figure 3.6). The linear filters derived analytically through spike-triggered average (Equations 3.3-3.13) and the linear filters following further numerical optimisation were compared, showing that the filters were largely identical before and after optimisation, with a median correlation coefficient of $r^2 = 0.93$. The optimised linear filters and nonlinearity parameters were used in all further analyses. Figure 3.6 shows the components of the model for one recording channel. As shown by the linear filters, which can be considered to be the electrical receptive fields (ERFs) for each cortical channel, this particular recording site was maximally activated by pulses on the top left edge of the stimulating array. The static nonlinearities are functions of the action of the linear filters on the stimulus vector. For this recording channel, the model prediction of the responses (Figure 3.6C) was in close agreement with the recorded responses shown in grey (mean $\pm$ SEM of the binned responses) (coefficient of determination $r^2 = 0.865$).

3.4.3 Validating Model Fit

For the averaged responses to stimuli to be a reasonable metric to fit the model, the stimulus response variability must be minimal. Figure 3.7A shows the measured EP power (mean $\pm$ SE) for all recording channels in response to 60 repetitions of a white noise stimulus pattern in one experiment. Only minor variation in responses was observed, and a clear and significant correlation was seen with the responses predicted by the model ($r^2 = 0.93$, $p < 0.0001$, FDR corrected permutation test, 10 million permutations). The same metric, but for a single cortical channel in response to 30 different white noise stimuli each repeated 60 times, is shown in Figure 3.7B. Again, only minor variation in

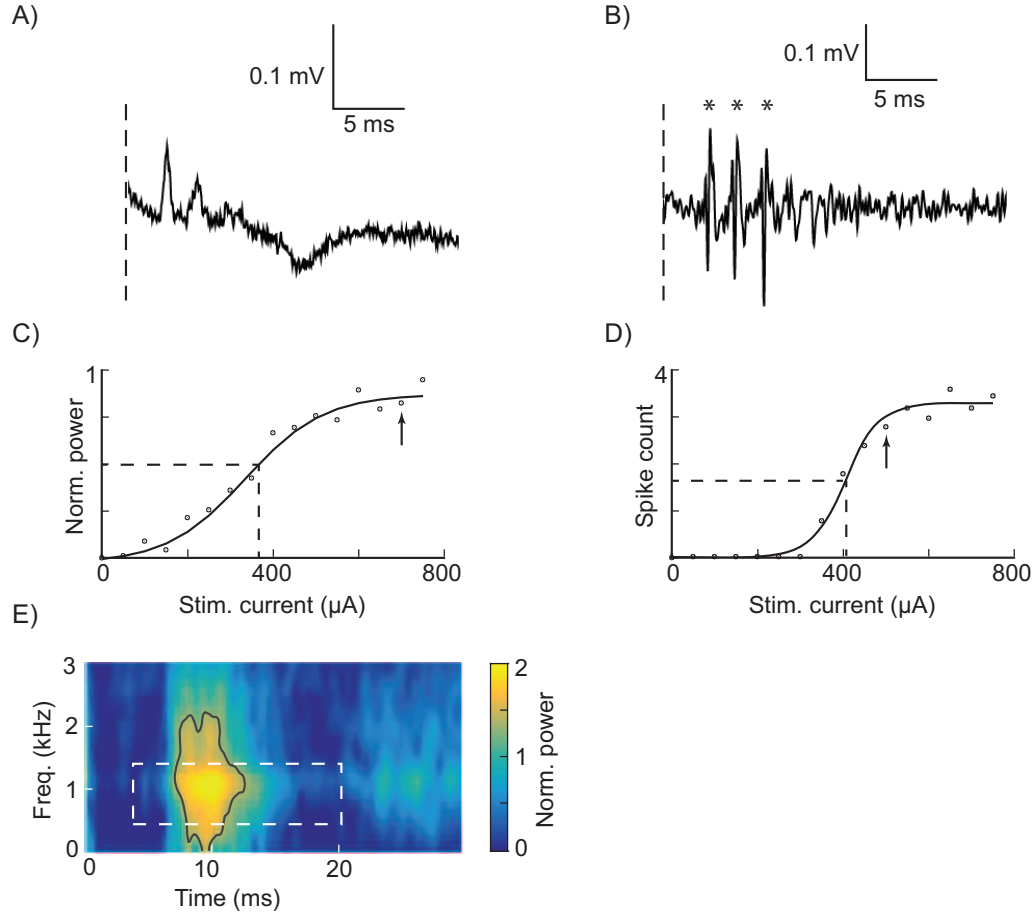


Figure 3.5: A) Raw response on one recording channel from a single electrode stimulus at 700 μA , showing a multi-peaked evoked potential; dashed line shows the end of the stimulus artefact. B) Filtered trace on a different recording channel, showing spiking (indicated by asterisks) in response to single electrode stimulation. C, D) Transfer functions of the power in the multi-peaked evoked potential (C) and spike count (D) versus stimulation amplitude, with a sigmoidal line of best fit. Dashed lines show the thresholds of these particular stimulating electrodes at 50% of maximum response. The arrows show the average normalised power and spike count for responses shown in panels A and B respectively. E) Power spectrum of the multi-peaked response shown in panel A. The black outline indicates significant increase in power from the baseline response. White dashed box indicates the time and frequency window within which the EP power is calculated.

response was observed, with a clear and significant correlation ($r^2 = 0.92$, $p < 0.0001$, FDR corrected permutation test, 10 million permutations). The stimuli used to fit the model comprised 3600 different white noise patterns, averaged over eight repeats. For one such white noise stimulus (Figure 3.8A), a comparison of the recorded and predicted responses

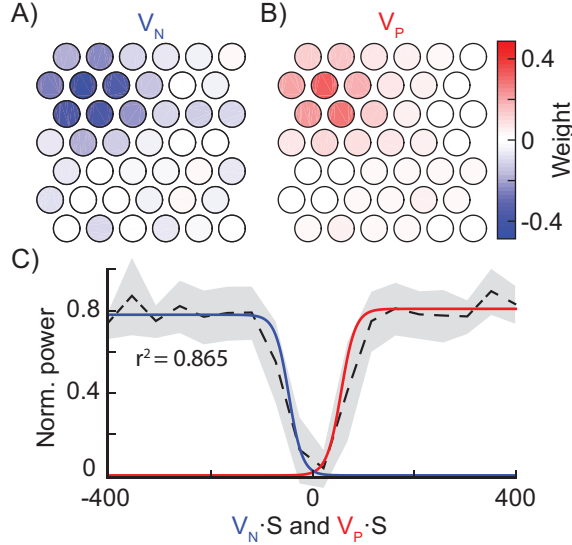


Figure 3.6: Model components for a single recording channel, as shown schematically in Figure 3.3, fitted with EP power in response to 5/6th of the white noise stimuli in one experiment. A) Spatial linear filters V_N and B) V_P , where each circle represents a stimulating electrode on the retinal array, and color refers to the strength of the linear filter. C) Static nonlinearities g_N (blue line) and g_P (red line). The y axis is normalised to the maximum average EP power recorded on any channel. Dashed black line shows the average recorded response to each of the binned generator signal levels (mean $\pm$ SEM, shown in grey) ($r^2=0.865$).

(Figure 3.8B) indicates that the model prediction of the response was very similar to the measured response, which was borne out by a coefficient of determination of $r^2 = 0.936$ (Figure 3.8C), with a strong overall correlation observed for this experiment ($r^2 = 0.819$, Figure 3.8D).

Similar correlations were found for all six cross-validated models for all eight experiments, with $0.55 < r^2 < 0.89$ ($n = 48$). Models fitted to MUA displayed significantly higher correlation coefficients (MUA vs. EP power: $r^2 = 0.77 \pm 0.01$ vs. 0.69 ± 0.02 , $p = 1.22 \times 10^{-4}$; Rank sum test) than those fitted to EP power. The average slope of the line of best fit for the models fitted to power was significantly closer to 1 (MUA vs. EP power: 0.88 ± 0.018 vs. 0.92 ± 0.13 , $p = 2.13 \times 10^{-4}$; Rank sum test) than those fitted to MUA.

Both types of models were able to predict the responses to at least 75% of the test set significantly better than chance (via bootstrap analysis), with the MUA model predicting significantly more than the EP power model (MUA vs. EP Power: $95.77\% \pm 0.57\%$ vs.

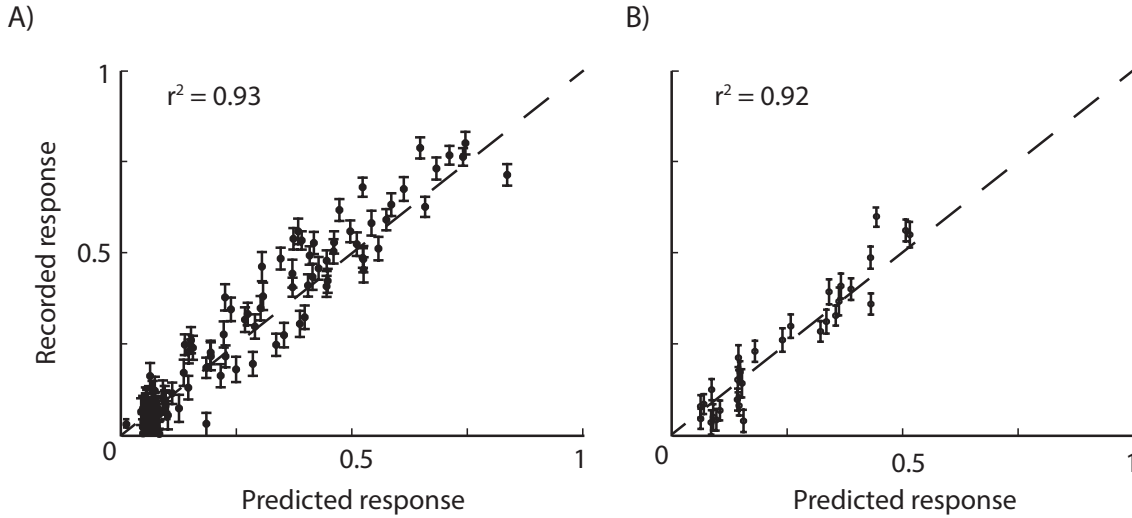


Figure 3.7: Mean and standard error of recorded responses ($n=60$ repetitions of each stimulus) plotted against the responses predicted by the model. Response measure is power in the multi-peaked EP. A) Responses on 88 cortical channels in one experiment to 60 repetitions of a single white noise stimulus. B) Responses on a single recording channel in the same experiment as in panel A, to 60 repetitions of 30 separate white noise stimuli.

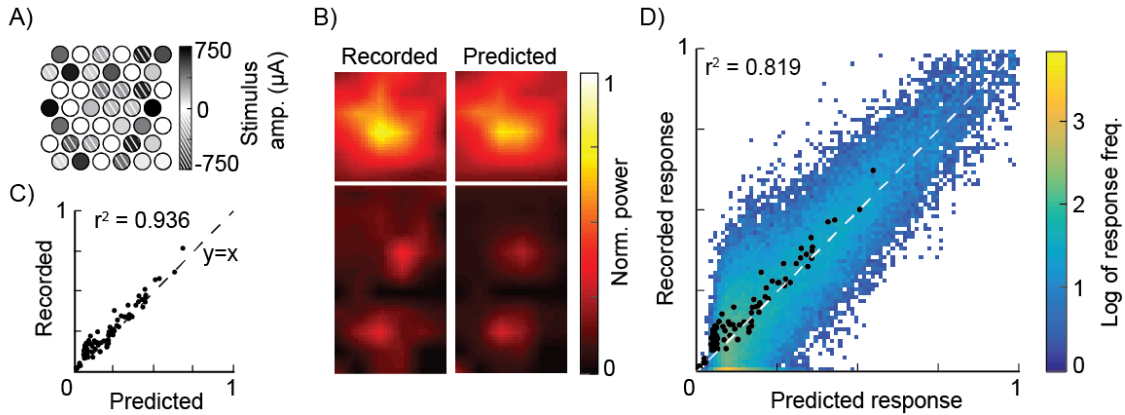


Figure 3.8: A) Example of a white-noise stimulus in one experiment with positive currents indicating anodic-first pulses and negative currents indicating cathodic-first pulses. B) Recorded and predicted EP power in the visual cortex across two recording arrays in response to the white noise stimulus shown in panel A (averaged over 8 repetitions). C) Correlation between the recorded response to the stimulus and the response predicted by the model. Each point represents the response measured on a single recording channel. Dashed line is $y=x$. D) Aggregated scatter plot for all 600 white-noise stimuli that were not used to fit the model for this experiment, with the log frequency of responses represented on the color scale. Black dots show the responses presented in panel C.

$92.70\% \pm 0.90\%$, $p = 4.08 \times 10^{-4}$; Rank sum test).

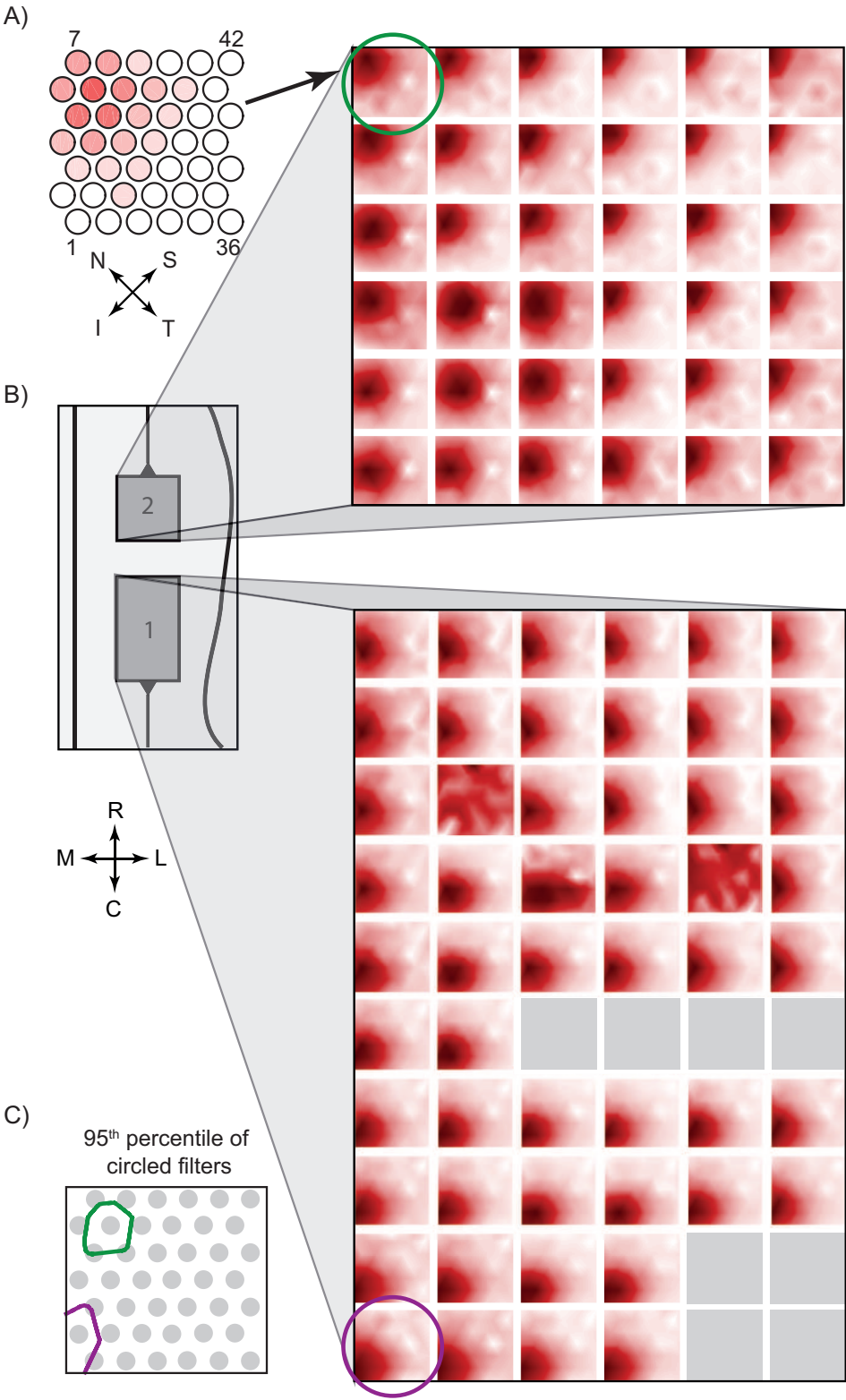
3.4.4 Electrical Receptive Fields

The linear filters of the model provided an estimate of the ERFs of each recording channel for simultaneous retinal stimulation. Figure 3.9A shows the ERF for stimuli with a net anodic-first effect (V_P), for the most rostro-medial recording site; this channel was most responsive to stimulation of the superior-nasal retinal electrodes.

The interpolated color maps depicting the linear filters at all cortical sites ($n = 96$) from this experiment (Figure 3.9B) display a clear preference to stimulation of superior-nasal retinal electrodes for more rostral recording sites, while caudal recording sites showed a preference for stimulation of electrodes located towards the inferior retina. The gradual movement of the ERFs between these two extremes indicates topographic mapping, and is likely to be retinotopic. Using these ERFs in combination with the static nonlinearities, it is possible to link regions of high activity to stimulus features. For example, the high power in the rostral (rostral direction shown in Figure 3.1B) sites in Figure 3.8B can be attributed to high stimulation amplitude on superior-nasal retinal electrodes.

Figure 3.9C more clearly illustrates the rostro-caudal movement of the linear filters, where contours represent the significant linear filter weights belonging to the most rostro-medial (green) and caudo-medial (purple) recording sites (circled in Figure 3.9B).

Figure 3.9: Linear filter maps for each recording site in one experiment. A) Linear filter of the most rostro-caudally located cortical site (marked with a green circle in panel B) prior to interpolation, as described in Figure 3.6. Only filters corresponding to anodic first stimulation (i.e., V_P) are shown. Electrode numbers correspond to those indicated in Figure 3.1A. S: superior retina, I: inferior retina, N: nasal retina, T: temporal retina. B) Interpolated color maps representing optimised linear filters for anodic first stimulation pulses (V_P) corresponding to each of the 96 recording sites. Schematic of cortical recording areas corresponding to each of the linear filter maps is also shown. Red indicates a strong effect of a particular stimulating electrode on the activity recorded on a given cortical site. Blank sites are those that did not record a monotonically increasing response to single electrode stimulation and were thus excluded from analyses. R: rostral, C: caudal, M: medial, L: lateral. C) Schematic of stimulating array showing significant areas of the linear filter maps for two cortical recording sites marked with green and purple circles (corresponding to rostro-medial and caudo-medial recording sites respectively) in panel B. The shift in the electrodes that have the strongest contributions to a channels activity with cortical channel position shows that the retinotopic organisation of the visual cortex is preserved with responses to simultaneous electrical stimulation of the retina.



The recording sites in this example were separated by a distance of approximately 7 mm in the cortex, which corresponds to approximately $14-23^\circ$ of visual angle using a cortical magnification factor of 0.3-0.5 mm/degree (Tusa et al., 1978). The peak weightings of the linear filters for these channels were separated by about 4 mm in retinal space, which subtends a visual angle of approximately 18° , and thus corresponds well with the recording channel separation.

While significant differences did occur between the spatial extent of the ERFs for stimuli with a net cathodic-first effect (V_N) and those with a net anodic-first effect (V_P) on a channel by channel basis when using the EP power based model (Sign test, 0.05 level of significance), these differences were not consistent across experiments. Two out of eight experiments had larger V_N filters than V_P filters; however, one experiment had significantly larger V_P filters than V_N filters. No differences occurred between MUA-based model ERFs. Similar inconsistent differences occurred when comparing the ERF size between the two model types (EP power based models: two experiments had larger V_N filters, three had larger V_P filters; MUA-based models: three experiments had larger V_N filters, three had larger V_P filters).

3.4.5 Predicting Responses to Electrical Pattern Stimuli

To examine whether a model fitted using responses to white noise stimuli could predict responses to non-white stimuli, measured responses to oriented pattern stimuli and single electrode stimuli were compared to responses predicted by the model. Three types of patterned stimuli were used: mixed-phase, anodic-first, and cathodic-first. Figure 3.10A shows the comparison between the recorded and predicted responses for the stimulus shown in Figure 3.2A, where the maximum current amplitude delivered was $295\mu\text{A}$. The responses predicted by the model corresponded well to the measured responses (Figure 3.10B, $r^2 = 0.89$) for this stimulus. To assess whether this effect extended to the entire set of oriented pattern stimuli, we calculated a coefficient of determination for each of the three pattern types for all experiments (Figure 3.11A). Coefficients corresponding to MUA models were significantly higher than those for EP power models for mixed-phase stimuli (MUA vs. EP power: $r^2 = 0.81 \pm 0.02$ vs. 0.69 ± 0.03 , $p = 0.016$; Rank sum test). There was no difference between the MUA model and EP power model for the other two stimulus types (MUA vs. EP power with anodic-first pulses: $r^2 = 0.65 \pm 0.07$ vs. 0.65 ± 0.05 , $p = 0.558$. MUA vs. EP power with cathodic-first pulses: 0.73 ± 0.06 vs. 0.73 ± 0.04 , $p = 0.457$). However, for the mixed-phase and cathodic-first stimulus types, the slopes of the lines of best fit for the models fitted to MUA were significantly lower than those fitted

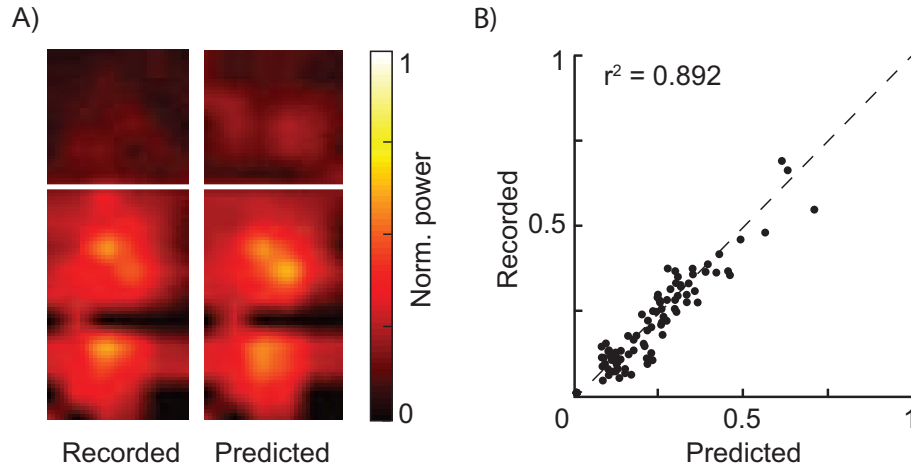


Figure 3.10: Recorded response and model-predicted response to the oriented pattern stimulus shown in Figure 3.2A (mixed phase first stimulus). Response is measured by the power in the EP, and shown over two recording arrays arranged as in Figure 3.9. The color map is normalised to the maximum recorded power. A) Recorded response (left), and predicted response (right). B) Correlation between the recorded and predicted responses ($n=88$ channels).

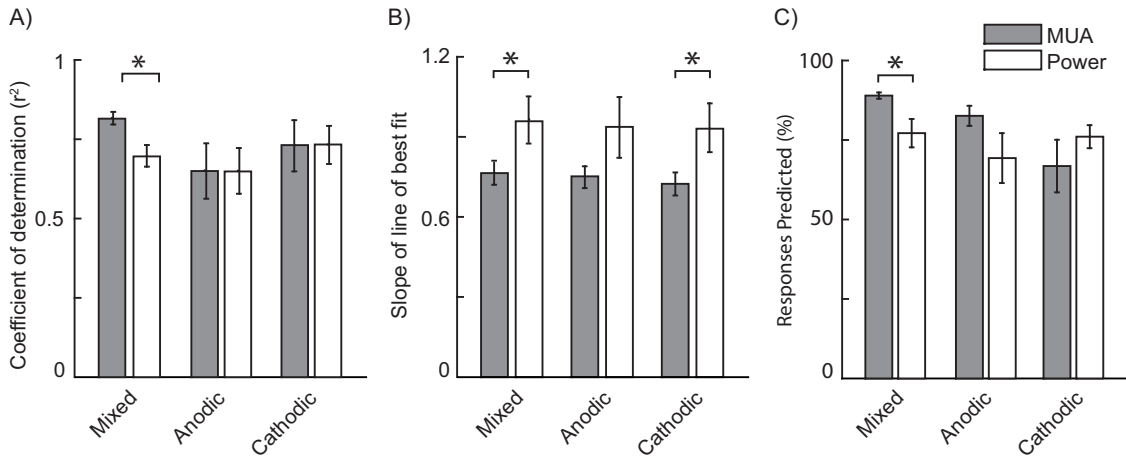


Figure 3.11: Comparison of the ability to predict responses to grating pattern stimuli between a spiking-based model (grey) and power-based model (white), averaged over all experiments and stimulation amplitudes (mean $\pm$ SEM) ($n = 40$). Asterisks represent a significant difference ($p < 0.05$; Rank sum test). A) Coefficient of determination between the model-predicted responses to oriented pattern stimuli and the recorded responses. B) Slope of the line of best fit for the correlation described in panel A. C) Percentage of responses to oriented pattern stimuli that were able to be predicted significantly using bootstrapping analyses.

to EP power (Figure 3.11B, MUA vs. EP power with mixed-phase pulses: 0.76 ± 0.05 vs. 0.96 ± 0.09 , $p = 0.02$. MUA vs. EP power with anodic-first pulses: 0.75 ± 0.04 vs. 0.94 ± 0.12 , $p = 0.08$. MUA vs. EP power with cathodic-first pulses: 0.72 ± 0.05 vs. 0.93 ± 0.10 , $p = 0.03$; Rank sum test).

While the percentage of responses that were able to be predicted significantly above chance was generally high for both types of response measures and all types of stimuli (via bootstrap analysis, Figure 3.11C), the model fitted to MUA was able to predict a significantly higher number of responses for both mixed-phase and anodic-first stimuli compared to the EP power model (MUA vs. EP power with mixed-phase pulses: $94\% \pm 2.1\%$ vs. $77\% \pm 5.2\%$, $p = 0.0024$. MUA vs. EP power with anodic-first pulses: $85\% \pm 5.4\%$ vs. $70\% \pm 7.0\%$, $p = 0.129$. MUA vs. EP power with cathodic-first pulses: $70\% \pm 5.8\%$ vs. $77\% \pm 6.1\%$, $p = 0.326$; Rank sum test).

When predicting responses to single electrode stimulation, coefficients of determination corresponding to EP power models were significantly higher than those corresponding to MUA models (MUA vs. EP power: $r^2 = 0.26 \pm 0.02$ vs. 0.37 ± 0.02 , $p = 0.016$; Rank sum test). Considering only responses to stimuli above threshold resulted in higher coefficients of determination, with no significant difference between response measures (MUA vs. EP power: $r^2 = 0.53 \pm 0.07$ vs. 0.56 ± 0.03 , $p = 0.16$; Rank sum test). However, both were still lower than those for multi-electrode stimulation. Despite being low, the majority of both MUA and EP responses to stimuli above threshold had r^2 values significantly higher than those of the corresponding bootstrapped distributions (MUA vs. EP power: $90.6\% \pm 1.20\%$ vs $92.76\% \pm 0.75\%$, averaged over above-threshold stimulus amplitudes and experiments). The slopes of the lines of best fit for the models fitted to MUA were significantly greater than those fitted to EP power (MUA vs. EP power: 0.40 ± 0.03 vs. 0.12 ± 0.01 , $p < 0.001$; Rank sum test), though both were lower than 1, indicating that both models predicted a larger response than what was recorded. The percentages of responses able to be predicted accurately by either model were also relatively low, and not significantly different between the model types (MUA vs. EP power: $53\% \pm 3.0\%$ vs. $45\% \pm 1.5\%$, $p = 0.208$; Rank sum test).

3.5 Discussion

We have demonstrated that cortical responses to simultaneous stimulation of the retina are repeatable and can be predicted by a simple linear-nonlinear (LN) model. We have shown that the model can be optimised using either multi-unit activity (MUA) or power

in the multi-peaked evoked potential (EP), and can be used to predict responses to types of stimulation that were not used to fit it. The optimised model also provides information about electrical receptive fields, including the relative effects of each stimulating electrode on every recording site. These ERFs show that the topographic mapping of cortical responses to single electrode stimulation (Elfar et al., 2009; Shivdasani et al., 2012; Wong et al., 2016) is maintained for simultaneous stimulation.

3.5.1 Comparison with Previous Studies

Studies have shown that simultaneous multi-electrode stimulation can extend the range of cortical responses possible from retinal implants beyond what is available with interleaved single electrode stimulation (Cicione et al., 2012; Matteucci et al., 2013; Dumm et al., 2014). These studies have characterised the responses of neurons to particular forms of simultaneous stimulation; however, these observations cannot be generalised to patterns for which the system has not been exposed. Response models recovered by white noise electrical stimulation go some way towards solving this issue, since white noise covers a wide range of possible inputs (Chichilnisky, 2001), and, as demonstrated by our results, can be generalised to non-white stimuli.

In terms of visual prostheses, investigations with *in vitro* preparations (Jepson et al., 2014b; Maturana et al., 2016) have shown that a piecewise linear model can predict the activation of a target cell in the retina; however, the method was limited to stimulation of small numbers of electrodes at fixed stimulation amplitudes. Maturana et al. (2016) successfully showed that a LN model, mathematically similar to our model, captured the responses of RGCs to simultaneous white noise stimulation of 20 electrodes. While the success of these models aligns with that of our LN model, several important distinctions should be made. In the present study, we showed that the LN model can be applied to responses recorded in the visual cortex that more closely approximate perception elicited by electrical stimulation of the human retina than activity recorded *in vitro* (von der Heydt and Peterhans, 1989; Gilbert and Wiesel, 1990; Salzman et al., 1990; Knierim et al., 1992; van Wezel et al., 1997). Additionally, we have demonstrated successful prediction of activity at multiple concurrent sites (up to 120 recording channels) in response to stimulation, as opposed to one or two cells at a time (Maturana et al., 2016 and Jepson et al., 2014b, respectively). Also, neither study investigated whether the suggested model performed equally well for stimuli that are non-white, as was shown for our model. Finally, we made use of the same clinical grade electrode arrays for stimulation as have been trailed in patients (Ayton et al., 2014; Shivdasani et al., 2014).

3.5.2 Comparison of MUA-based and EP power-based models

Two activity measures in the visual cortex were used to fit separate LN models: MUA and EP power. These activity measures correspond to two different types of cortical processing: MUA reflects the spiking activity of multiple neurons within a radius of 150-300 μm from the electrode tip (Gray et al., 1995; Henze et al., 2000); the EP contains both supra-threshold and sub-threshold components, the latter of which extends out over a larger area (Fregnac et al., 1996). The electrically evoked potentials seen in our study were found to be in a relatively high frequency band compared to traditional local field potentials, and they exhibited a characteristic, positive-going, multi-peaked shape. A number of studies have investigated the origins of these evoked potentials and the factor that influence them (Chang, 1950; Malis and Kruger, 1956; Schoolman and Evarts, 1959; Doty and Grimm, 1962; Burke et al., 1985; Mitzdorf, 1985).

Burke et al. (1985) suggested that the first two peaks of this multi-peaked response are due to conduction from Y and X type retinal ganglion cells, respectively (Enroth-Cugell and Robson, 1966; Stone, 1983). Mitzdorf (1985) found that the polarity of the peaks was dependent on the cortical depth of their recording electrode. Thus, the constant polarity of the peaks in our study may be attributed to the fact that all recording electrodes on the penetrating array were the same length (1 mm) and were thus likely to be recording from the same cortical layer. More recently, the EP has been shown to be a viable alternative to MUA when comparing percepts from visual prostheses to those from light stimulation, since a similar EP is present in photic stimulation, albeit with delayed peak latencies due to retinal processing (Nakauchi et al., 2005; Sun et al., 2011). Our findings expand on this, to show that both measures of activity can be the basis of a model for characterising cortical responses to simultaneous stimulation of the retina. Both model types were able to predict the vast majority of white noise stimuli that were presented in all experiments. In addition, both models displayed high correlation coefficients between recorded and predicted cortical responses to retinal stimuli that were not white, and were able to predict the majority of responses to these stimuli. No consistent differences were found between different receptive field sizes. However, given that we have recorded multi-unit activity as opposed to activity from single neurons, it is difficult to make any conclusions regarding the significance of this finding. Some differences were present between the models however; the MUA model performed significantly better than the EP power model at predicting responses to white noise, mixed-phase first stimuli and anodic-phase first stimuli. This may be due to a greater signal to noise ratio for the MUA responses than EP power, which suggests that, for a model based on EP power, the number of repetitions for each stimulus should be

increased.

Neither model performed well in predicting responses to single electrode stimulation. This may be attributed to the lower thresholds observed when stimulating across several electrodes simultaneously (Shivdasani et al., 2012), such as during white-noise stimulation. This is supported by our results since the model predicted larger responses to single electrode stimulation than were observed. However, both models could predict responses to supra-threshold single electrode stimulation, suggesting that the retinotopic organisation is preserved. From a clinical stand-point it is therefore possible that, even if the highest safe amplitude of single electrode stimulation is unable to elicit a percept, simultaneous stimulation of nearby electrodes could elicit a response in the required area.

In the present study, recording channels were only considered for analyses if they exhibited a monotonic increase in response with increasing stimulation current on any single retinal electrode. As a result, a significantly greater number of sites were excluded from the MUA analyses than from EP power analyses. This likely reflects the requirement that, for recording MUA, the recording site should be in close proximity to spiking cells, whereas estimating EP power does not require direct proximity to cells to register an increase. Thus, while the MUA model predicted cortical responses more accurately than the EP power model, the latter had the advantage of characterising a greater number of recording channels. This suggests that the activity measure on which to train the model could be altered dependent on the requirements of the application, and time available to record responses.

Clinically, the EP power would be a more attractive cortical measure as currently it is possible to record these EPs with less invasive methods than those required to record MUA; i.e., penetrating electrodes for MUA vs. scalp electrodes. Additionally, EPs have been shown to be more robust for the long term, retaining information even when spikes are lost on the same electrode (Flint et al., 2012). Thus, the EP power is an attractive measure for use with human patients, since it would eliminate the need for a further, invasive procedure, and remain informative in the long term.

3.5.3 Considerations for Future Applications

The model described in this study is purely spatial in nature owing to the sparse timing used between stimulus pulses. A similar model has been employed previously to model the spatio-temporal dynamics of neurons responding to visual stimulation of the retina (DeAngelis et al., 1993). We expect that by modifying our model to include a temporal

dimension in our linear filters, as by Chichilnisky (2001) and Kameneva et al. (2015), the model could be extended to predict temporal dynamics of cortical responses during repetitive, high-rate stimulation, such as perceptual fading. While there is strong evidence suggesting that perceptual fading could be attributed to desensitisation observed in retinal ganglion cells (Freeman and Fried, 2011), it could also be attributed to neural mechanisms within central visual structures. Since, in human trials, stimulation rates for suprachoroidal prostheses have ranged between 50 and 500 pulses per second (pps) (Ayton et al., 2014; Shivdasani et al., 2014) and in other neural systems stimulation rates may be even higher (e.g., stimulation at up to 2000 pps has been studied in cochlear implants (Galvin III and Fu, 2005)), further investigation of the characteristics of adaptation would be of particular interest. However, in pursuit of this goal, one would have to overcome the hurdle of the stimulation artefact present in the recordings. Our stimulus pulses were 1 ms per phase and biphasic, evoking cortical responses lasting approximately 20 ms. The use of stimulus rates higher than 50 pps would not allow analysis of activity with each pulse in the stimulus train without contamination by the stimulus artifact of subsequent stimulus pulses. However, evidence suggests that desensitisation of retinal ganglion cells and perceptual fading can also occur at rates lower than 50 pps (Freeman et al., 2011; Fornos et al., 2012). At such rates, it would be possible to remove artefacts with the same technique that we have used (Heffer and Fallon, 2008) and analyse the responses between pulses. The model we propose could then be readily extended to incorporate spatiotemporal ERFs (Chichilnisky, 2001).

Our model also provides an intriguing option for investigation of the effect of photoreceptor degeneration on responses to electrical retinal stimulation. Photoreceptor degeneration in a rat model has been shown to be accompanied by an increased activation threshold to electrical stimulation (Suzuki et al., 2004; O’Hearn et al., 2006) as well as an increase in baseline spiking (Pu et al., 2006; Stasheff, 2008). This may be a contributing factor to the inconsistencies found in the clinical results of simultaneous stimulation. Photoreceptor degeneration also leads to remodeling of the retina (Jones et al., 2003; Marc et al., 2003) and cortex (Gilbert and Wiesel, 1992; Kaas et al., 1990). Thus, it is likely that the electrical receptive fields of cortical neurons would be different in appearance to those described here.

Another factor that should be considered in future applications of this method is the effect of anesthesia on cortical activity. Given that anesthesia has been found to reduce the signal-to-noise ratio of visual responses in the cat visual cortex (Livingstone and Hubel, 1981) and preserve the main tuning characteristics of V1 neurons in monkeys (Lamme

et al., 1998) and mice (Niell and Stryker, 2010), we expect that the responses to electrical stimulation in an awake cat would be stronger but have similar tuning properties and, therefore, be well suited for application of this method.

The model we have presented provides novel insights into the effects of interactions between electrodes during simultaneous electrical retinal stimulation. It is expected that with some adjustments, this model could provide a potential approach for choosing optimal stimulation patterns for eliciting specific neural activity (for example, activity more akin to that elicited by visual stimulation). This would entail first fitting the model to responses of the system in question to spatial white noise stimulation. Then, a second optimisation algorithm would be used to compute stimulation patterns that would be likely to produce a desired cortical response. In this way, the model could be used to ultimately increase the resolution of the implant.

3.6 Conclusion

In this study, we have shown that cortical responses to simultaneous stimulation of the retina are consistent and repeatable. We investigated the effects of electrode interactions of retinal stimulation by creating a linear-nonlinear model of visual cortex responses. We showed that the model could accurately predict spiking neural responses to both white-noise stimulation and patterned stimulation. The model could also be based on an alternative measure of cortical activity, namely power in the evoked potentials, suggesting that, for clinical applications, a less invasive measure could be applicable. The model we describe provides an effective means of understanding the spatial interactions of retinal stimulation at the level of the cortex, showing that they are predominantly linear and the electrical receptive fields of cortical recording channels are topographically organised. The predictive success of our model shows promise for efficiently determining optimal stimulation paradigms for shaping neural activity with a neural prosthesis.

Chapter 4

Neural responses to multi-electrode stimulation of healthy and degenerate retina

4.1 Abstract

Simultaneous stimulation of multiple retinal electrodes in normally sighted animals shows promise in improving the resolution of retinal prostheses. However, the effects of simultaneous stimulation on degenerate retinæ remain unknown. Therefore, we investigated the characteristics of cortical responses to multi-electrode stimulation of the degenerate retina. Four adult cats were bilaterally implanted with retinal electrode arrays in the suprachoroidal space after unilateral adenosine triphosphate (ATP) induced retinal photoreceptor degeneration. The extent of global photoreceptor degeneration was determined from the electroretinogram a-wave amplitude at 7 to 19 weeks post-ATP injection. Multi-unit activity was recorded from both hemispheres of the visual cortex. Responses to single- and multi-electrode stimulation of the ATP-injected and fellow control eyes were characterised and compared. ATP-injected eyes displayed changes consistent with mid- to late-stage photoreceptor degeneration and remodeling. Responses to multi-electrode stimulation of the ATP-injected eyes exhibited shortened latencies, lower saturated spike counts, and higher thresholds compared to stimulation of the fellow control eyes. Electrical receptive field sizes were significantly larger in the ATP-injected eye than in the control eye, and positively correlated with the extent of degeneration. These results show that significant differences exist between cortical responses to stimulation of healthy and

degenerate retinæ. Furthermore, our results highlight the importance of using a retinal degeneration model when evaluating the efficacy of novel stimulation paradigms.

4.2 Introduction

Retinitis Pigmentosa is a leading degenerative disease of the visual system that causes photoreceptor loss and eventual blindness (Sharma and Ehinger, 1999; Friedman et al., 2004). Experimental approaches to treat these conditions include retinal prostheses (Ayton et al., 2014; Humayun et al., 1996; Zrenner et al., 2011), cortical prostheses (Lewis et al., 2015), optogenetic therapies (Barrett et al., 2014), and stem-cell treatments (Uy et al., 2013). This work focuses on retinal prostheses, which provide a sense of vision by electrically stimulating surviving neurons of the retina. Although clinical trials have shown that retinal prostheses can elicit useful percepts (Ayton et al., 2014; Velikay-Parel et al., 2009; Luo and da Cruz, 2014; Stingl et al., 2015; Rachitskaya and Yuan, 2016), spatial resolution is a limiting factor, particularly for implants located far from the target cells such as with the suprachoroidal approach (Ayton et al., 2014; Fujikado et al., 2011).

Animal studies suggest that spatial resolution and numbers of distinct percepts can be improved with appropriate stimulation strategies (Cicione et al., 2012; Jepson et al., 2014b; Matteucci et al., 2013; Dumm et al., 2014; Spencer et al., 2016). Such strategies apply simultaneous stimulation across multiple electrodes, with interactions between electrodes altering the electrical field at the retina to reduce the spread of activation (Jepson et al., 2014b; Spencer et al., 2016) (“current focusing”) or activate groups of cells between electrodes (Dumm et al., 2014) (“current steering”). However, stimulation strategies demonstrated in healthy retinæ cannot necessarily be extrapolated to degenerate retinæ (O’Hearn et al., 2006; Suzuki et al., 2004; Ye and Goo, 2007; Chan et al., 2008; Cho et al., 2016). Consequently, the large body of knowledge derived from animal studies is difficult to translate into clinical practice. This highlights a need to comprehensively evaluate the responses of degenerate retinæ to electrical stimulation (Aplin et al., 2016a).

Studies in blind human patients have investigated concurrent stimulation of pairs (Humayun et al., 1996; Rizzo et al., 2003a; Shivdasani et al., 2014), rows (Benav et al., 2010; Humayun et al., 1999; Zrenner et al., 2011; Caspi et al., 2009), or blocks of several electrodes (Horsager et al., 2010; Rizzo et al., 2003b; Fornos et al., 2012). The evoked percepts from these types of stimulation strategies have been reported to be variable across trials and patients, suffering from low predictability and dissimilarities between studies (Freeman et al., 2011). It is unknown whether stimulation strategies that successfully focus

or steer current in healthy retinæ can provide meaningful improvements when applied to degenerate retinæ.

In this study, we investigate cortical responses to simultaneous stimulation of multiple retinal electrodes in a feline model with unilateral adenosine triphosphate (ATP) induced photoreceptor degeneration. This work builds upon a previous study which showed that glial changes in the ATP-injected retina significantly influenced the efficacy of single electrode stimulation (Aplin et al., 2016a). The cat visual system has long been studied as an analogue for human vision (Hubel and Wiesel, 1998), making the photoreceptor degeneration model ideal for the development of retinal prostheses (Aplin et al., 2016a, 2014, 2016b). The similarity in size of the cat eye to the human eye also enables clinically-relevant electrode size and positioning, with a similar level of photoreceptor degeneration and remodeling to that seen in humans with Retinitis Pigmentosa (Aplin et al., 2016b). Blindness was unilaterally induced by intravitreal injection of ATP in a single eye, allowing comparison of healthy and degenerate retinæ in the same animal (Aplin et al., 2016a). As such, the ATP-injected cat model is ideal for investigating cortical responses to simultaneous stimulation of multiple retinal electrodes, which previous *in vivo* studies have not explored. As a first step towards understanding the reasons behind the variability seen in patients and to determine how current focusing and steering can be applied to degenerate retinæ effectively, the aim of this study was to examine how loss of photoreceptors influences the cortical responses to multi-electrode stimulation.

4.3 Methods

This study used bilateral implantation of retinal electrode arrays in the suprachoroidal space of adult cats with unilateral ATP-induced retinal degeneration. Neural responses were recorded from both hemispheres of the visual cortex. Responses to single- and multi-electrode stimulation of the ATP-injected and fellow control eyes were characterised and compared. Treatment of animals complied with the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research, and the National Health and Medical Research Council’s ‘Australian Code of Practice for the Care and Use of Animals for Scientific Purposes’ (2013) and the ‘Prevention of Cruelty to Animals Act’ (1986 and amendments). The study was approved by the Bionics Institute Animal Research and Ethics Committee (Project #14/304AB).

4.3.1 Intraocular Injection of ATP and Clinical Assessments

Five adult cats were used in this procedure. Anesthesia for both ATP injections and clinical assessments was induced via a subcutaneous injection containing ketamine (20 mg/kg, Ilium Ketamil, Troy Laboratories, Australia) and xylazine (2 mg/kg, Ilium Xylazil-20, Troy Laboratories). Pupils were dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride; Hartmann's solution (2.5 ml/kg/h) was used to rehydrate for recovery. The procedure for inducing unilateral retinal degeneration has been previously described in detail. One eye was injected with a 200 μ L solution of 3 parts sterile saline (0.9% NaCl) and 1 part Dexamethasone (4mg/ml), containing approximately 0.2M (11mM vitreous concentration) adenosine tri-phosphate disodium salt (ATP, Sigma-Aldrich) to induce photoreceptor degeneration. The fellow eye was a non-injected control. Multiple ATP injections were required to induce a sufficient level of degeneration at the area centralis. Degeneration was monitored over the course of the induced retinal degeneration at approximately 2 weeks after each injection and at 7 to 19 weeks after the final ATP injection.

Clinical assessments of retinal structure and function were performed as described elsewhere (Aplin et al., 2014). Under anesthesia, a full-field flash electroretinogram (ERG; Espion, Diagnosys LLC, USA) was used to record the retinal response to stimulus intensities from 0.001 to 10 cd.s/m² after 20 minutes of dark-adaptation, however only the combined rod-cone maximal ERG response (10 cd.s/m²) is reported. The a-wave amplitude was used to measure photoreceptor function. Spectral domain optical coherence tomography (OCT; Spectralis HRA+OCT, Heidelberg Engineering GmbH, Germany) and color fundus photography (TRC-50Dx, Topcon Medical Systems, USA) were used to assess retinal structure. Line scans were used to confirm outer nuclear layer (ONL) dropout at the area centralis and to ensure the retinal array was placed at an area of degeneration, since the ATP degeneration model does not produce uniform photoreceptor loss. Sufficient photoreceptor degeneration was classified as at least 50% reduction in ERG a-wave amplitude along with accompanying ONL dropout at the area centralis assessed by OCT. We waited a minimum of 12 weeks between the final ATP injection and the acute electrophysiology experiments in order to maximise photoreceptor degeneration (Aplin et al., 2014). One animal developed a large retinal detachment 2 weeks after the first ATP injection and was not used further in this study. Hereafter, animals are referred to by numbers 1 through 4 based on the level of photoreceptor degeneration apparent in the ATP-injected eye, with 1 indicating the least amount of degeneration and 4 the most.

4.3.2 Acute Electrophysiology Experiment Setup

Cats were pre-medicated with a dose of Medetomidine (0.012 mg/kg, intramuscular (i.m.)), ketamine (8 mg/kg, i.m.), and methadone (0.4 mg/kg, i.m.). Anesthesia was induced and maintained with an intravenous infusion of Propofol (24 mg/kg). A solution of Hartmann's and Methadone (0.25 mL of 10 mg/ml Methadone in 250 mL of compound sodium lactate, 0.05 mg/kg/hour) was also delivered intravenously. A tracheotomy was performed and the animal was ventilated with 100% oxygen (20-25 breaths/minute) (Model 6025, Ugo Basile, Italy). Dexamethasone (0.3 mg/kg, i.m.) and Clavulox (10 mg/kg, s.c.) injections were given daily. Core body temperature was maintained at 37°C and physiological indicators, including expired CO₂, O₂ saturation, heart rate, and blood pressure, were continuously monitored throughout the experiment. The experiments were typically conducted over three to four days, after which the animal was terminated, by using an overdose of intravenous sodium pentobarbitone (60 mg/kg; Troy Laboratories).

4.3.3 Stimulating Electrode Array

Once anaesthetised, animals were bilaterally implanted with suprachoroidal electrode arrays using a previously described surgical procedure (Shivdasani et al., 2012). The arrays consisted of flexible medical grade silicone substrates with 7 rows x 6 columns of platinum electrodes (600 µm diameter, 1 mm pitch) and a helical platinum-iridium cable (Villalobos et al., 2012). A lateral canthotomy was performed and the choroid was exposed via a scleral incision. A pocket was created between the sclera and choroid into which the electrode array was inserted and sutured in place (Villalobos et al., 2012).

4.3.4 Cortical Electrophysiology

Following suprachoroidal array implantation, both hemispheres of the visual cortex were exposed with a bilateral craniotomy and penetrating microelectrode arrays (6 × 10 channels, 1 mm length, 400 µm spacing, Blackrock Microsystems, USA) were implanted. Optimal microelectrode array positions were determined by first recording evoked potentials (EPs) from the cortical surface in response to retinal stimulation (Cicione et al., 2012; Dumm et al., 2014).

Neural signals were sampled at 30 kHz (Cerebus Neural Processing System, Blackrock Microsystems) with offline stimulus artefact removal (Cicione et al., 2012; Heffer and Fallon, 2008; Shivdasani et al., 2012) and band-pass filtering (third-order Butterworth fil-

ter, 300-5000 Hz). Multi-unit spiking activity (MUA) was detected by threshold crossings (at four times the root mean square of a 60 s moving time window).

For each biphasic pulse the period from 3-20ms post-stimulus was analysed, which is considered to include spikes as a result of both direct stimulation of retinal ganglion cells (RGCs) and indirect activation of the inner retina (Boinagrov et al., 2014). The spontaneous spiking rate on each channel was determined from the number of spikes in the 500 ms prior to each stimulus, which was subtracted to account for variation in the stimulus-independent activity.

4.3.5 Visual Stimulation

Cortical responses to flashes (1.5 cd.s/m^2 with a duration of $10 \mu\text{s}$ at 1 Hz) were delivered using a FLYSYS FlashLamp System (Tucker-Davis Technologies, USA) with the lamp guide placed approximately 2.5 cm in front of each eye individually (with a patch covering the fellow eye).

4.3.6 Single Electrode Electrical Stimuli

All electrical stimuli consisted of charge-balanced biphasic current pulses with 1 ms phase width and $25 \mu\text{s}$ interphase gap at a 1 Hz presentation rate. Electrical stimulation was provided by a multi-channel stimulator (Tucker Davis Technologies: RZ2 base station and IZ2 multichannel stimulator) commanded by a custom-made MATLAB (MathWorks version 2014b) interface. Electrodes were first individually stimulated with cathodic-first pulses, 0-750 μA stimulus amplitude, in 50 μA steps, with 10 repetitions of each amplitude. For each stimulating electrode/recording channel combination, a sigmoid curve was fitted to the mean spike-rate (3-20 ms window) as a function of stimulation amplitude. A P50 current of the sigmoid was calculated as the stimulus amplitude at which the spike rate reached 50% of the maximum (Cicione et al., 2012). For each stimulating electrode, the lowest P50 current across all recording channels was used in the generation of the multi-electrode simultaneous stimuli.

4.3.7 Multi-electrode Simultaneous Electrical Stimulation

Multi-electrode stimulation consisted of temporally sparse (1 Hz), spatially white electrical pulse patterns delivered simultaneously across the stimulating electrodes in each eye separately. This form of stimulation is suitable for estimating several key parameters

of the cortical response via fitting to a linear-nonlinear model as described previously (Halupka et al., 2016) and summarised below. The stimulus amplitude for each electrode was sampled from a zero-mean Gaussian distribution, with the standard deviation equal to the P50 current for that electrode. For stimulating electrodes where a sigmoid could not be reliably fitted to responses to single electrode stimulation, and therefore a P50 current could not be determined, the standard deviation was scaled by the average P50 current on all other electrodes. The zero mean of the Gaussian distribution meant that a mix of cathodic-first and anodic-first waveforms were presented in each pattern (negative amplitudes as cathodic-first). A total of 3150 multi-electrode stimulation patterns were generated separately for each eye in all experiments. Since the distribution of stimulus amplitudes for each electrode was dependent the threshold of that electrode, the 3150 patterns presented to the healthy retina were different to those presented to the degenerate retina. Each pattern was presented eight times and the responses averaged.

4.3.8 Linear-Nonlinear Model

To characterise differences in cortical responses to multi-electrode stimulation in both the healthy and degenerate retinæ, a linear-nonlinear model was fitted to the Gaussian white noise stimulation responses (Maturana et al., 2016; Halupka et al., 2016; Sekhar et al., 2016). This model provides information about the response of each cortical recording site to stimulation of multiple electrodes, including which electrodes significantly affect each channel, and the nonlinear characteristics, such as response thresholds and saturation levels. The model comprises two spatial linear filters ($\mathbf{V}_P$ and $\mathbf{V}_N$), followed by two parallel static nonlinearities (g_P and g_N) for each recording channel. An estimate of the spike count (R_{est}) in response to a stimulus vector at time t ($\mathbf{s}_t$) is given by

$$R_{\text{est}} = g_P \left(\mathbf{s}_t^{\text{tr}} \mathbf{V}_P \right) + g_N \left(\mathbf{s}_t^{\text{tr}} \mathbf{V}_N \right), \quad (4.1)$$

where tr is the matrix transpose. Two filters were required to account for net anodic-first ($\mathbf{V}_P$ and g_P) or net cathodic-first ($\mathbf{V}_N$ and g_N) stimulation (where net anodic-first means that the sum of the biphasic pulses across all electrodes, weighted by $\mathbf{V}_P$, is positive). The spatial linear filters, recovered using spike-triggered covariance analysis and normalised by dividing by their L-2 norm, serve to project the high-dimensional stimulus (M=42 electrodes) into a single-dimensional subspace, termed the ‘generator signal’ subspace. The spatial linear filters can be interpreted as electrical receptive fields (ERFs) of a population of neurons at each cortical recording channel. To determine the significance of each elec-

trode's contribution to the ERF, a control distribution was generated for both $\mathbf{V}_P$ and $\mathbf{V}_N$ by repeating the fitting procedure 1000 times on randomly time-shifted responses. Electrodes with linear filter values larger than the root mean square of the randomly generated distribution were considered significant. The size of each ERF is then given by

$$\begin{aligned} D_P &= \frac{\sum_{j=1}^M v_j^P d_j}{\sum_{j=1}^M v_j^P} \\ D_N &= \frac{\sum_{j=1}^M v_j^N d_j}{\sum_{j=1}^M v_j^N}, \end{aligned} \quad (4.2)$$

where d_j is the distance of each electrode j to the center of mass of the significant electrodes and v_j^P and v_j^N are the weights given by $\mathbf{V}_P$ and $\mathbf{V}_N$, respectively. ERF sizes were converted to degrees of visual angle using a 4.4 degrees/mm conversion factor for the cat eye (Vakkur et al., 1963).

The static nonlinearities, which account for nonlinear response characteristics such as thresholds and saturation, were recovered by projecting the stimuli onto the linear filters to recover generator signal values $x_P = \mathbf{s}_t^{\text{tr}} \mathbf{V}_P$ and $x_N = \mathbf{s}_t^{\text{tr}} \mathbf{V}_N$, then fitting a sigmoidal function to the binned generator signal values and their respective responses (spike count averaged over repeated trials). The static nonlinearities g_P and g_N are fitted using the logistic equations

$$\begin{aligned} g_P(x_P) &= \frac{y_P}{1 + \exp(-b_P(x_P - a_P))} \\ g_N(x_N) &= y_N - \frac{y_N}{1 + \exp(-b_N(x_N - a_N))}, \end{aligned} \quad (4.3)$$

where y_P and y_N are the saturation spiking levels in response to net anodic-first and net cathodic-first stimulation, respectively, a_P and a_N are the P50 levels, and b_P and b_N represent the slopes at P50 of each sigmoid. The summation of the outputs of these sigmoid functions provided a predicted response (R_{est}) for each cortical channel for any stimulus vector, as defined in Eq. 4.1.

To determine the accuracy of the model in characterising cortical responses, we compared the responses predicted by the fitted model to the actual recorded responses for all 3150 multi-electrode patterns. Only channels that exhibited a coefficient of determination (r^2) of at least 0.5 were considered in further analyses.

4.4 Results

4.4.1 Arrays Were Positioned Over ATP Induced Photoreceptor Degenerated Regions

ATP injections caused photoreceptor degeneration unilaterally in four subjects (Figure 4.1), consistent with previous publications (Aplin et al., 2014, 2016a). The color fundus photographs (Figure 4.1A) from the ATP-injected eyes show widespread mottled pigmentation indicating broad but variable degeneration both across an individual retina and between subjects. The degeneration was more extensive in subjects 3 and 4. Additionally, there was a marked attenuation of inner retinal vessels in subject 4, likely occurring in response to inner retinal remodeling following extensive photoreceptor degeneration (Aplin et al., 2016b). The implant position relative to the area centralis (Figure 4.1B) was asymmetric between eyes in subjects 2 and 3, with the array in right eye underlying the area centralis and that in the left eye being >4 disc diameters ($>6\text{mm}$) from the area centralis. The other two subjects (1 and 4) had implants positioned near the area centralis in both eyes, although the right eye implants were relatively nasal to the left eye implants. There was ONL degeneration overlying electrodes (Figure 4.1C) in the three subjects where electrodes were visible on SD-OCT scans, although regional variability of degeneration is evident (indicated by asterisks). The array was positioned too far temporally in subject 2 to obtain images of retina overlying the array, although since the degeneration appears widespread (Figure 4.1A), there is likely to have been degeneration overlying the array in this subject as well. The inner retinal layers were generally well-conserved, although there were signs of mild inner retinal thinning in the two subjects with the greatest ONL degeneration (3 and 4), likely due to retinal remodeling (Aplin et al., 2016b). The reduction in the a-wave of combined rod-cone ERG responses (10 cd.s/m^2) in all ATP-injected eyes relative to the healthy fellow eyes was 50-85% (Figure 4.1D), indicating a significant global reduction in photoreceptor function.

4.4.2 Cortical Responses to Electrical Stimulation of the Degenerate Retina Had Shorter Latencies

Following confirmation of sufficient unilateral photoreceptor degeneration, suprachoroidal stimulating arrays were bilaterally implanted and cortical responses to single- and multi-electrode stimulation of healthy and degenerate retinæ were recorded on a total of 476 recording channels (over all experiments a total of 480 channels were implanted, but 4

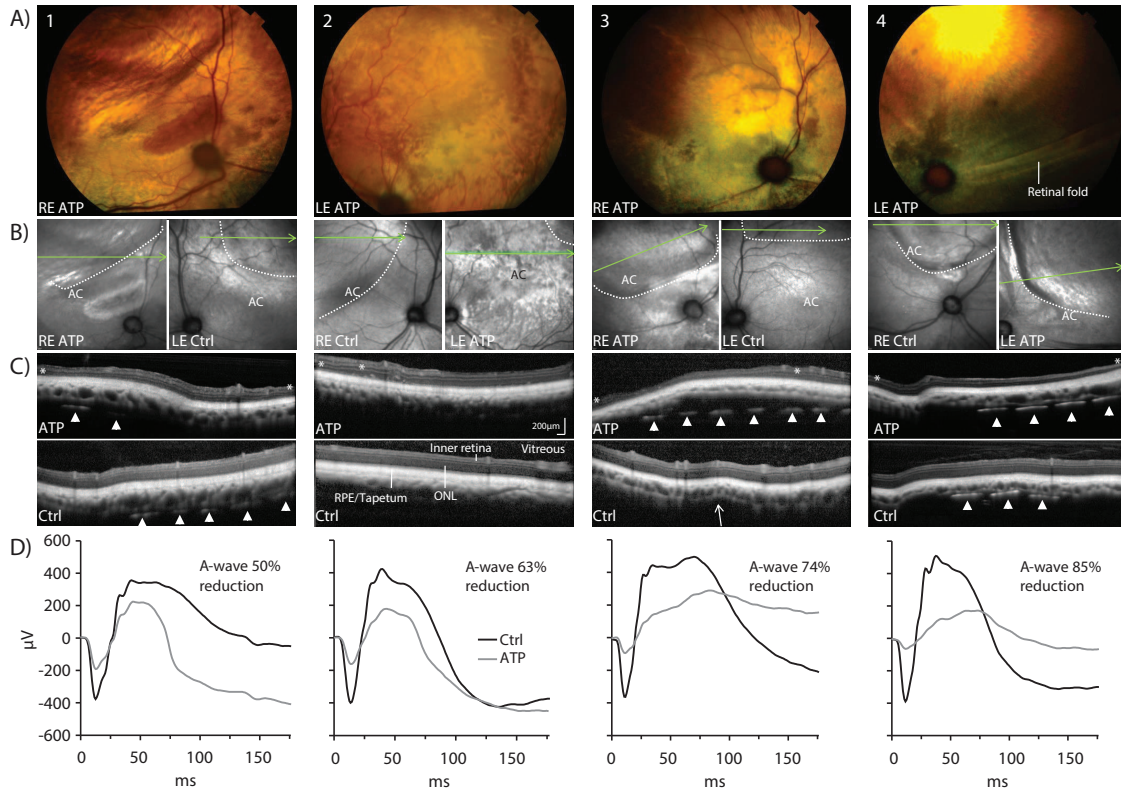


Figure 4.1: A) Color fundus photographs in the ATP-injected eye for each subject showing mottled pigmentation consistent with widespread but variable photoreceptor degeneration across the retina (LE: left eye, RE: right eye, ATP: ATP-injected eye, Ctrl: fellow control eye). Subject numbers (1 to 4) are shown on each panel. B) Infrared images from both ATP-injected and healthy fellow control eyes for each subject indicating implant position (dotted lines) relative to the area centralis (AC). The green arrow indicates the position of the SD-OCT scan. C) SD-OCT images in both eyes for each subject demonstrated thinning of the outer nuclear layer (ONL) in the ATP-injected eyes at locations between the asterisks. There was variability in ONL drop-out across the retina within an eye and also between subjects. Where visible, the locations of electrodes are indicated by arrowheads. There was ONL degeneration overlying electrodes in the three subjects where electrodes are visible. The array was too far temporal in subject 2 to obtain images of retina overlying the array. The silicone edge of the implant is indicated (white arrow) in one eye (subject 3). D) Combined rod-cone ERG responses (10 cd.s/m²) in each subject showed a global reduction in the a-wave amplitude (photoreceptor function) in all ATP-injected eyes by 50-85% relative to the healthy fellow eyes.

were inoperative).

Deterioration of the cortical response to visual stimulation of the blind eye was confirmed with light flashes to both eyes of each animal. Figure 4.2A shows a peri-stimulus time histogram (PSTH) of the MUA response to visual stimulation of the normal and blind eyes in subject 4, smoothed with cubic interpolation. Clear early (photoreceptor mediated) and late (possibly due to cortical feedback) response peaks could be distinguished in the flash response from the normal eye ($p < 0.001$ both peaks prior to smoothing, Rank sum test vs. baseline with Bonferroni correction, 0-50 ms and 50-200 ms windows), while flashing the blind eye yielded no response above baseline for either peak ($p = 0.04$ and $p = 0.22$ for the early and late peaks respectively, Rank sum with Bonferroni correction). All other animals also exhibited significant early and late response peaks from flashing the control eye ($p < 0.001$ for all comparisons). However, the strengths of response of the ATP-injected eyes varied in significance, with significant early and late responses ($p < 0.001$ for all comparisons) recorded in the two animals that exhibited less pronounced degeneration (1 and 2). Neither response peak was significant in subject 3 with 74% retinal degeneration ($p = 0.51$ and $p = 0.06$ for the early and late peaks, respectively).

Electrical stimulation of both degenerate and healthy retinæ elicited robust MUA (Figure 4.2B). PSTHs of responses to single-electrode stimulation (Figure 4.2C) and multi-electrode stimulation (Figure 4.2D), collated over all stimulus levels and cortical channels, showed that both stimulus types were able to elicit early cortical responses within the first 20 ms regardless of retinal health ($p < 0.001$, Rank sum). For these early responses, firing rates were markedly different between healthy and degenerate retinæ and between single and multi-electrode stimulation.

Late spiking components (> 20 ms) to electrical stimulation, which are likely network mediated, were observed from stimulation of the healthy retina but were largely or completely absent when stimulating the degenerate retina (response significantly greater than baseline for single electrode stimulation in 30-100 ms window: $p < 0.001$ healthy retina, $p = 0.14$ degenerate retina; for multi-electrode stimulation: $p < 0.001$ healthy retina, $p = 0.06$ degenerate retina, Rank sum).

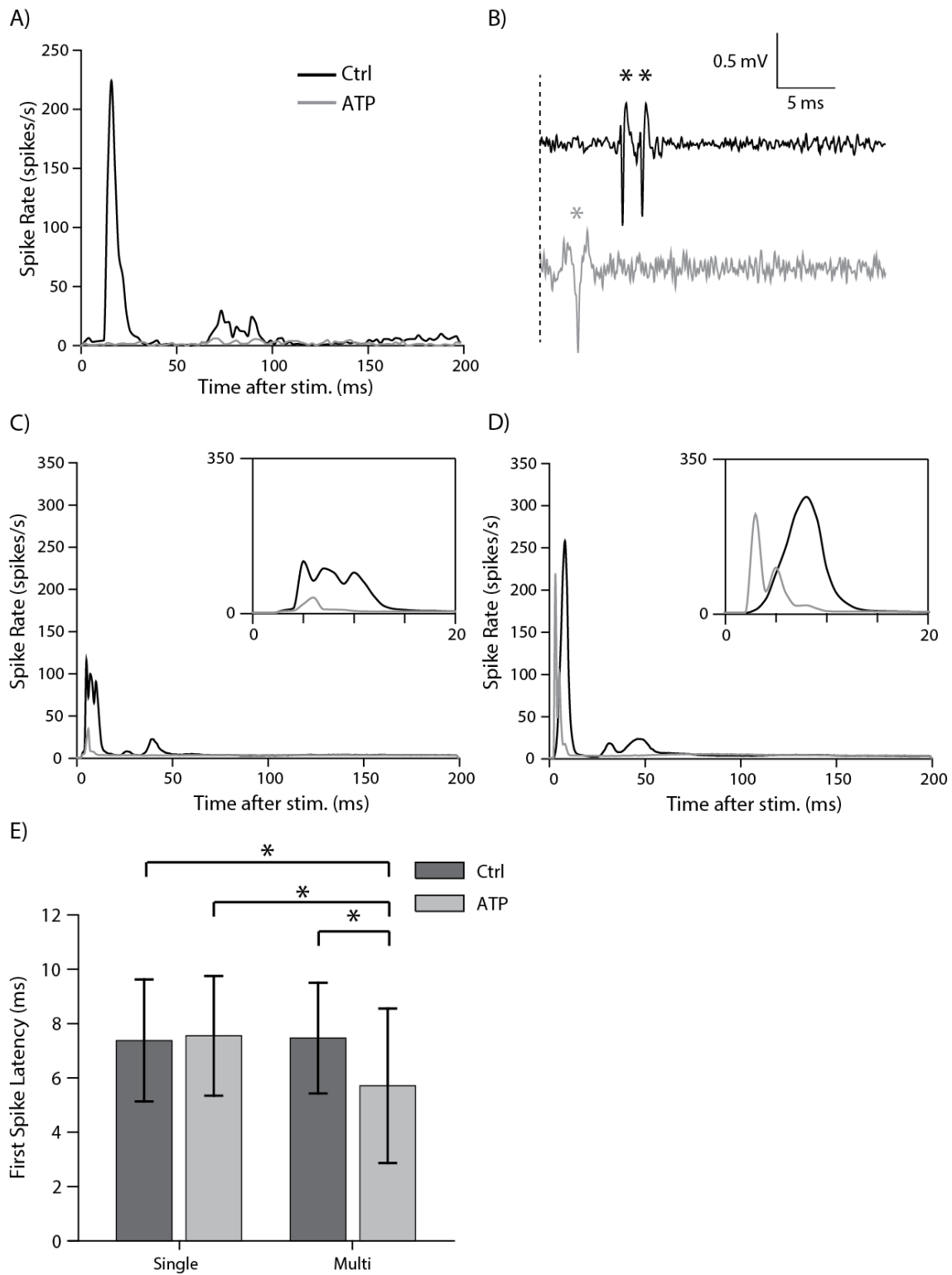


Figure 4.2: A) PSTHs in response to flashes directed at ATP-injected eye (grey line) and fellow control eye (black line), averaged over all cortical channels and 10 repetitions, for subject 4 ($n=120$ channels $\times$ 10 repetitions, bin width=2ms). Cubic interpolation was used to smooth all PSTHs. B) Filtered trace on two recording channels from multi-electrode stimulation showing spiking (indicated by asterisks). Dashed line indicates the beginning of the stimulus pulse. C, D) Smoothed PSTHs of responses to (C) single-electrode and (D) multi-electrode stimulation, averaged over all cortical sites and stimuli, in both the ATP-injected eye (grey line) and the fellow control eye (black line), in subject 4. Inset panels show a close-up view of the time period 0-20 ms post-stimulus. E) First spike latency for cortical channels that exhibited at least one spike per trial averaged over all experiments. Error bars indicate mean $\pm$ SD, markers (*) indicate $p < 0.001$.

Across the four animals, the early response was consistently significantly above baseline ($p < 0.001$ for both retinæ and stimulus types, Rank sum) and late responses were not significant for stimulation of the degenerate retina ($p = 0.17$ for single electrode stimulation, $p = 0.29$ for multi-electrode stimulation, Rank sum). For the population, the first spike latency averaged across all cortical channels that exhibited at least one spike per trial (Figure 4.2E) was found to depend significantly on the interaction between stimulus type and retinal health status. In particular, multi-electrode stimulation of the ATP-injected eye resulted in significantly shorter latencies than any other stimulus type/retina status combination ($p < 0.001$ for all comparisons, two-way ANOVA with Bonferroni correction). This result was also present and significant for each animal individually ($p < 0.001$ for all comparisons). There was no significant difference between first spike latencies to single- versus multi-electrode stimulation of the control eyes ($p = 0.12$, two-way ANOVA with Bonferroni correction; Figure 4.2E), or between single-electrode stimulation for either eye status ($p = 0.047$, two-way ANOVA with Bonferroni correction). These results were also present in all animals individually, except for subject 4 with the greatest photoreceptor degeneration (85%), which exhibited significantly shorter latencies to single-electrode stimulation of the ATP-injected eye than in the control eye ($p < 0.001$).

4.4.3 Retinal Degeneration Leads to Higher Cortical Thresholds and Lower Saturated Spike Rates

To more directly compare responses of single- and multi-electrode stimulation, we converted both single-electrode and multi-electrode stimuli into a generator signal by projecting the stimuli onto the linear filters, defined by our linear-nonlinear model fitted to multi-electrode stimulation responses (see Methods).

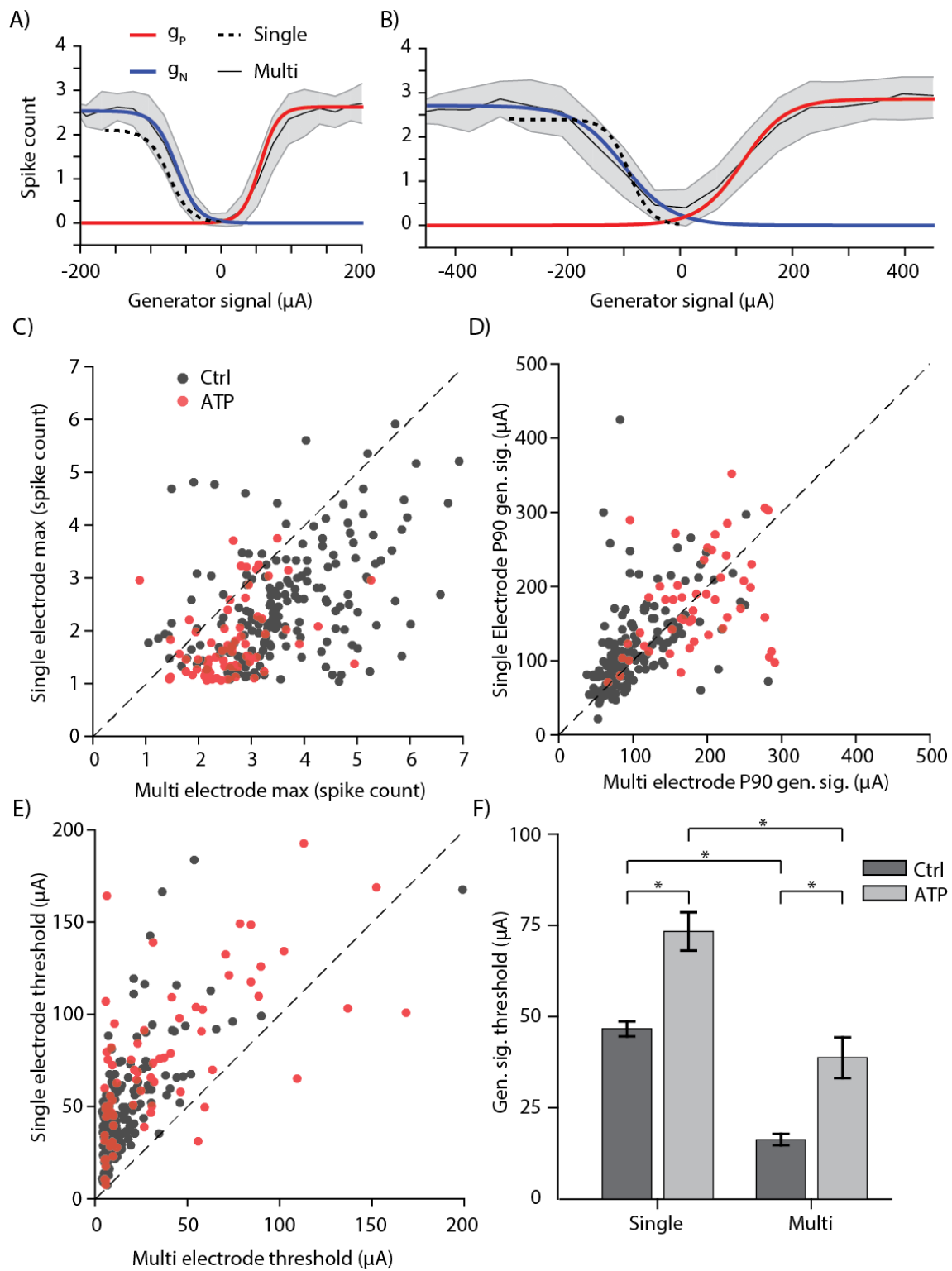


Figure 4.3: A,B) Examples of multi-electrode static nonlinearities g_N (blue line) and g_P (red line) for the control eye (A) and ATP-injected eye (B) for one cortical channel in subject 2. Solid black line shows the average recorded response to each of the binned generator signal levels (mean $\pm$ SEM, shown in grey). Dashed black line shows the sigmoid fitted to responses to cathodic-phase-first single electrode stimulation. C) Saturated spike count, (D) generator signal value at 90% of saturated spike count (P90), and (E) activation threshold (generator signal at $3 \times$ standard deviation of baseline spike rate) for multi-electrode stimulation versus single electrode stimulation in response to stimulation of the control (black markers) and ATP-injected eyes (red markers) for all animals collated (n=184 for control eye, n=72 for ATP-injected eye). F) Comparison of thresholds collated across experiments. Markers (*) indicate a 0.001 level of significance. Error bars indicate mean $\pm$ SE.

We then plotted the responses as functions of this common generator signal to reveal non-linear relationships (examples shown in Figures 4.3A and B). Only cortical channels that responded to both multi-electrode stimulation and at least one single electrode were included in this analysis (control eye: n=184, ATP-injected eye: n=72). For responses to single-electrode stimulation (dashed black lines), only the stimulating electrode eliciting the lowest P50 current for each channel was used in the comparison. Across the population, saturation spike levels (90% of sigmoid maximum), generator signal amplitudes at saturation (P90 generator current), and thresholds, defined as the generator signal value required to evoke a firing rate of at least 3 standard deviations above baseline, were compared. This definition of threshold (as opposed to a fixed point on the sigmoid) mitigated the impact of dynamic range differences observed between single and multi-electrode stimulation.

Population metrics indicated that multi-electrode stimulation elicited significantly higher (p<0.001, two-way ANOVA with Bonferroni correction) saturated spike levels than single-electrode stimulation in the control (mean $\pm$ SD, single-electrode: 2.52 ± 1.04 , multi-electrode: 3.61 ± 1.15) and ATP-injected (mean $\pm$ SD, single-electrode: 1.81 ± 0.72 , multi-electrode: 2.61 ± 0.75) eyes (Figure 4.3C). Furthermore, multi-electrode stimulation of the healthy retina resulted in higher saturation levels than that of the degenerated retina (Figure 4.3C, p<0.001, two-way ANOVA with Bonferroni correction).

This result was generally consistent on an individual basis; however, subject 1 showed no significant difference between eyes for saturation responses to multi-electrode stimulation (p=0.8). Subject 4 was the only animal for which single electrode stimulation elicited higher saturated spike rates in the control eye compared to the degenerate eye (p=0.002), with all others exhibiting no significant difference.

The amplitude of the generator signal value at which the saturated spike count was

reached (P90 generator current) was significantly lower when stimulating the control (mean $\pm$ SD, single-electrode: 112.1 ± 57.6 ; multi-electrode: 99.0 ± 45.6) vs. the ATP-injected (mean $\pm$ SD, single-electrode: 177.9 ± 66.4 ; multi-electrode: 185.3 ± 60.2) eyes ($p < 0.001$ for both single- and multi-electrode stimulation, two-way ANOVA with Bonferroni correction) on a population basis (Figure 4.3D). No significant difference was observed between single- and multi-electrode stimulation P90 generator currents for either the control ($p = 0.14$, two-way ANOVA with Bonferroni correction) or the ATP-injected eye ($p = 1.0$, two-way ANOVA with Bonferroni correction). Individual animal statistics were consistent with that of the population.

Multi-electrode stimulation also resulted in significantly lower thresholds compared to single-electrode stimulation regardless of retinal health status (control eye: $p < 0.001$; ATP-injected eye: $p = 0.0019$, two-way ANOVA with Bonferroni correction) on a population basis (Figure 4.3E,F). Furthermore, stimulation of the control eye resulted in significantly lower thresholds compared to the ATP-injected eye for both stimulus types (single-electrode: $p = 0.004$; multi-electrode: $p = 0.002$). These results were generally consistent for most animals individually; however, subject 1 showed no significant threshold difference between eyes for either single-electrode stimulation ($p = 1.0$) or multi-electrode stimulation ($p = 0.07$).

4.4.4 Retinal Degeneration Results in Increased Electrical Receptive Field Sizes

Interactions between electrodes during multi-electrode stimulation mean that the activity of a particular cortical channel can be affected by sub-threshold stimulation of distant electrodes, thereby possibly limiting resolution. This effect can be measured by the electrical receptive field (ERF) size for each cortical channel. The linear filters of the linear-nonlinear model characterised by white noise stimulation can be interpreted as ERFs, and indicate the relative strength of the effect of each stimulating electrode on a cortical site. Generally, ERFs were found to be spatially localised, with a central peak in electrode efficacy that diminished to negligible level further from the centre in both the control (Figures 4.4A,B) and ATP-injected (Figures 4.4C,D) eyes. However, ERFs were noticeably larger in the ATP-injected eyes. ERFs were only compared for channels with a correlation coefficient of 0.5 or greater for the fit of the model to the responses (control eye: $n = 163$ contralateral, $n = 87$ ipsilateral; ATP-injected eye: $n = 114$ contralateral, $n = 43$ ipsilateral). ERFs for channels meeting this criterion in subject 1 are shown in Figures 4.4E and F for the healthy retina (87 channels) and degenerate retina (36 channels), respectively. Across the

population, distinct differences in ERF size between the healthy and degenerate retinae (Figure 4.4G) were found. Specifically, the ERF size in response to stimulation of the ATP-injected eye was significantly larger than for the fellow control eye for both the contralateral and ipsilateral hemispheres (contralateral: $p < 0.001$; ipsilateral: $p < 0.001$, Rank sum), with a larger variance in size. No significant difference in ERF size was evident between different hemispheres when stimulating either retina (healthy: $p = 0.34$; degenerate: $p = 0.20$, Rank sum). In each animal individually, ERFs were also significantly larger in the ATP-injected eye than the fellow control, and a trend was evident between a-wave amplitude reduction and median ERF size across both the contralateral and ipsilateral channels (Figure 4.4H). The ERF sizes for the control eyes were not significantly different between experiments ($p > 0.0083$ for all comparisons, Rank sum test with Bonferroni correction for 6 comparisons), while for the degenerate retinae all comparisons were significant ($p < 0.0083$ for all comparisons, Rank sum test with Bonferroni correction for 6 comparisons).

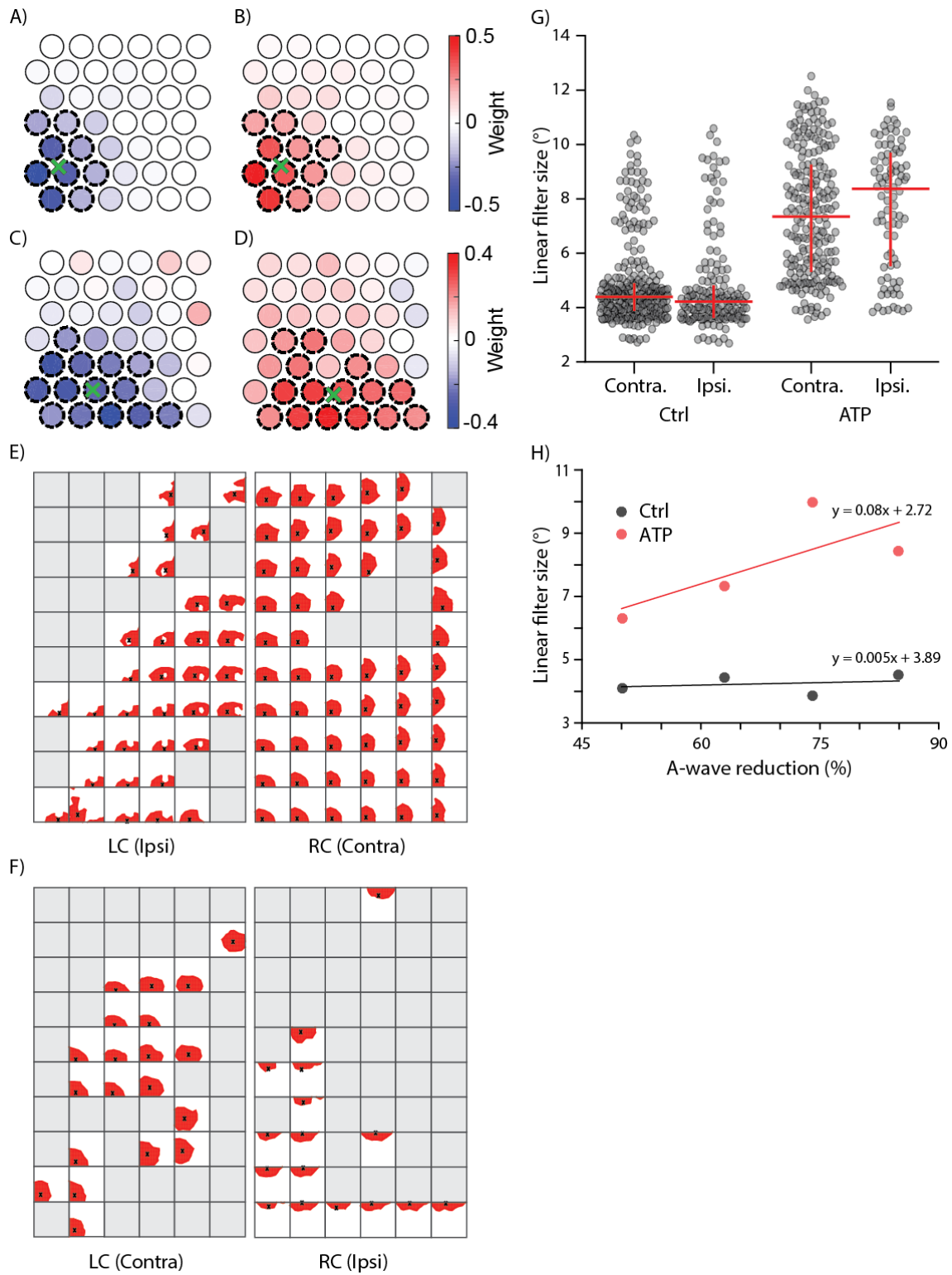


Figure 4.4: A,B) Examples of ERFs in response to net anodic-first (A) and cathodic-first (B) multi-electrode stimulation of the control eye. C,D) Similar data for stimulation of the ATP-injected eye. Dotted outlines indicate significant electrodes, green crosses indicate the ERF center. E,F) Significant ERF areas for all 120 cortical channels in subject 1, in response to stimulation of the control eye (E) and ATP-injected eye (F). Red indicates significance, and the center of each ERF is marked with a black cross. Grey sites are those for which a linear-nonlinear model was not acceptably fitted ($r^2 < 0.5$); they were therefore excluded from ERF analysis. LC=left cortex, RC=right cortex. G) ERF sizes (both VP and VN) for the control and ATP-injected eyes for well-fitted cortical sites, collated over all experiments (250 of each VP and VN for the control eye, 157 of each VP and VN for the ATP-injected eye), split into ipsilateral and contralateral locations. Red lines indicate the medians, first quartile and third quartile of the linear filter sizes for the healthy and degenerate retinae, collated over all experiments. H) Relationship between the median ERF size and percentage a-wave reduction of the ERG, for the ATP-injected (red) eyes with line of best fit. The size of ERFs in the fellow control eyes (black) is shown vertically in line with the respective degenerate eye for comparison.

4.4.5 Retinal Degeneration Influences Spatial Extent of Cortical Activation

The ERFs and threshold levels indicate the ability of electrical stimulation of the retina to activate populations of neurons near each cortical channel. Here we quantify and compare the spatial extent and characteristics of cortical activation. Multi-electrode stimulation in the healthy retina (Figures 4.5A,B) and the degenerate retina (Figures 4.5C,D) typically resulted in responses in both cortical hemispheres, with a stronger contralateral response. However, the response was more widespread and stronger when stimulating the healthy retinae, with multiple scattered peaks of activation. To compare the spatial extent of responses across each recording array for multi-electrode stimulation, the spike count measured on each channel was normalised by dividing by the maximum count recorded on that channel. A channel was then considered ‘active’ if its normalised spike rate was greater than 3 times the standard deviation of the baseline rate. The response size collated over the population, calculated as the percentage of active cortical channels, was larger in the contralateral hemisphere (Figure 4.5E) compared to the ipsilateral hemisphere (Figure 4.5F) for both healthy and degenerate retinae ($p < 0.001$, median difference permutation test, 100,000 samples). In addition, when analysing each hemisphere separately, responses to stimulation of the healthy retina were on average larger in extent compared to those from stimulation of the degenerate retina ($p < 0.001$, permutation test

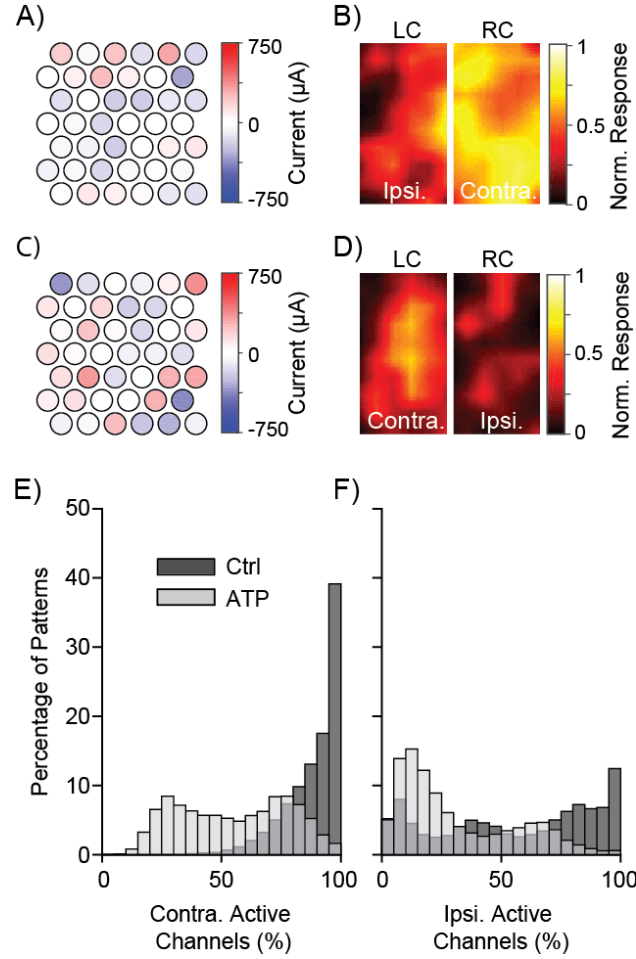


Figure 4.5: A,C) White noise pattern stimuli and B,D) resulting cortical responses with spike counts normalised to each channel's own maximum spike count in subject 1. First row is the stimulus and response of the healthy retina (left eye in this animal, A and B), second row is the degenerate retina (right eye in this animal, C and D). For each response, both sides of the cortex are shown: ipsilateral and contralateral to the eye being stimulated. LC=left cortex, RC=right cortex. E,F) Percentage of channels on the side of cortex contralateral or ipsilateral to the stimulated retina, with a spike count greater than 3 standard deviations of the baseline level, when stimulating the control eye (dark grey), as compared to the ATP-injected eye (light grey), collated over all animals.

for the difference in medians, with 100,000 samples). With the exception of the ipsilateral responses in subject 3, for which there was no significant difference between eyes ($p=0.1$), these results were consistent and significant for each animal individually ($p<0.001$ for all other comparisons).

4.5 Discussion

Multi-electrode stimulation has shown promise in improving visual outcomes for retinal implant recipients. Previous studies have shown that simultaneous stimulation with multiple electrodes can increase the number of percepts by activating groups of cells between electrodes (Dumm et al., 2014) and increase resolution by inhibiting current spread (Jepson et al., 2014b; Spencer et al., 2016) compared to single-electrode stimulation. However, these studies have involved normally sighted animals, limiting the clinical applicability of their findings. We find several important differences exist between responses evoked by stimulation of the degenerate eye and normal eye, including response latency, saturated spike rate, activation threshold, ERF size, and cortical spread. While we have only performed experiments in a small number of animals, with varying levels of degeneration, our results provide support for the hypothesis that the extent of retinal degeneration is the primary underlying reason for the variable results observed in human clinical trials of multi-electrode stimulation.

4.5.1 Reduced Response Latency to Multi-Electrode Stimulation of the Degenerate Retina

The degree of photoreceptor degeneration as measured by a-wave amplitude reduction was less in this cohort compared to previous work, where at least 70% reduction was reported for all animals at 12 weeks post-injection (Aplin et al., 2016a). While this highlights the inherent variability in the ATP blinding technique, in the present study, sufficient photoreceptor degeneration was classified as at least 50% reduction with accompanying ONL dropout at the area centralis, and all implanted animals met this constraint. A significant reduction in response latency to multi-electrode stimulation of the degenerate retina was measured in all experiments when compared to single-electrode stimulation or stimulation of the fellow control retina (Figure 4.2E). It is possible that these differences are due to the higher total currents delivered to the degenerate retina during multi-electrode stimulation (Jensen and Rizzo III, 2008; Stett et al., 2000; Tsai et al., 2009). The multi-electrode stimuli were sampled from Gaussian distributions with standard deviations equal to single electrode P50 currents. These P50 currents were higher for the ATP-injected retinæ, resulting in generally higher multi-electrode current amplitudes (standard deviation of multi-electrode stimulation over all animals was 191.3 μ A for control eye, 247.9 μ A for ATP-injected eye). This factor would not influence the latencies seen with single-electrode stimulation, since identical current amplitudes were delivered

to both retinæ. This is also consistent with a previous study by Aplin et al. (2016a), where with single electrode stimulation, no differences in latencies were found between normal and degenerate eye stimulation. However, we also found shorter latencies for single electrode stimulation of the ATP-injected eye compared to the control eye of subject 4. Given that identical stimulation amplitudes were used in both eyes during single electrode stimulation, this difference likely stems from degeneration of the retina. It is possible that the observed mild thinning of the inner retina seen using OCT could cause a reduction in response latency by reducing the distance between the electrodes and surviving retinal cells. However, this can only be confirmed with histological analyses which are beyond the scope of this study. Alternatively, changes in signaling pathways during degeneration may have affected the latency of responses. Jensen and Rizzo III (2008) reported a reduction in response latency for particular RGC types in *rd1* mice retinæ compared to those in wild-type retinæ in response to subretinal stimulation, possibly due to degeneration preferentially affecting the ON-pathway. Considering the complexity of information pathways within the retina it is difficult to interpret the exact cause of the latency decrease that we have observed, particularly given that the recording electrodes are far removed, and stimulating from the suprachoroidal space results in extensive current spread. However, these results suggest that further investigation into the temporal characteristics of responses in degenerate retina, particularly in *in vitro* preparations, is warranted. These data suggest that the higher charge delivered to the degenerate retina with multi-electrode stimulation, possibly coupled with retinal thinning, results in reduced response latency. Since temporal response accuracy may be central to the success of retinal prostheses (Fried et al., 2006), the interaction between high overall charge delivered across multiple electrodes simultaneously, and thinned retina due to degeneration, should be considered in future studies.

4.5.2 Nonlinear Response Characteristics Vary with Stimulation and Retina Types

Response thresholds to simultaneous stimulation of multiple electrodes were lower than those seen for single-electrode stimulation for both healthy and degenerate retinæ (Figures 4.3E,F). This is in agreement with clinical (Horsager et al., 2010; Wilke et al., 2011b), pre-clinical (Shivdasani et al., 2012), and modelling (Wilke et al., 2011a) studies. The latter shows that multi-electrode stimulation from the suprachoroidal space penetrates the retina more effectively than single-electrode stimulation (Wilke et al., 2011a), which could account for the lower thresholds. Previously, increased thresholds in the ATP-injected

feline model have been found to correlate with a decrease in retinal ganglion cell density and the extent of retinal gliosis above an electrode (Aplin et al., 2016a). Given that the local degree of degeneration influences threshold, the simultaneous stimulation of multiple electrodes overlying retinal areas with differing degrees of degeneration, confounds comparisons to single-electrode responses. Therefore, spatially uneven degeneration or gliosis are possible explanations for the lack of difference in thresholds between the control eye and the ATP-injected eye found in the animal with the lowest percentage a-wave reduction (subject 1).

Saturated spike rates were found to be higher for multi-electrode stimulation than for single-electrode stimulation (Figure 4.3C), which is in agreement with a previous *in vivo* study in normally sighted felines (Shivdasani et al., 2012). It is possible that the discrepancy in maximum spike level was due to single-electrode stimulation not actually reaching saturation, and that with higher stimulation amplitudes the spike rate would further increase. However, this is not likely given that the generator current required to reach saturation (P90) was generally similar between stimulus types (Figure 4.3D). Therefore, the more likely explanation is that spatial interactions between simultaneously stimulated electrodes activates individual neurons lying intermediate between electrodes that were not activated by single-electrode stimulation alone. This could be further investigated by performing spike sorting to characterise the number of individual neurons exhibiting a response to each stimulus type (Jepson et al., 2014b), but this is outside the scope of this study. Increased spike rates could be the reason for brighter percepts reported by human subjects in response to multi-electrode stimulation clinically (Horsager et al., 2010).

We also found that for the majority of our cohort, saturated spike levels for single electrode stimulation were similar between control and ATP-injected eyes; however, the animal with the most advanced retinal degeneration exhibited lower maximum spiking rates in response to stimulation of the ATP-injected eye than the control. This is likely due to retinal remodeling, but it is difficult to determine why a similar effect was not seen in the other animal with extensive degeneration (subject 3). Histology could provide further insight into these findings and will be addressed in a subsequent study.

4.5.3 Larger Electrical Receptive Fields and Smaller Activated Area for Stimulation of the Degenerate Retina

Simultaneous stimulation of multiple electrodes results in predominantly linear interactions between the elicited electric fields (Halupka et al., 2016). The extent over which

these interactions occur for a particular cortical location is represented by the size of the electrical receptive field (ERF) associated with that site. We found that ERFs for stimulation of the degenerate retina were significantly larger than for the healthy retina (Figure 4.4G). It is unlikely that the size discrepancy was due to different array placements between animals or eyes given that no differences were found in ERF sizes of healthy retinæ between animals. Instead, the increased ERF sizes suggest that in order to evoke a cortical response, a larger area of the degenerate retina must be recruited. This is possibly caused by changes in retinal cell excitability and density that occur during degeneration (Chan et al., 2008; Cho et al., 2016; Aplin et al., 2016b). In rd10 mice, the excitability of different RGCs has been reported to be variable, with some cells displaying similar thresholds to those in the wild-type, and others characterized by higher thresholds (Cho et al., 2016). If a similar variability in RGC thresholds were to occur in the degenerate feline retina, this could explain the enlarged ERFs. If a larger area of the retina were required to be stimulated in order to elicit a response at each cortical site, spatially-white electrical stimulation (with both anodic-phase first and cathodic-phase first pulses on different electrodes) in the degenerate retina would have a decreased probability of evoking cortical activity compared to the normal retina. Therefore, this is in agreement with our finding that in general, stimulation of the degenerate retinæ elicits smaller response areas in the cortex than stimulation of the healthy retinæ (Figure 4.5E). This outcome could not have been predicted based on the work of Aplin et al. (2016a), who found that in the same animal model selectivity of cortical activity evoked by single electrode stimulation at threshold was similar between normal and degenerate eyes. Thus, these data highlight the complex interactions that occur with multi-electrode stimulation, and the need for validation of sophisticated stimulation strategies in animal models exhibiting retinal degeneration.

Our data also suggest that a trend exists between more degeneration and larger ERFs. To further investigate this, the linear-nonlinear model presented here could be used at various intervals over the period of retinal degeneration of a single animal to assess changes in response characteristics with disease progression. Comparing these changes with *in vitro* investigations of individual cell thresholds at similar time points could help elucidate the physiological mechanism behind our findings. It is also possible that ERF sizes are mediated by remodeling elsewhere on the visual pathway. Visual deprivation in the form of retinal lesions (Darian-Smith and Gilbert, 1995) or rearing with one eye shut (Ganz et al., 1968) has been shown to increase the size of visual receptive fields in the cortex but not the lateral geniculate nucleus. While this was not possible to assess in the current study, changes in retino-cortical connections between the ATP-injected and fellow

control eyes could be investigated in future through the injection of tracers to assess the degree of retino-cortical remodeling (Darian-Smith and Gilbert, 1995; Antonini and Stryker, 1993) and then the retinae extracted to compare the extent of retinal remodeling. Such a study would also serve to explore the cortical targets of retinal projections. This has been extensively studied in normally-sighted felines, showing that the majority of cells located nasally relative to the area centralis project to the contralateral hemisphere. Additionally, some temporally located cells, particularly those close to the area centralis, also project to the contralateral hemisphere (Tassinari et al., 1997; Illing and Wässle, 1981; Wässle and Illing, 1980; Payne, 1994). Our results suggest that this organization is maintained during degeneration, since, for stimulating arrays located across the midline, contralateral responses were larger by area than ipsilateral for multi-electrode stimulation of both degenerate and healthy retinae. However, Aplin et al. (2016a) showed that single-electrode stimulation of the degenerate eye elicited larger areas of response in cortex ipsilateral to the degenerate retina. This difference may have been due to the stimulating arrays being located more temporally than those in the present study, and therefore recruiting a greater number of cells that project to the ipsilateral hemisphere. However it is also possible that the more extensive degeneration of ATP-injected eyes in the study by Aplin et al. (as measured by percentage a-wave reduction) was accompanied by reorganization of the visual pathway downstream of the retina.

The increased ERF sizes affect the applicability of current focusing and steering strategies, particularly if ERFs are also more diffuse, as strategies may require discrete ERF peaks between adjacent electrodes (Jepson et al., 2014b). Application of a linear-nonlinear model as described here and in previous studies (Maturana et al., 2016; Halupka et al., 2016) is a promising avenue towards not only identifying response characteristics, but also for optimizing stimulation patterns to shape cortical activity. This could be achieved by fully or partially inverting the model. Full model inversion would involve using a nonlinear optimization algorithm to compute stimulation patterns that would be likely to produce a desired cortical response. Alternatively, a more efficient approach would involve target ‘responses’ being identified in the generator signal subspace. From here, stimuli associated with these patterns could be retrieved via a linear transformation (multiplication by the pseudo-inverse of the linear filter matrix). Shaping cortical responses in this way could improve the efficacy of retinal prostheses.

4.6 Conclusion

We have shown that significant differences exist between cortical responses to single-electrode and multi-electrode stimulation of the healthy and degenerate retina. We show that multi-electrode stimulation in the degenerate retina results in increased retinal penetration and lower thresholds compared to single-electrode stimulation. This could prove invaluable for prostheses located far from target cells (such as suprachoroidal prostheses) in retinæ with already reduced cell counts. However, large electrical receptive field sizes may negatively impact the applicability of current focusing and steering strategies. These results highlight that simultaneous stimulation paradigms trialed in normally sighted models do not yield the same results in degenerate models, and indicate that the variability in results seen for simultaneous stimulation in clinical trials may be attributed to the extent of retinal degeneration.

Chapter 5

Cortical Activity Shaping Via Partial Model Inversion

5.1 Abstract

Interactions that occur between simultaneously stimulated electrodes of neural prostheses have long been considered detrimental to the efficacy of such devices. However, some research has suggested that these interactions could be harnessed to improve patient outcomes. Here, we investigate the use of a linear-nonlinear model to generate multi-electrode stimulation patterns in the retina with the aim of modulating cortical activity. Temporally sparse, spatially white current pulses were delivered across 42 electrodes of a suprachoroidal stimulating array implanted in an anaesthetised, normally-sighted adult cat. Multi-channel, multi-unit spiking activity recorded in the visual cortex was used to constrain a linear-nonlinear model. The model was partially inverted to generate new multi-electrode stimulus patterns to achieve desired patterns of cortical activity. Stimuli resulting from the model inversion could evoke responses that were distinct from those achieved with single electrode stimulation, and responses were well predicted by the model. Furthermore, the spatial characteristics of responses, including size and centre location, could be controlled in repeatable and topographically organised ways. We have demonstrated that a linear-nonlinear model can be used to generate novel stimulation patterns for shaping neural activity. Our results demonstrate the first successful application of current steering using more than three electrodes in a retinal prosthesis. Additionally, our method is efficient and easily scalable to more stimulating and recording electrodes. The method shows promise for improving the efficacy of retinal prostheses.

5.2 Introduction

Neural activity shaping seeks to control the spatial and/or temporal profile of activity in a population of neurons through the application of multi-electrode stimulation. The goal of activity shaping is to accurately select the region of surrounding tissue that will be activated in response to stimulation. Activity shaping strategies can generally be divided into two categories: those that aim to direct current to particular parts of the tissue to elicit distinct responses that cannot be activated by single electrode stimulation (“current steering”) or those that aim to reduce current spread and thereby increase selectivity (“current focusing”).

Current steering has been used successfully in a range of neural structures, including the cochlea (van den Honert and Kelsall, 2007; Bonham and Litvak, 2008), spinal nerve trunk (Grill Jr and Mortimer, 1996), deep brain areas (Keane et al., 2012; Martens et al., 2011) and the retina (Dumm et al., 2014; Jepson et al., 2014b). The retina would particularly benefit from activity shaping strategies given the close proximity of the electrodes to target neurons and need for large numbers of electrodes (Stingl et al., 2013). Current steering in the retina offers the promising possibility of evoking a larger range of percepts without increasing the number of physical electrodes by combining stimulation from nearby electrodes. However the process of mapping the large range of stimulus combinations with their perceptual effects is a time consuming process. Unsurprisingly, since the time required for this process increases with the number of stimulating electrodes, previous studies in retinal prostheses have been limited to computational modeling (Moghaddam et al., 2014; Loizos et al., 2016) or steering between only two or three electrodes (Jepson et al., 2014b; Dumm et al., 2014). More importantly, approaches that attempt neural activity shaping without any knowledge of the way the specific neural population responds are prone to error, particularly as the number of electrodes increases.

An alternative approach, which has only been applied in *in vitro* preparations so far, uses an empirical model to predict stimulation patterns that will elicit desired responses (Jepson et al., 2014b). This approach captures the interactions between electrode that are specific to a given retina and implant. In clinical practice, this would involve an optimisation algorithm for computing the optimal pattern for selective stimulation of target neurons. However, more issues arise with this approach, including the choice of the target pattern of activity, whether it can be achieved within current injection limitations, and complexity of nonlinear optimisation algorithms with large numbers of stimulating and recording electrodes. For example, certain desired activity patterns may only be achievable

with stimulation current amplitudes in excess of stimulator or safety limitations.

Here, we present an efficient approach for generating activity shaping stimulus patterns that are within current limitations, by partial inversion of the linear-nonlinear model described in Chapters 2 and 3. This process utilises the underlying characteristics of cortical responses to multi-electrode stimulation to define a subspace of achievable responses based on responses to white noise stimulation. We describe the method used to invert the model and generate new stimuli online. The observed responses to the new stimuli are accurately predicted by the linear-nonlinear model, and are spatially distinct from those evoked by single electrode stimulation. Furthermore, we show that the spatial characteristics of the cortical activity can be modulated by multi-electrode stimulation in a reliable, predictable way. This preliminary study, in one *in vivo* experiment in a feline model, demonstrates a potential highly novel approach to current steering in the retina.

5.3 Methods

The experiment was performed in one normally-sighted, adult cat. All procedures were approved by the Bionics Institute Animal Research Ethics Committee (Projects 14/304AB).

5.3.1 Anesthesia and Surgery

The procedure for anaesthesia and surgical insertion of the suprachoroidal stimulating array and cortical recording arrays has been described in detail in Chapter 2. Briefly, the animal was anaesthetised with ketamine (intramuscular, 20 mg/kg) and xylazil (subcutaneous, 2 mg/kg). Anesthesia was maintained over the experimental period of 4 days with continuous intravenous infusion of sodium pentobarbitone (60 mg/kg/hr) and Hartmanns solution (sodium lactate, 2.5 mL/kg/hour). Dexamethasone (intramuscular, 0.1 mg/kg) and Clavulox (subcutaneous, 10 mg/kg) injections were given daily to minimise brain swelling and infection. Vitals were continuously monitored throughout the experiment.

The suprachoroidal array (Figure 3.1A) consisted of a biocompatible silicone substrate with 7 rows x 6 columns of 600 μm diameter electrodes arranged hexagonally with 1 mm center-to-center spacing (Villalobos et al., 2012). The array was inserted and sutured into place approximately 15 mm into the suprachoroidal space following a lateral canthotomy, scleral incision, and dissection of a pocket between the sclera and choroid (Villalobos et al., 2012).

Following a craniotomy to expose the visual cortex contralateral to the implanted eye, two penetrating microelectrode arrays (6 x 10, 1 mm length, 400 μm spacing, Blackrock Micro., USA) were implanted (Figure 3.1B). The position of arrays on the visual cortex was determined based on the location that exhibited the lowest thresholds of evoked potentials in response to retinal stimulation (Cicione et al., 2012; Dumm et al., 2014).

5.3.2 Electrical Stimuli

Electrical stimuli were delivered with a 128-channel IZ2 stimulator (Tucker-Davis Technologies, Alachua, FL, USA). All stimuli used during the experiment were biphasic charge-balanced pulses with 1 ms phase width, 25 μs interphase gap, and 1 Hz presentation rate, as described in Chapters 2 and 3. Due to restrictions of the stimulator, the amplitude of each pulse was limited to 700 μA .

Single Electrode Stimuli

Each electrode was stimulated individually with cathodic-first biphasic pulses with 0-700 μA stimulus amplitude in 50 μA steps. Responses were averaged over 10 repetitions of each stimulus. Sigmoid curves were fitted to the spike-rate versus stimulation amplitude function for each stimulation electrode/recording electrode combination. The threshold of each stimulating electrode was defined as the lowest stimulus amplitude that elicited 50% of the maximum firing rate on any recording electrode (Shivdasani et al., 2012).

White Noise Stimuli

Electrical pulses were delivered simultaneously across all stimulating electrodes, and the responses were used to fit the parameters of a linear-nonlinear model. The patterns are termed “white-noise” since the stimulus amplitude for each electrode was sampled from a zero-centered Gaussian distribution with standard deviation equal to the threshold for that electrode. If a sampled current exceeded the 700 μA limitation, it was discarded and a replacement drawn. A mix of anodic-first and cathodic-first polarity pulses were used in each pattern, with anodic-first corresponding to a positive number drawn from the Gaussian distribution and cathodic-first corresponding to a negative number. A total of 3600 unique white-noise stimulation patterns were presented, each repeated eight times, and responses averaged for analysis.

Inversion Stimuli

Inversion stimuli were generated via the process described in Section 5.3.5. This process resulted in 1452 individual, multi-electrode stimulus patterns. The patterns were each presented to the retina 16 times in randomised order and responses were averaged for analysis. A higher number of repetitions was used for the inversion stimuli to reduce the impact of noise on the analysis of response profiles.

5.3.3 Data Preprocessing

As described in detail in Chapters 2 and 3, cortical activity was analysed as multi-unit activity (MUA); stimulus artefacts were removed offline using techniques described by Heffer and Fallon (2008), followed by band-pass filtering (third-order Butterworth filter, 300-5000 Hz). MUA was detected as spikes crossing a threshold of four times the root mean square of a moving 60 s window. The number of threshold crossings occurring 3-20 ms after the stimulus was counted, from which the baseline spiking rate in the 500 ms prior to the stimulus pulse was subtracted to account for stimulus-independent activity. The time-window of 3-20 ms was chosen since activity in this time frame is considered to be a result of direct retinal ganglion cell activation and indirect activation of the inner retina (Boinagrov et al., 2014).

5.3.4 Model Estimation

In Chapters 2 and 3, a linear-nonlinear model fitted to the responses to Gaussian white noise stimulation was described in detail. The purpose of this model was to capture the spiking rate of multiple locations in the visual cortex in response to simultaneous stimulation of multiple electrodes in the retina. The same approach was used to here to estimate the model used for inversion, and is briefly summarised below. The model consisted of two stages. First, the stimulus was projected onto two spatial linear filters ($\mathbf{V}^P$ and $\mathbf{V}^N$), which are the electrical receptive fields (ERFs) of each cortical channel. Next, the resulting “generator signal” was transformed by static nonlinearities (g^P and g^N) that account for the nonlinear characteristics of neurons. Therefore, the spike count ($r_{i,t}$), at cortical recording site $i \in \{1 \dots K\}$ in response to a stimulus vector at time $t \in \{1 \dots T\}$ ($\mathbf{s}_t$) was estimated by

$$\hat{r}_{i,t} = g_i^P \left(\mathbf{v}_i^P \mathbf{s}_t \right) + g_i^N \left(\mathbf{v}_i^N \mathbf{s}_t \right), \quad (5.1)$$

where $\mathbf{s}_t$ is an $M \times 1$ dimensional vector containing the stimulation current applied at time t for each stimulating electrode $j \in \{1 \dots M\}$ and $\mathbf{v}_i^P$ and $\mathbf{v}_i^N$ are the i^{th} rows of $\mathbf{V}^P$ and $\mathbf{V}^N$, respectively (i.e., the ERFs for the i^{th} cortical channel). Since the stimulation current on each electrode was limited in amplitude to $\pm 700 \mu\text{A}$, the space of possible stimulating patterns is M -dimensional and bounded in all dimensions by $\pm 700 \mu\text{A}$.

The linear filters were found by applying principal component analysis to the stimuli that resulted in a response. The resulting eigenvectors in the stimulus space represented stimulus patterns associated with the largest amount of variance in the data. Projecting the stimuli onto the first eigenvector served to split the stimuli and resulting responses into net anodic-first stimuli (those with a positive projection) and net cathodic-first stimuli (those with a negative projection). The spike triggered averages of these stimulus subsets were $\mathbf{V}^P$ and $\mathbf{V}^N$ respectively. The static nonlinearities g^P and g^N (shown in Eq. 3.14 and 3.15) were then recovered by projecting the stimuli onto the linear filters (converting them into generator signals) and fitting a sigmoidal function to the binned generator signal values and their respective responses. We used a Levenberg-Marquardt nonlinear least squares algorithm in MATLAB 2014b (Mathworks, Inc. USA) to find the optimal parameters of g^P and g^N .

Following the fitting of the model to the responses to white-noise stimuli, the model was partially inverted and used to generate new stimuli, with the goal of shaping cortical activity. In order to achieve this, the model was first simplified using the assumption that the linear filters $\mathbf{V}^P$ and $\mathbf{V}^N$ were sufficiently similar that we could approximate that $\mathbf{V}^P = \mathbf{V}^N = \mathbf{V}$ (discussed further in Section 5.4.1). Thus, the $K \times M$ dimensional linear filter $\mathbf{V}$ served to map between the stimulus $\mathbf{s}_t$ ($M \times 1$) and generator signal $\mathbf{x}_t$ ($K \times 1$):

$$\mathbf{x}_t = \mathbf{V} \mathbf{s}_t, \quad (5.2)$$

$$\mathbf{s}_t = \mathbf{V}^+ \mathbf{x}_t, \quad (5.3)$$

where $\mathbf{V}^+$ is the pseudo-inverse of linear filter matrix $\mathbf{V}$. Since the generator signal space X is higher dimensional than the stimulus space S , $\mathbf{V}^+$ acts as an inverse on the subspace of X that S maps onto.

The estimated activity shown in Eq. 5.1 was then given by

$$\hat{r}_{i,t} = g_i^P(x_{i,t}) + g_i^N(x_{i,t}). \quad (5.4)$$

Using this definition, a response r_t on one recording channel could be elicited by two

possible generator signal values, one associated with a net-anodic first stimulus and the other with a net-cathodic first stimulus. By extension, a response pattern recorded over K channels is associated with 2^K possible generator signal patterns, corresponding to all the possible anodic first and cathodic first combinations, each of which can be converted into a stimulus via Eq. 5.3. While only a subset of these stimulus patterns is possible given the constraints on stimulus current, this subset will likely still be numerous, growing exponentially with K , and computationally expensive, or even prohibitive, to evaluate.

We instead propose a partial model inversion, where target patterns of activity are defined in the generator signal subspace within which each pattern is associated with a response and can be efficiently and linearly mapped to a stimulus.

5.3.5 Generating stimulus vectors via partial model inversion

Ideally, a target generator signal would be associated with a perceptual pattern. However, in the present study, it was not possible to relate patterns of cortical spiking activity to visual percepts. Instead, generator signal targets were created by sparsely sampling the generator signal subspace that is accessible via electrical stimulation $\tilde{X} \subset X$. In principle $\tilde{X}$ is the set of all $x \in X$ such that $x = Vs$ for some stimulus in the stimulus space $s \in S$. Hence, sparse sampling was achieved by creating generator signal patterns from linear combinations of the leading eigenvectors of the subspace.

The subspace was defined by the linear filter matrix $\mathbf{V}$, which comprised concatenated linear filter row vectors $\mathbf{v}_i$ and had dimensions $K \times M$:

$$\mathbf{V} = \begin{bmatrix} \mathbf{v}_1 \\ \vdots \\ \mathbf{v}_K \end{bmatrix}. \quad (5.5)$$

It should be noted that while the generator signal subspace $\tilde{X}$ is theoretically K -dimensional (equal to the number of recording electrodes), $\mathbf{V}$ is effectively rank degenerate. This occurs because many of the rows of $\mathbf{V}$ (i.e., ERFs) are similar, since nearby cortical recording locations are affected by stimulation of the same retinal electrodes.

Prior to singular value decomposition (SVD) we subtracted the mean from $\mathbf{V}$. Em-

pirical mean $\mathbf{u}$ was a $K \times 1$ dimensional vector,

$$u_i = \frac{1}{M} \sum_{j=1}^M v_{i,j}, \quad (5.6)$$

and was subtracted from each row of $\mathbf{V}$,

$$\widetilde{\mathbf{V}} = \mathbf{V} - \mathbf{u}\mathbf{h}, \quad (5.7)$$

where $\mathbf{h}$ is a $1 \times M$ vector of ones. This had the effect of diminishing the eigenvalue of the leading eigenvector, but had little effect of the remaining eigenvectors.

The covariance matrix is then

$$\mathbf{C} = \widetilde{\mathbf{V}}\widetilde{\mathbf{V}}^{tr}. \quad (5.8)$$

The directions of maximum variance of the generator signal subspace are given by the eigenvector matrix of $\mathbf{C}$, denoted $\hat{\mathbf{P}}$,

$$\hat{\mathbf{P}} = [\hat{\mathbf{p}}_1, \dots, \hat{\mathbf{p}}_K], \quad (5.9)$$

where

$$\hat{\mathbf{p}}_i = \frac{\mathbf{p}_i}{\|\mathbf{p}_i\|_2}. \quad (5.10)$$

Here, $\hat{\mathbf{p}}_1$ is the principal eigenvector (corresponding to the largest eigenvalue) and is a column vectors with K elements. The corresponding eigenvalues are denoted λ_i ,

$$\boldsymbol{\lambda} = \begin{bmatrix} \lambda_1 \\ \vdots \\ \lambda_K \end{bmatrix}. \quad (5.11)$$

The higher order eigenvectors were associated with low signal-to-noise ratios, which would lead to unpredictable outcomes if used for activity shaping. Further they potentially require large amplification to have an impact on the generator signal due to their small eigenvalues, possibly leading to unfeasibly large currents. Therefore, a subset of leading eigenvectors were chosen as basis vectors for the new lower-dimensional space. The relative strength of the eigenvalues was used to estimate the number of dimensions required to represent approximately 95% of the variance in the space. For the present study, five

dimensions were used. In order to span the space and generate new stimulus patterns that elicited responses with the most variance, linear combinations of the five basis vectors were formed,

$$\mathbf{x}_{\text{new}} = \mathbf{u} + \sum_{m=1}^5 l_m \hat{\mathbf{p}}_m, \quad (5.12)$$

where l_m ($m \in \{1, 2, 3, 4, 5\}$) is a scalar term that determines the relative contribution of each associated basis vector $\hat{\mathbf{p}}_m$ and $\mathbf{u}$ is the mean defined in Eq. 5.6. The addition of the mean served to centre the matrix $\widetilde{\mathbf{V}}$ and caused the resultant stimuli to have means proportional to $\mathbf{u}$, rather than 0, as would have occurred without mean addition. This effectively confines the stimulus solutions to one “orthant” (the analogue in n-dimensional Euclidean space of a quadrant in the plane) in the stimulus space. In doing so, the degeneracy of the double-sided sigmoidal nonlinearity is avoided. In order to explore the effect of different relative contributions of the basis vectors, two strategies were employed to determine the value of l_m .

Strategy 1 The relative absolute contribution of each basis vector was equal, such that

$$l_m = \begin{cases} -1 \\ 0 \\ 1 \end{cases} \quad \forall m = \{1, 2, 3, 4, 5\}, \quad (5.13)$$

Strategy 2 Basis vectors with larger eigenvalues were scaled down in each pattern relative to those with smaller eigenvalues, such that

$$l_m = \begin{cases} -1/\lambda_m \\ 0 \\ 1/\lambda_m \end{cases} \quad \forall m = \{1, 2, 3, 4, 5\}, \quad (5.14)$$

Therefore, within each strategy, there were 3^5 possible combinations of basis vectors. With the exception of the null case, all possible combinations were trialled, resulting in 242 patterns per strategy. The resulting generator signal patterns were then mapped to the stimulus space and weighted by γ ,

$$\mathbf{s}_{\text{new}} = \gamma \mathbf{V}^+ \mathbf{x}_{\text{new}}. \quad (5.15)$$

Within each strategy, three approaches to setting the weight γ were trialled, labeled

a-c.

- a) γ was calculated separately for each stimulus, such that the average generator signal across the K channels for each pattern was equal to the mean threshold of g^P across channels (termed a^P as per Eq. 3.14). This approach served to maximise the dynamic range of the expected response across the array, such that most channels would yield responses without saturating. Thus,

$$\gamma = \frac{\frac{1}{K} \sum_{i=1}^K a_i^P}{\frac{1}{K} \sum_{i=1}^K x_{\text{new},i}}. \quad (5.16)$$

In the event that this choice of γ resulted in a current amplitude in excess of the stimulator maximum (700 μA), γ was set such that the greatest current amplitude across the array was equal to the maximum.

- b) γ was set such that the greatest current amplitude across the stimulating array for the pattern was 100 μA .
- c) γ was set such that the greatest current amplitude across the stimulating array for the pattern was 600 μA .

The values of 100 μA and 600 μA in approaches *b* and *c* respectively were chosen arbitrarily to investigate different scaling techniques. A notable implication of all three approaches was that the value of γ varied across stimuli.

This process resulted in two strategies, given by Eqs. 5.13 and 5.14, each with 242 unique stimulating patterns, each weighted by three scales for γ . These are summarised in Table 5.1 and hereafter will be referred to by a number and letter combination; e.g., “1.a”, meaning Strategy 1 with weighting ‘a’.

Therefore, 6×242 individual stimulus patterns were presented to the retina and responses were averaged over 16 repetitions of each pattern.

Since the method is limited by the accuracy of the model, only recording channels with sufficient accuracy in estimating channel responses were used in the SVD analysis. Accuracy was determined by comparing the responses predicted by the model to the actual recorded responses for all 3600 white noise patterns. Only channels that exhibited a correlation between the observed and predicted activity with a coefficient of determination of $r^2 \geq 0.5$ were considered in the analysis. In the present study, the recording channels

Table 5.1: Overview of inversion stimulus strategies.

Strategy 1	
Basis vectors not scaled (Eq. 5.13).	
1.a	Average generator signal equal to mean threshold of g_P (Eq. 5.16).
1.b	Maximum current amplitude across stimulating array = 100 μA
1.c	Maximum current amplitude across stimulating array = 600 μA
Strategy 2	
Basis vectors scaled by eigenvalues (Eq. 5.14).	
2.a	Average generator signal equal to mean threshold of g_P (Eq. 5.16).
2.b	Maximum current amplitude across stimulating array = 100 μA
2.c	Maximum current amplitude across stimulating array = 600 μA

from both implanted cortical arrays were combined in the SVD analysis; however, it would also be possible to perform SVD on each array separately.

5.3.6 Data Analysis

Gaussian Fitting for Response Characterisation

We found that the great majority of responses were spatially localised on each recording array around a single centre. Therefore, to characterise the spatial profile of the observed cortical responses, 2D Gaussian profiles were fitted to the observed responses on each planar recording array separately using a Trust-region nonlinear least-squares algorithm ('lsqnonlin') in MATLAB (Mathworks, Inc. USA) to find the optimal parameters. The Gaussian profiles were characterised by a general 2D Gaussian function,

$$f(x, y) = A \exp \left(- \left(a(x - x_o)^2 + 2b(x - x_o)(y - y_o) + c(y - y_o)^2 \right) \right) \quad (5.17)$$

with

$$\begin{aligned} a &= \frac{\cos^2 \theta}{2\sigma_x^2} + \frac{\sin^2 \theta}{2\sigma_y^2} \\ b &= -\frac{\sin 2\theta}{4\sigma_x^2} + \frac{\sin 2\theta}{4\sigma_y^2} \\ c &= \frac{\sin^2 \theta}{2\sigma_x^2} + \frac{\cos^2 \theta}{2\sigma_y^2}, \end{aligned}$$

where x_o and y_o are the horizontal and vertical distances, respectively, in millimetres, of the centre of the Gaussian from the most medio-caudal corner of each array, A is the amplitude of the response, θ is the angle of rotation in radians of the Gaussian, and σ_x and σ_y are the degree of spread of the Gaussian along an axis rotated by θ relative to the recording array. The correspondence of each 2D Gaussian profile with the observed response was quantified by the coefficient of determination (r^2). A permutation test with 100,000 resamples of each optimal Gaussian profile was used to construct a sampling distribution of the r^2 values separately for each response pattern. Gaussian profiles and their associated observed responses with an r^2 value in the top 5% of the distribution were considered significant, and used in further analysis. Additionally, Gaussians with centre locations falling outside of or between the recording arrays were discarded.

Cluster Analysis

For responses with well fitted 2D Gaussian profiles, the centre of the Gaussian (x_o and y_o) and the area of the response (given by the percentage of recording channels with a spike count greater than 3 standard deviations of the baseline level for that channel) was calculated. Thus, 242 values for each measure were found for Strategies 1.a and 2.a separately. Cluster analysis was used to determine which basis vectors resulted in a significant change to any of these measures. For each basis vector ($\hat{\mathbf{p}}_m$), clusters were labeled based on the relative contribution of that vector ($\mathbf{l}_m$), resulting in three clusters. To evaluate how well the points were clustered, a Davies-Bouldin index (Davies and Bouldin, 1979) was calculated. The significance of the index value was determined by permutation test ($n = 1 \times 10^6$) at 0.05 level of significance.

5.4 Results

During the experiment, 120 electrodes on two arrays were inserted into the visual cortex, of which 119 were functional. Multi-unit activity in response to single electrode stimulation was recorded, from which thresholds for each stimulating electrode were found. White noise stimuli were generated from Gaussian distributions having standard deviations equal to the thresholds. These stimuli were presented to the animal and the responses used to fit the linear-nonlinear model. Partial inversion was performed on the model to generate inversion stimuli. However, only channels that exhibited a correlation between the observed and predicted activity with a coefficient of determination of $r^2 \geq 0.5$ were considered in the SVD analysis due to the requirement that the model be accurate. 100 channels out of

a possible 120 passed this criterion (therefore, $K = 100$). The inversion stimuli were then presented to the same animal approximately 10 hours after the last white noise stimulus presentation.

5.4.1 Feasibility of Model Approximation

The generation of inversion stimuli required the assumption that the linear filters $\mathbf{V}^P$ and $\mathbf{V}^N$ were approximately equal in amplitude, with opposite sign. Figure 5.1 shows a comparison of the linear filter values for all elements of both matrices. Each point represents the effect of anodic first stimulation of a stimulating electrode j on cortical channel i ($v_{i,j}^P$) versus the effect of cathodic first ($v_{i,j}^N$). A strong correlation was apparent between the two linear filter matrices ($r^2 = 0.925$), indicating that the assumption was reasonable. The figure also shows that the filters are more similar for larger amplitudes than for smaller amplitudes.

5.4.2 Generator Signal Subspace Reduction

Singular value decomposition was performed on the linear filter matrix $\mathbf{V}$ to find the eigenvectors and eigenvalues of $\widetilde{\mathbf{V}}\widetilde{\mathbf{V}}^{tr}$. The eigenvectors represent the directions of maximum variance in the generator signal space, and their associated eigenvalues are proportional to the amount of variance that is correlated with each eigenvector. A reduced dimensional subspace was used such that only eigenvalues accounting for 95% of the variance of the generator signal subspace were considered. Figure 5.2A shows a scree plot of the sorted eigenvalues. The plot has a steep slope with a large difference between the first three eigenvalues, followed by an elbow in the curve. The variance in the generator signal space that is accounted for by each eigenvector is shown in Figure 5.2B, where 95% of the variance of the space is represented by the first five eigenvectors. Therefore, these were chosen as the basis vectors of the reduced dimensional subspace.

The basis vectors are shown in Figures 5.3A-E. The eigenvectors have been reshaped into matrices such that the channel numbers relate to their locations on the recording arrays. While the SVD was completed over both recording arrays together (100 channels in total), for visualisation purposes, the recording arrays are shown here separated by a thick black line. Figures 5.3A and B show that the first and second eigenvectors primarily represent the level of activity on the caudal and rostral arrays, respectively. The third eigenvector (Figure 5.3C) further modulates the location of activity on the rostral array between the rostro-medial and caudo-lateral corners. The impact of the two remaining

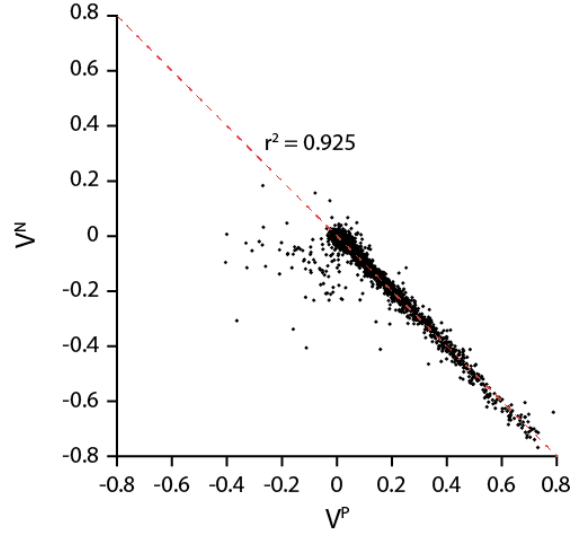


Figure 5.1: Comparison of values in linear filter matrix $\mathbf{V}^P$, which indicates the effect of net anodic-first stimulation on each cortical channel, versus the corresponding values in $\mathbf{V}^N$, which indicates the effect of net-cathodic first stimulation (Pearson's correlation coefficient $r^2 = 0.925$). Each marker represents $v_{i,j}^P$ versus $v_{i,j}^N$ for $i \in \{1 \dots K\}$ and $j \in \{1 \dots M\}$, $n = 4200$. Red dashed line represents $y = -x$.

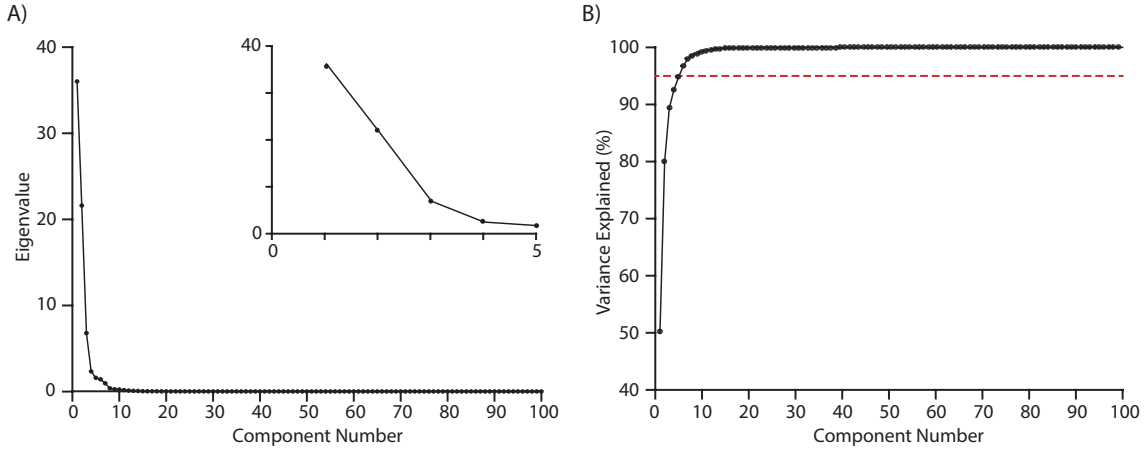


Figure 5.2: A) A scree plot of the eigenvalues λ_i in Eq. 5.11 (in descending order) associated with the basis vectors $\hat{\mathbf{p}}_i$, with magnified view shown inset. B) Cumulative percentage of variance in the generator signal space that is explained by each of the eigenvectors, with a red dashed line indicating the 95% cutoff point.

eigenvectors is more complex than that of the first three; however, both are spatially well organised and far from random, appearing to modulate activity both medio-laterally and rostro-caudally on both recording arrays. Figures 5.3F-G show the result of projecting the matrices from Figures 5.3A-E into the stimulus space via left-multiplication with the pseudo-inverse $\mathbf{V}^+$ (see Methods for explanation of pseudo-inverse). It should be noted that, for the purposes of visualisation, the mean $\mathbf{u}$ has not been added to each eigenvector prior to projection, as per Eq. 5.12. The patterns in the stimulus space are spatially similar to the associated eigenvectors in panels A-E. This is expected, given that $\mathbf{V}^+$ accounts for the electrical receptive fields of each cortical site. The patterns in the stimulus space also highlight that the cortical recording area is particularly sensitive to stimulation on the left hand half of the stimulating array, likely due to retinotopic organisation.

These conclusions on the impact of each eigenvector are supported by the result of a 2D Fourier transform of the generator signal basis patterns (for each recording array separately), shown in Figure 5.4. The bright centre with low amplitude surrounds of the caudal array for P_1 (Figure 5.4A) and the rostral array of P_2 (Figure 5.4B) shows that eigenvectors 1 and 2 mainly modulate the average generator signal amplitude on these arrays. The low amplitude centre on the other panels (P_3 and P_5 rostral, P_4 caudal) indicates that the average generator signal amplitude across these is zero. The rostral array of P_3 has a bright vertical strip, while that of P_4 has a horizontal strip, reflecting the axis that the respective eigenvectors mainly modulate. The analysis also shows that only the lowest spatial frequencies can be modulated using the eigenvectors.

5.4.3 Model Prediction of Responses

Linear combinations of the five basis vectors were formed as per Eq. 5.12. A graphical example of this process is shown in Figure 5.5A.

The resulting generator signal has been transformed into a stimulus (as per Eq. 5.15), shown in Figure 5.5B, and scaled by γ , such that the average generator signal across the K channels is to equal the mean threshold across the channels (Eq. 5.16). The response observed from stimulation with this pattern was very similar to that predicted by the linear-nonlinear model (Figure 5.5C), with a correlation coefficient of $r^2 = 0.822$.

The correlation coefficients between the observed and predicted responses were also compared for all other inversion stimuli trialled. Figure 5.6 shows the r^2 values for strategies 1.a and 2.a. The r^2 values for Strategy 2 (where basis vectors were normalised by their respective eigenvalues) were generally higher than those of Strategy 1 (where basis vectors

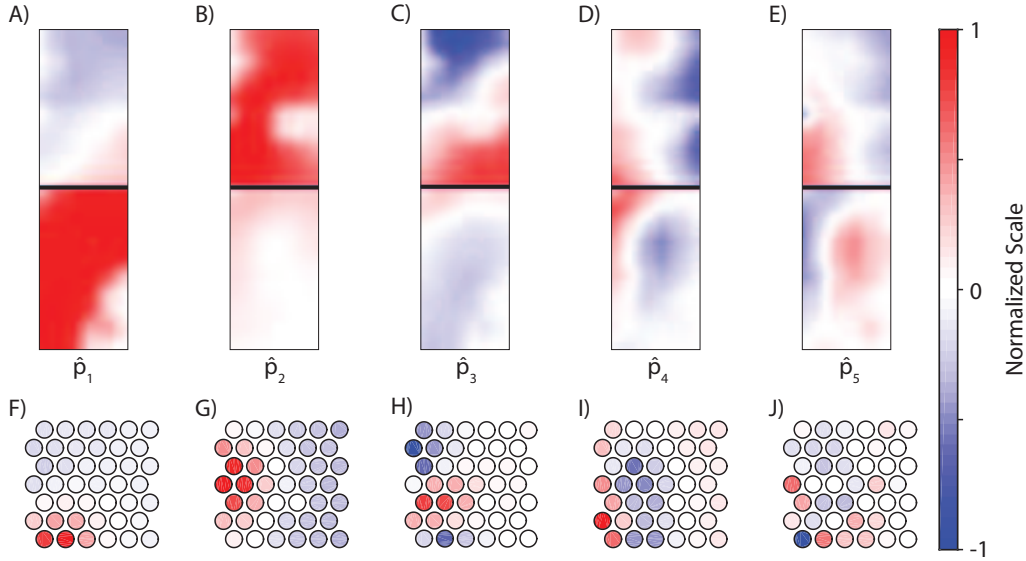


Figure 5.3: A-E) Leading eigenvectors from Eq. 5.9, reshaped into matrices such that channel numbers relate to their locations on the recording arrays. Eigenvectors were computed over all channels at once; however, for each panel of A-E, the rostral array (top) and the caudal array (bottom) are shown separated by a black line. F-J) Projection of each basis vector in the stimulus space via left-multiplication with $\mathbf{V}^+$. The for purpose of visualisation each panel has been normalized by the maximum of the rectified values, giving an overall range of values in all figures (“Normalized Scale”) from -1 to 1.

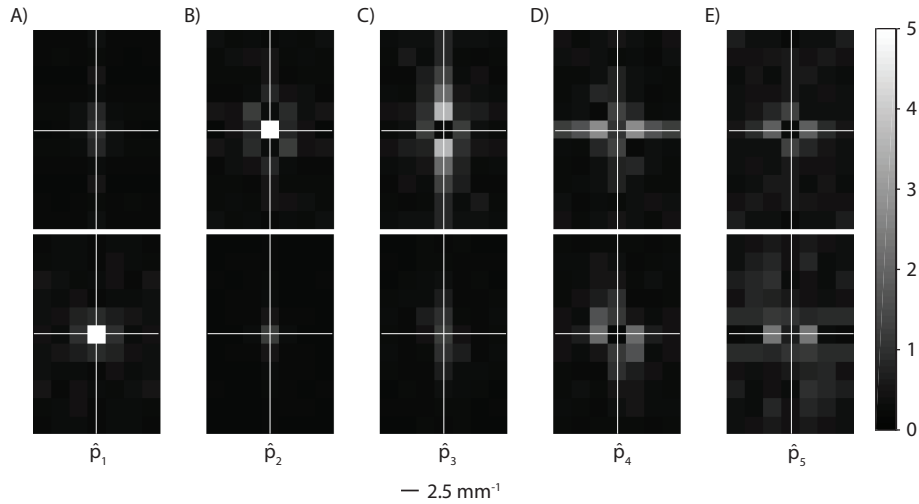


Figure 5.4: 2D Fourier analysis of the generator signal basis matrices shown in Figure 5.3 (prior to interpolation). For each panel, the rostral array is shown at the top, and the caudal array at the bottom; the Fourier transform has been completed separately for each array. The intersection of the white lines on each panel indicates (0,0), with scale shown below the figure.

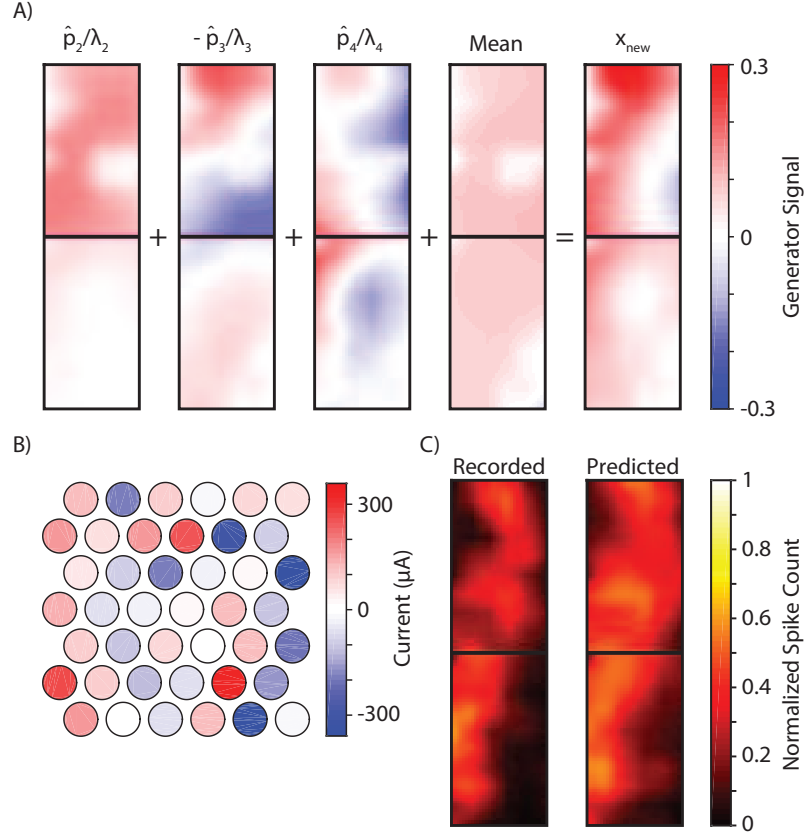


Figure 5.5: Example of the process of constructing a generator signal and associated stimulus via combination of basis vectors, given in Eq. 5.12. This example corresponds to Strategy 2.a, such that eigenvectors are normalised by their respective eigenvalues, with $l_m = [0, \frac{1}{\lambda_2}, \frac{-1}{\lambda_3}, \frac{1}{\lambda_4}, 0]$. A) Target generator signal as a sum of the basis vectors using Strategy 2.a. B) Corresponding stimulus pattern, and C) recorded and predicted cortical responses to the stimulus. The correlation coefficient between the recorded and predicted responses shown is $r^2 = 0.822$.

were not normalised), despite some particularly high values in Strategy 1. However, one particular cluster of Strategy 2 values was noticeably lower than the rest (highlighted in red in Figure 5.6). This cluster was associated with stimuli with $l_4 = \pm \frac{1}{\lambda_4}$ and $l_5 = \frac{1}{\lambda_5}$ (i.e., $\pm \hat{p}_4$ and a positive amount of $\hat{p}_5$). While no other relationships were found between combinations of eigenvectors and correlation coefficients, it is possible that this combination of more than two eigenvectors resulted in lower (or higher) r^2 values; however, this has not been investigated.

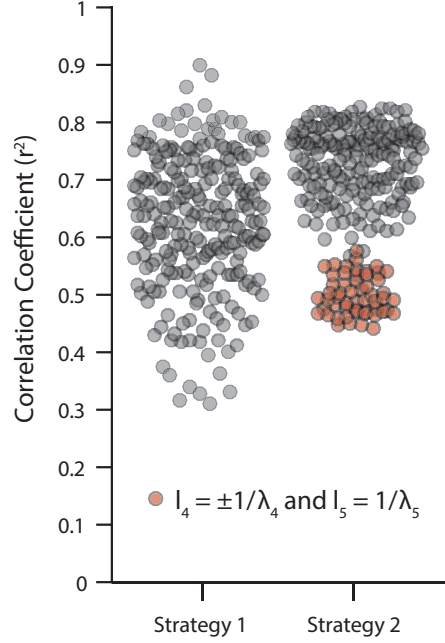


Figure 5.6: Correlation coefficients between the observed response and the response predicted by the linear-nonlinear model (Eq. 5.1) for all patterns for Strategies 1.a and 2.a ($n=242$ in each group). Each marker represents the r^2 value for a particular response pattern. The responses to stimuli where $l_4 = \pm \frac{1}{\lambda_4}$ and $l_5 = \frac{1}{\lambda_5}$ are highlighted in red.

5.4.4 Spatial Characteristics of Responses to Inversion Stimuli

Of particular interest when considering the success of the current method of stimulus generation is the characteristics of observed responses. A stimulation strategy that is able to generate repeatable, distinct responses is desirable. Inspection of the eigenvectors shown in Figure 5.3 suggests that $\hat{\mathbf{p}}_1$ and $\hat{\mathbf{p}}_2$ primarily modulate the level of activity on the caudal and rostral arrays, respectively. The effect of these basis vectors on the recorded responses was investigated by examining the percentage of channels with spike counts greater than three standard deviations of the baseline level in response to each stimulus, referred to as the “active area” in Figure 5.7.

Here, the active area on the rostral recording array versus that for the caudal array is shown, in response to Strategy 1.a stimuli. The points are coloured according to the weight (l) of $\hat{\mathbf{p}}_1$ and $\hat{\mathbf{p}}_2$ (Figs. 5.7A and B, respectively). Figure 5.7A shows that markers corresponding to stimuli where $l_1 = 1$ (yellow markers) are generally associated with a higher active area on the caudal array than when $l_1 = -1$ (blue markers). This is

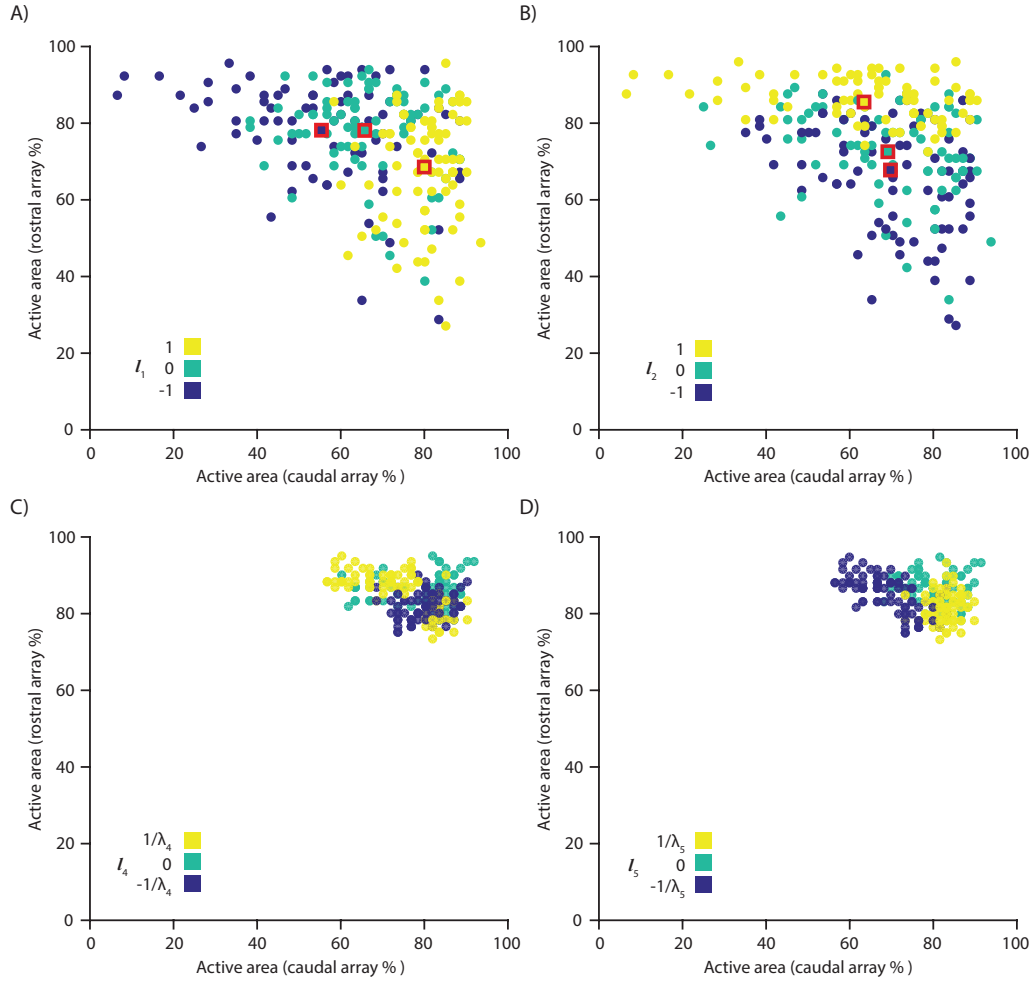


Figure 5.7: Comparison of the active area on each recording array as a function of the contributing eigenvector ratios. Each marker represents the active area in response to one stimulus pattern from Strategy 1.a (A and B) or 2.a (C and D). The location of each marker indicates the percentage of active channels on the the lower (caudal) and upper (rostral) array, shown on the x and y axes, respectively, in response to each stimulus. ‘Active’ refers to channels with a spike count greater than three standard deviations of the baseline level. Marker colours represent the scale of the 1st (A), 2nd (B), 4th (C), and 5th (D) eigenvectors used to generate the stimuli ($n = 242$ in each panel). Three additional square red markers on panels A and B show the mean location of each of the three clusters, with centre colour indicating the associated cluster.

particularly evident by considering the mean location of each cluster (indicated by red squares with coloured centres in Figs. 5.7A). This indicates that the active area on the

caudal array is modulated by l_1 (the weight of $\hat{\mathbf{p}}_1$) with $l_1 = 1$ resulting in a larger active area. In the same way, Figure 5.7B shows that markers corresponding to $l_2 = 1$ up-modulate the active area of the rostral array, while $l_2 = -1$ down-modulates the active area.

To determine whether the points in Figure 5.7 were significantly clustered, the Davies-Bouldin index of each set of points was calculated and compared to a permuted distribution. This showed that, in Strategy 1.a, grouping based on the weight of both $\hat{\mathbf{p}}_1$ and $\hat{\mathbf{p}}_2$ resulted in significant clusters, with $p < 0.0001$ for both comparisons (permutation test, $n = 1 \times 10^6$). However, for Strategy 2.a, the clusters for $\hat{\mathbf{p}}_1$ and $\hat{\mathbf{p}}_2$ were not significant ($p = 0.92$ and $p = 0.52$, respectively). Repeating the same test of response area clustering for the remaining basis vectors revealed that no other basis vectors resulted in significant clusters for Strategy 1. However, for Strategy 2, grouping based on the weightings of $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ resulted in significant clusters ($p < 0.0001$ for both comparisons). The effect of these basis vectors on the active area is shown in Figs. 5.7C and D. The relationship between these two basis vectors and the active area of response is more complex than that of $\hat{\mathbf{p}}_1$ and $\hat{\mathbf{p}}_2$ for Strategy 1.a. The cluster representing $l_4 = \frac{1}{\lambda_4}$ in Figure 5.7C is noticeably non-convex, with two separate parts to the cluster. This is likely due to an interaction with $\hat{\mathbf{p}}_5$, since the rightmost cluster of yellow points in Figure 5.7C overlaps with that of Figure 5.7D, which represent $l_5 = \frac{1}{\lambda_5}$. It should also be noted that the overall active area is more tightly clustered at higher values than that for Strategy 1.a. No significant clusters were found for $\hat{\mathbf{p}}_3$ in either Strategy 1 ($p = 0.135$) or Strategy 2 ($p = 0.245$).

To efficiently characterise and compare the movement of the spatial activity profile within an array, 2D Gaussians were fitted to the observed responses to single electrode and inversion stimuli. Only Gaussians with significant coefficients of determination ($p < 0.05$, permutation test with $n = 100,000$), and centre location lying within the recording array dimensions were considered. Additionally, for single electrode stimulation only responses to stimulation above threshold were used. Change in the centre locations of the fitted Gaussian functions (horizontal and vertical components given by x_o and y_o , respectively, in Eq. 5.17) indicate the degree to which the response can be spatially shifted. Figure 5.8A shows the centre locations of the inversion stimuli (including strategies 1.a-c and 2.a-c) and single electrode stimuli (on all operational electrodes, at current amplitudes above threshold) overlaid on a schematic of the recording arrays.

On the rostral (top) array, the centre positions for responses to both single electrode and inversion stimuli were generally confined to an arc traversing the lateral half of the recording array. On the caudal (bottom) array, responses for the inversion stimuli were

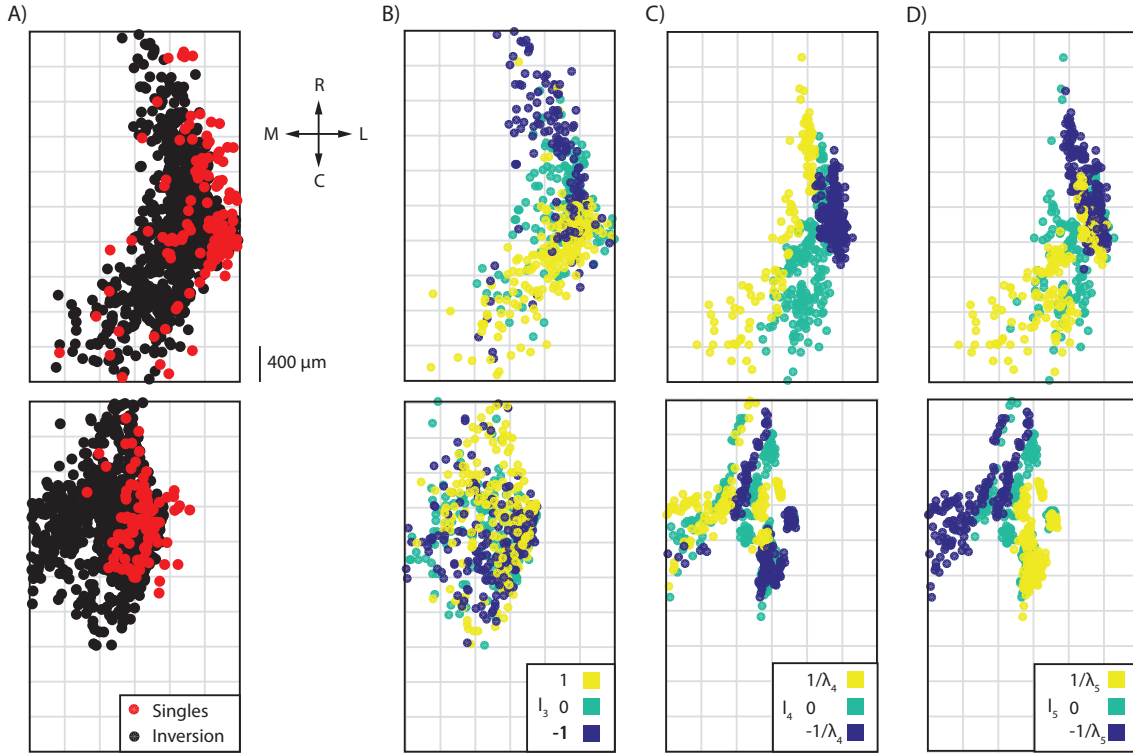


Figure 5.8: Centers of the Gaussians fitted to recorded responses, overlaid on a schematic of the recording arrays, where both recording arrays are shown; cortical directions and scale are shown to the right of panel A. R: rostral, L: lateral, C: caudal, M: medial. A) Response centres for both single electrode stimulation of all electrodes (42 electrodes, at current amplitudes above threshold, at $50\mu A$ steps, shown in red, $n = 133$ on top array, $n = 139$ on bottom array) and inversion stimuli using strategies 1.a-c and 2.a-c (well fitted patterns shown in black, $n = 1078$ on top array, $n = 1161$ on bottom array). B-D) Response centres for inversion stimuli, colour represents the scale of $\hat{p}_3$ (B), $\hat{p}_4$ (C) and $\hat{p}_5$ (D) in the generator signal associated with the stimuli. Panel B shows responses using strategies 1.a-c ($n = 167$ on top array, $n = 197$ on bottom array), while panels C and D both show strategies 2.a-c ($n = 234$ on top array, $n = 238$ on bottom array).

generally located in the rostro-medial quarter of the array, while those for single electrode stimulation were confined to a narrow strip in the rostral half of the array. On the rostral array the centres for single electrode and inversion stimuli spanned a similar area, though inversion stimuli responses were distributed evenly between separate clusters of single electrode responses. In contrast, on the caudal array, inversion stimuli elicited responses with a noticeable medial shift compared to single electrode responses.

To characterise the effects of particular eigenvectors on the centre position, panels B-D show subsets of the black markers from panel A, colour coded to represent the contribution of $\hat{\mathbf{p}}_3$, $\hat{\mathbf{p}}_4$, and $\hat{\mathbf{p}}_5$, respectively, to the generator signal associated with the stimulus (basis vectors 1 and 2 did not display clustering relating to the centre position and are therefore not shown). The markers in all panels show clustering according to colour, indicating that these three basis vectors affect the position of the response centre. The significance of the clusters was evaluated separately for the medio-lateral and rostro-caudal centre positions (x_0 and y_0 , respectively), as located on a plane described by the position on the rostral array (y axis) and caudal array (x axis), using the Davies-Bouldin index. This analysis showed that for Strategy 1, only $\hat{\mathbf{p}}_3$ was associated with any significant clustering for response position ($p = 0.009$) and then only for the rostro-caudal position (y_0). The effect of $\hat{\mathbf{p}}_3$ is shown in Figure 5.8B, where points with $l_3 = 1$ cause the response centre to shift more caudal on the rostral array and more rostral on the caudal array, while those with $l_3 = -1$ lead to the opposite. The analysis also showed that for Strategy 2, $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ were associated with significant clustering of both medio-lateral and rostro-caudal centre positions (medio-lateral: $p < 0.0001$ for both $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$; rostro-caudal: $p < 0.0001$ for $\hat{\mathbf{p}}_4$, $p = 0.013$ for $\hat{\mathbf{p}}_5$).

The effects of these basis vectors are shown in Figs. 5.8C and D, and Figure 5.9. Figure 5.8C shows that $\hat{\mathbf{p}}_4$ appears to shift the centre position either medially (when $l_4 = \frac{1}{\lambda_4}$) or laterally (when $l_4 = -\frac{1}{\lambda_4}$), while Figure 5.8D shows that $\hat{\mathbf{p}}_5$ shifts the response either rostrally (when $l_5 = \frac{1}{\lambda_5}$) or caudally (when $l_5 = -\frac{1}{\lambda_5}$). On the caudal array, $\hat{\mathbf{p}}_5$ acts to shift the response medio-laterally; however, the effect of P_4 is not as obvious. Overall, the effect on the centre position of $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ is clearly more complex than that of $\hat{\mathbf{p}}_3$, and also indicated that the two might interact. Therefore, Figure 5.9 shows the centre movement coloured to highlight the interaction between $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$.

Here, points are coloured according to the combination of $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ weightings (l_4 and l_5), and arranged such that each point represents the centre location of the response on the caudal array (x-axis) and rostral array (y-axis), as opposed to each array being shown separately as per Figure 5.8. It is apparent from the clear separate clusters in Figure 5.9A that the medio-lateral location of the response centre on both the rostral and caudal arrays can be controlled by combining $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ in different amounts. For example, when $l_4 = \frac{1}{\lambda_4}$ and $l_5 = \frac{-1}{\lambda_5}$, the response centre on the caudal array is shifted towards the medial edge of the recording array. The rostro-caudal centre position is also controllable (Figure 5.9B); however, the overlap between clusters indicates that different combinations $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ do not elicit unique vertical centre locations.

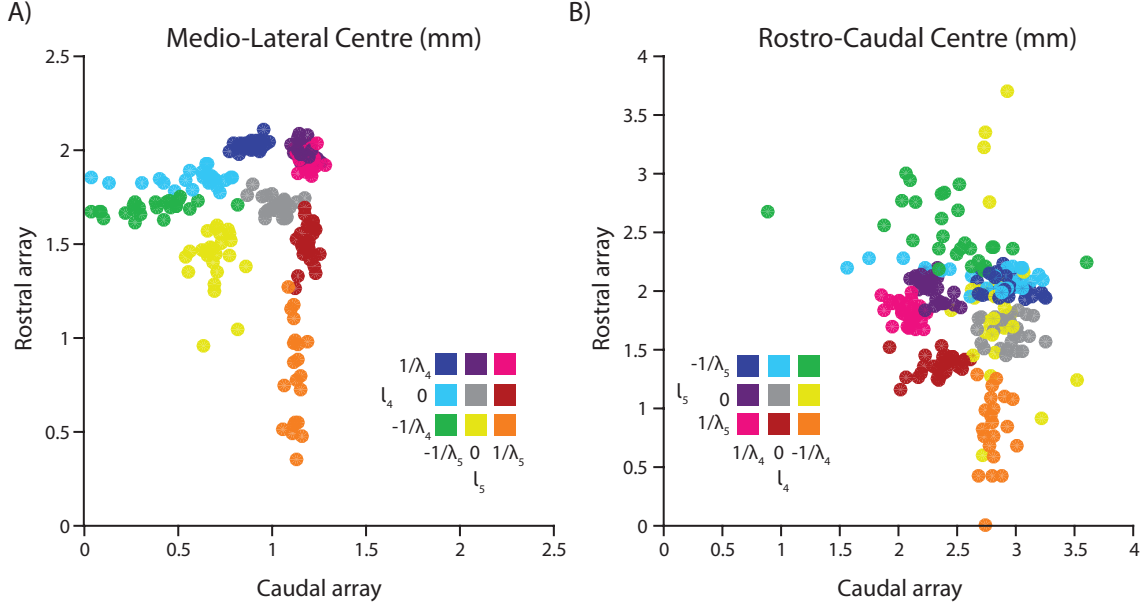


Figure 5.9: Horizontal (A) and vertical (B) shift of the Gaussian centers, as measured in mm from the medio-caudal corner of the lower (caudal) array and upper (rostral) array, shown on the x and y axes, respectively. Only inversion stimuli utilising Strategy 2.a for the normalised basis vector weightings are shown. Colours represent the combined weightings (l_4 and l_5) of the 4th and 5th eigenvectors ($\hat{p}_4$ and $\hat{p}_5$) used to generate the stimuli ($n = 234$ on both A and B).

5.5 Discussion

We have demonstrated that a linear-nonlinear model fitted to cortical responses to spatial white noise can be partially inverted to generate stimulus vectors that evoke predicted responses. Stimuli were created by sparsely sampling a reduced generator signal subspace, which represents the projection of the stimuli onto the electrical receptive fields. We showed that the basis vectors are associated with certain response patterns, and linear combinations of the vectors result in predictable changes in the spatial response profile. Furthermore, the evoked responses are able to be shifted beyond the area achievable with single electrode stimulation, as well as interpolating within it. Therefore, this method could increase the range of responses available from stimulation of the retinal prosthesis, presumably resulting in an expansion of the library of possible visual percepts attainable by patients.

5.5.1 Application of Partial Model Inversion to Activity Shaping

Activity shaping is generally applied in one of two ways: current focusing or current steering. The goal of current focusing is to stimulate regions of the tissue that are narrower than that evoked by traditional single-electrode stimulation (Cicione et al., 2012; Matteucci et al., 2013; Wong et al., 2009; Spencer et al., 2016), while the goal of current steering is to direct current to parts of the tissue between physical electrodes (Dumm et al., 2014; Jepson et al., 2014b). In the present study, we demonstrated a current steering method to shape cortical responses to multi-site electrical stimulation of the retina. A linear-nonlinear model was fitted to the cortical responses to multi-electrode retinal stimulation, and linear combinations of the basis vectors of the reduced generator signal space were transformed into stimulus patterns. We showed that this method can predictably and reliably alter the spatial profile of responses over 120 recording locations. The responses that were elicited by these inversion stimuli were well predicted by the linear-nonlinear model, although correlations between recorded and predicted responses were noticeably lower for the strategy involving no normalisation of basis vectors (Strategy 1). This is possibly related to the greater number of channels recording a significant response for the strategy involving normalisation of the basis vectors by their associated eigenvalues (Strategy 2) compared to Strategy 1 (shown by Figure 5.7). Channels that did not have a significant response were still compared in the r^2 analysis, and given that there is a lower signal-to-noise ratio at lower spike counts, this noise could have negatively affected correlation coefficients. For Strategy 2, patterns created from linear combinations involving $+\hat{\mathbf{p}}_5$ (particularly when combined with $\pm\hat{\mathbf{p}}_4$) had lower correlation coefficients, indicating that the responses to these patterns were more difficult to predict. This is possibly due to basis vectors with lower eigenvalues (i.e., $\hat{\mathbf{p}}_5$) being associated with a lower signal-to-noise ratio in their responses. However, the well-defined movement of observed response spatial profiles associated with all combinations of $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ suggests that the responses themselves are not heavily affected by noise, but rather the response predictions. Interestingly, the r^2 values seen here are similar to those shown for responses to patterned stimuli in Chapter 2. Given that the model has been shown to very accurately predict the responses to white noise stimuli, the slightly lower correlation coefficients for patterned and inversion stimuli indicate a limitation in the ability of the model to predict responses to non-white stimuli. This could possibly be mitigated by further optimisation of model parameters with non-white stimuli.

We characterised the spatial profiles of responses by fitting 2D Gaussian functions to the activity heat map recorded in response to each stimulus. This method provides a

measure of the centre of the evoked activity that isn't skewed towards the centre of the recording array, as is the case for a centre of mass measure. However, there are limitations inherent in the measure, such as the inability to characterise multiple response peaks on each array. Therefore, it is possible that the basis vectors trialled here had further impact on the spatial profile of responses that were not able to be accurately identified with this measure.

We showed that the general impact of each basis vector on responses could be inferred from examination of the vectors themselves. For example, $\hat{\mathbf{p}}_1$ and $\hat{\mathbf{p}}_2$ modulate the overall level of response on the caudal array and rostral array, respectively, which is reflected by the appearance and Fourier transforms of the basis patterns. This was similar for the remaining three basis vectors, which modulated the rostro-caudal centre position ($\hat{\mathbf{p}}_3$, $\hat{\mathbf{p}}_4$, and $\hat{\mathbf{p}}_5$) and subtle movements in the medio-lateral axis ($\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$). We found that $\hat{\mathbf{p}}_4$ and $\hat{\mathbf{p}}_5$ could also modulate the area of response on each array for Strategy 2. This shows that the impact of each basis vector was heavily dependent on the strategy used to combine them; vectors associated with large eigenvalues ($\hat{\mathbf{p}}_1$, $\hat{\mathbf{p}}_2$, and $\hat{\mathbf{p}}_3$) were most effective when not normalised by those eigenvalues (i.e., Strategy 1), but the opposite was true for vectors with smaller eigenvalues. It would be of interest in future studies to investigate other normalisation strategies that might better account for this inequality in representation, such as normalising by the square-root of the eigenvalue. However, our results clearly indicate that the spatial profile of responses can be predictably modulated by specific combinations of stimulating electrodes during multi-electrode stimulation. Additionally, we showed that the responses can be shifted to areas of the stimulating array beyond what is achievable with single electrode stimulation, thereby increasing the library of responses available to a retinal prosthesis user.

Mean subtraction was applied in order to avoid the degeneracy of the double-sided nonlinearity by sampling from one orthant of the subspace. However, the method by which this was achieved (subtracting the mean from the linear filter matrix $\mathbf{V}$) may not be the optimal approach, since a separate scaling process was then required to maximise the dynamic range of responses across the array (Eq. 5.15). Such scaling may skew the relative proportions of eigenvectors associated with a stimulus. Instead, we propose that the generator signal subspace sampling could be mapped about a different mean; e.g., a mean equal to the generator signal threshold for each recording channel. Further details on this proposed method, and a description of how it may be achieved, are discussed in Appendix A.

In the present study, only channels displaying a coefficient of determination between

the observed responses and the responses predicted by the linear-nonlinear model of at least 0.5 were used in the SVD analysis. This constraint was set to ensure that the linear filter for each channel was representative of the actual electrical receptive field. If this criterion were to be relaxed it is possible that the addition of poorly fitted channels to the linear filter matrix would increase the matrix rank artificially, resulting in basis vectors that are not representative of the observed responses. Alternatively, the level of 0.5 may be too conservative, and might be lower if it were to be based on a significance level. Therefore, in future studies it would be of interest to investigate the sensitivity of the results to this criterion, and whether a more considered approach to setting the cut-off may be more appropriate.

5.5.2 Comparison to Other Current Steering Studies

Current steering presents the opportunity to alter excitation patterns in response to electrical stimulation, and has been shown to be effective in a range of neural stimulation devices. Current steering has been used extensively in the cochlear implant, where one particular outcome has been the creation of ‘virtual electrodes’ intermediate between physical electrodes, by stimulating two or more electrodes simultaneously, to increase the number of effective channels (Brendel et al., 2009; Firszt et al., 2007; Landsberger and Srinivasan, 2009). Current steering has also been used in the cat sciatic nerve to selectively activate any of four motor fascicles using a four contact self-sizing spiral cuff electrode (Tarler and Mortimer, 2004). In these applications, however, it can be time-consuming to test all possible stimulation configurations, particularly for prostheses with large number of electrodes. Even with only four electrodes, time constraints precluded Tarler and Mortimer (2004) from attempting current steering in one third of their experiments.

This is a particularly limiting factor when considering the application of current steering in retinal prostheses, with their large number of electrodes and two dimensional arrangement. Dumm et al. (2014) demonstrated current steering between two electrodes by changing the ratio of current applied on either one. This resulted in a shift of the centre of evoked cortical activity. This shift is akin to that seen in the present results, where combinations of different eigenvectors resulted in repeatable shifts in centre position. A key aspect of the study by Dumm et al. (2014) was the ability to shift the centre position to a location between two physical electrodes, indicating that a wider range of phosphenes could be evoked using the method. Our work expands on this concept, showing that stimulation of multiple electrodes simultaneously can shift the response beyond the area achievable with single electrode stimulation, as well as interpolating within it. Our method

also excels in terms of efficiency. By using white noise stimulation across all electrodes on the array, we were able to quickly characterise the cortical response to multi-electrode stimulation in a way which is readily scaled, ameliorating time restrictions relating to the number of electrodes. In contrast, Dumm et al. (2014) observed unexplained differences between the few electrode combinations that were trialled, indicating that it might not be possible to extrapolate the results to other electrode and current amplitude combinations.

Jepson et al. (2014b) employed a similar approach to ours, showing that a piecewise linear model could predict the relative activation of a small number of target cells in the *in vitro* retina in response to simultaneous stimulation of three electrodes. By inverting this model, they were able to closely predict the optimal combination of three electrode currents for activation of a single cell without activating a nearby cell. While this work is encouraging since it indicates that subtle but specific changes can be induced in neural responses with current steering, responses were only recorded from the target cell and one other nearby cell at a time. Therefore, it was not possible to infer the impact of the stimulation on other cells nearby. Since we recorded from 120 channels in the cortex at once, we were able to better surmise this impact and demonstrated the scalable nature of our method. Additionally, activation of a single cell *in vitro* may not result in perceivable phosphenes, particularly if natural patterns of stimulation are not also mimicked in surrounding cells. Therefore, our method, where responses are recorded in the cortex (and are therefore more closely related to perception) may be superior in terms of translating our findings to clinical outcomes.

5.5.3 Considerations for Clinical Application

The objective of this study is to develop a simple model that can be used to generate optimal stimulation patterns, with the goal of improving outcomes for patients with retinal prostheses. An important question, therefore, is how the strategy described herein could be applied clinically. While the present method has several positive aspects, including generalisability to large numbers of electrodes and efficiency of approach, it also has the requirement that neural responses are recorded in response to stimulation in order to fit the linear-nonlinear model. As we have discussed previously (Subsection 3.5.2), the invasive nature of recording multi-unit activity means that it is not an ideal measure for clinical applications. However, our model could also be fitted to other neural response measures, including the power in the evoked potentials as shown in Chapter 3. Therefore, it may be possible to fit the linear-nonlinear model to evoked potentials recorded through less invasive means, such as scalp electrodes. We have shown here that such a model can be

partially inverted to generate stimulation patterns likely to produce a particular cortical response. However, the choice of a target cortical response is particularly nontrivial, given that cortical activity is not necessarily representative of perceptual outcome.

One possible application of the method we have presented here is to extend the library of phosphenes available with a retinal prosthesis beyond those evoked by single electrode stimulation. To do this, single electrode stimuli could be projected into the generator signal subspace. The target generator signals would then be intermediate between the locations of the single electrode stimuli generator signals, or areas that are not reached by single electrode stimulation but are shown to be possible with multi-electrode stimulation. Stimuli associated with these locations could then be presented to the patient, who could provide feedback on the perceptual result of the stimulus, as per previous clinical trials (Sinclair et al., 2016; de Balthasar et al., 2008; Nanduri et al., 2012; Zrenner et al., 2008; Wilke et al., 2011b). Stimuli that generate different or useful percepts could then be included in the library of available patterns.

5.5.4 Conclusion

Previous studies have shown that simultaneous stimulation of multiple electrodes is a promising strategy for increasing the resolution and range of responses of neural prostheses. This is the first study to demonstrate successful application of current steering using more than three stimulating electrodes in a retinal prosthesis. Using a linear-nonlinear model, we have shown that stimuli can be generated that will elicit a target response. Our method is also efficient and easily scalable to large numbers of electrodes. The method shows promise for improving perceptual efficacy of retinal implants.

Chapter 6

Conclusion

6.1 Summary

Clinical studies of retinal implants have reported that simultaneous stimulation of multiple electrodes evokes phosphenes more complex than when electrodes are sequentially stimulated (Zrenner et al., 2008; Rizzo et al., 2003b; Wilke et al., 2011b). This is due to interactions that occur between concurrently driven electrodes, which detrimentally affect spatial resolution. However, both *in vitro* and *in vivo* studies suggest that interactions between electrodes may be harnessed to increase resolution (Jepson et al., 2014b) or to generate activity that could not be evoked by single electrode stimulation (Dumm et al., 2014).

The large selection of possible electrode combinations and stimulation paradigms that arise when simultaneous stimulation is considered in an *ad-hoc* manner makes the application of such strategies intractable. The work presented here is motivated by the need to better understand the effects of electrode interactions on cortical responses and investigate whether those interactions can be harnessed to improve device efficacy. To this end, recordings of visual cortex responses to electrical stimulation of the retina were used to develop a quantitative model that could predict cortical responses to arbitrary stimulus patterns, thereby making multi-electrode stimulation tractable. The model was subsequently used to characterise responses to simultaneous stimulation of multiple electrodes in both normal and degenerate retina. Furthermore, the use of the model for generating optimal stimulation patterns to shape cortical activity was investigated, with a view to improving prosthetic vision.

In Chapter 3, a linear-nonlinear model fitted to cortical responses to white noise stimulation of suprachoroidal retinal electrodes was described. The recovered model accurately predicted cortical responses to both white and patterned stimulation. Importantly, the model was shown to be successful for both multi-unit activity and evoked potentials, suggesting that the model could also be used clinically with non-invasive cortical recording methods. In addition to predicting responses, the two main components of the model (the linear filters and static nonlinearities) provided important information about the properties of responses to multi-electrode stimulation. In particular, the linear filters showed the relative effects of each stimulating electrode on each cortical site and are, therefore, representative of electrical receptive fields (ERFs) for multi-electrode stimulation. These ERFs showed that the retinotopic organisation seen for single electrode stimulation of the retina is maintained with simultaneous stimulation of multiple electrodes.

While the results of Chapter 3 were influential in developing an understanding of cortical responses to multi-electrode stimulation of the healthy retina, further investigation was required in order to determine whether the degenerate retina would respond in a similar way. Previously, characteristics of responses to electrical stimulation in the normal and degenerate retina have been compared using *in vitro* and *in vivo* studies, but only with single electrode stimulation (Weitz et al., 2015; Aplin et al., 2016a; Chen et al., 2006; Lorach et al., 2015; Siu and Morley, 2008b). Results from clinical studies with blind individuals have been variable, with low predictability and dissimilarities of perceived phosphenes between studies (Freeman et al., 2011). The retina is known to undergo significant morphological and physiological changes with Retinitis Pigmentosa (Jones et al., 2016). Therefore, in Chapter 4, we used a similar stimulation paradigm to that used in Chapter 3 in unilaterally blind cats to explore the responses to multi-electrode stimulation in the degenerate retina and test the applicability of the model to long-term blind retina. A particularly interesting aspect of this study was the use of unilaterally blind, bilaterally implanted animals, enabling responses to stimulation of the normal retina and degenerate retina to be compared in the same animal. A number of differences were found, including shorter response latencies to multi-electrode stimulation of the degenerate retina than the normal retina, larger activation thresholds and decreased cortical spreads. Cortical responses from stimulation of both the normal and degenerate retinas could also be predicted accurately by the linear-nonlinear model. The application of the model allowed for ERF sizes of the normal and degenerate retina to be compared, showing that ERF sizes in the degenerate retina were larger than in the normal retina. This means that, in a degenerate retina, electrode interactions may be significant over a larger range of inter-electrode distances. Additionally, large ERF sizes may negatively impact the applicability

of current steering and focusing strategies (discussed further in Section 6.2.3).

Finally, in Chapter 5, the possibility of shaping cortical responses by using spatial patterns of current injection in the retina was investigated. In order to obtain a proof of concept, this experiment was conducted in a normal sighted animal. Particular components of activity that accounted for the majority of response variability to stimulation with white noise were isolated. By partially inverting the linear-nonlinear model developed in Chapter 3, stimulus patterns associated with linear combinations of these activity components were found. Cortical responses resulting from these stimulus patterns were well predicted by the linear-nonlinear model. In addition, responses could be reliably elicited in regions of the cortex that could not be accessed with single electrode stimulation. A particularly influential aspect of this study was that the spatial characteristics of the responses were tightly linked to key aspects of the stimuli identified via analysis of responses to white noise stimulation. This showed that cortical activity could be shaped with simultaneous multi-electrode stimulation of the retina and that current could be steered in a controllable manner by altering the stimuli.

To summarise, this thesis showed that not only are cortical responses to simultaneous stimulation of multiple electrodes repeatable and predictable, but that electrode interactions are predominantly linear in nature. Additionally, while the degenerate retina responds to multi-electrode stimulation in a number of significantly different ways compared to normal retina, responses could still be accurately predicted. The present work presents a method by which cortical activity can be shaped with current steering between multiple electrodes in the retina. The following section presents a number of aspects of the thesis that could be further developed in future studies or should be taken into consideration when considering clinical applications.

6.2 Future Research Directions

6.2.1 Spatiotemporal Linear-Nonlinear Model

The linear-nonlinear model we have presented is wholly spatial in nature. Since responses used to characterise the model were averaged over the 3-20 ms post-stimulus and consecutive biphasic pulses were presented at a rate of 1 Hz, it is unlikely that any temporal interactions occurred between pulses. As discussed in Chapter 3, it would be possible to extend the model to predict temporal dynamics of cortical responses to high-rate, repetitive stimulation by including a temporal dimension in the linear filters of the model.

This would be particularly interesting in light of the promising clinical applications of the present work since, in human trials, stimulus rates for retinal prostheses have ranged between 5 and 500 pulses per second (pps) (Stingl et al., 2015; Fornos et al., 2012; Ayton et al., 2014; Shivdasani et al., 2014). When temporal properties of perceptual responses have been specifically investigated, it has been reported that, at pulse rates of up to 20 pps, the temporal dynamics of percepts between subjects vary substantially, with many reporting fading over time (Fornos et al., 2012). Fading of percepts in relation to pulse rate has been reported epiretinally (Fornos et al., 2012), subretinally (Zrenner et al., 2011), and supra-choroidally (Sinclair et al., 2016). These findings show that understanding the temporal dynamics of responses is key to designing efficacious stimulation strategies. Therefore, it would be of interest to include a temporal dimension in the model in order to study temporal interactions, and fading of percepts.

Sekhar et al. (2016) recently showed that, at rates of stimulation that usually induce fading, the retina can be activated by consecutive, random amplitude, subthreshold stimulation pulses on a single electrode, presumably by integrating them over time. The authors suggest that such subthreshold stimulation could be a possible avenue for combating perceptual fading. However, the effect of high rate, sub-threshold, multi-electrode stimulation on cortical responses remains an open question. It would be of interest to reduce the standard deviation of the Gaussian white noise stimuli used in our method while increasing the stimulation rate to at least 20 Hz, such that a single stimulus pattern would not activate the cortex (i.e., using stimulus patterns below the multi-electrode threshold) but consecutive stimuli integrated over time would evoke activity. This would help to determine whether sub-threshold stimulation results in reduced cortical response fading.

In pursuit of a spatiotemporal linear-nonlinear model, the issue of the stimulation artefact that is present in recordings would have to be addressed, as discussed in Chapter 3. The same method of artefact removal we used in the present work (Heffer and Fallon, 2008) could be used for pulse rates of up to 50 Hz, which would be sufficient to further investigate the findings of Fornos et al. (2012) and Zrenner et al. (2011) in an *in vivo* model. Other reliable artefact suppression techniques would be required to handle artefacts when using higher stimulation rates and multiunit data.

Including a temporal dimension in the method of activity shaping presented in Chapter 5 would mean that the basis vectors describing the main modes of response would have both spatial and temporal dimensions. If the higher frequency stimulation has a significant effect on the temporal dynamics of the response, in addition to the existing effect of stimulation on the spatial characteristics of the response, then it would be expected that

a greater number of basis vectors would be required to account for 95% of the response variance. The method we used to sample the subspace was tractable with the use of five basis vectors; however, the addition of more basis vectors would exponentially increase the time required to sample the subspace. Therefore, if this were to be attempted, the method of subspace sampling would need to be adjusted. One option for this would be to remove stimuli involving a null amount of any basis vector. This would not be ideal, since it may preclude the discovery of interactions between components, but it would drastically reduce the number of possible combinations of basis vectors while still allowing for the effect of each basis vector to be investigated. For example, for five basis vectors, this would reduce the number of combinations in strategy 1.a from 242 to 32, or would allow eight basis vectors to be investigated in 256 stimulus patterns. Alternatively, the subspace could be sampled about a chosen operating point using Gaussian white noise, as suggested in Appendix A. This would remove the strict dependence between number of stimulus patterns and basis vectors. However, it could complicate the analysis of the relationship between spatial characteristics and basis vectors, and without a large number of stimulus patterns the subspace could be inadequately sampled.

6.2.2 Cortical Recording Area Limitation

A limiting aspect of the work presented in this thesis is the area of the cortex that could be recorded. In all of the studies presented herein, planar penetrating recording arrays with 6×6 or 6×10 electrodes were implanted into exposed portions of the visual cortex. In Chapters 3 and 5, combinations of one of two of these arrays were placed contralateral to the implanted eye. A single 6×10 electrode array spanned 4 mm in the rostral-caudal direction and 2.4 mm in the medio-lateral direction, corresponding to approximately $5^\circ \times 3^\circ$ of visual angle, using a cortical magnification factor of 0.8 mm/degree (Tusa et al., 1978). However, two stimulating electrodes located close to the area centralis and separated by 1 mm subtend a visual angle of approximately 4° (Hubel and Wiesel, 1959), which is comparable to the visual angle covered by the entire recording array. Therefore, it is unsurprising that recording arrays located in an area of cortex representing the area centralis don't show a large degree of movement between ERF centres.

It would be preferable in future experiments to record from a greater area of the cortex, possibly with wider-spaced recording electrodes. However, given that part of the retina stimulated by the electrode array may be represented on the medial sulcus of the cortex (Tusa et al., 1978), this may not be possible with planar recording arrays. An approach to mitigating this limitation is the simultaneous use of a planar recording ar-

ray located on the gyrus and a multi-shank probe with multiple recording sites on each shank (referred to as a Michigan array (Bai et al., 2000)) inserted down a sulcul bank. Alternatively, variable-depth penetrating electrodes in a microdrive system (e.g., 32-96 channel microdrives by Gray Matter Research, Montana, USA) could be used. By positioning such a system over the hemisphere contralateral to the implanted eye, electrodes could be inserted to variable depths in the cortex, such that the sulcus could be sampled along with the exposed portion of the cortex. Alternatively, flexible, high-density electrode arrays have been successful in mapping the visual cortex of cats (Viventi et al., 2011). Such arrays record micro-electrocorticographic signals, which can in some cases provide cortical activity information comparable to MUA (Andersen et al., 2004). The particular advantage of these arrays is that they can be inserted into the sulci of the brain with minimal tissue damage (Viventi et al., 2011). Since we have shown here that our model can be successfully characterised using both MUA and EP activity, it is likely that micro-electrocorticographic signals could also be used. Recording from a larger area of the cortex would result in a greater range of ERFs. In addition, with the ability to manipulate responses over a wider area of the cortex, it is likely that more basis vectors would account for response variance in the activity shaping approach of Chapter 5.

6.2.3 Impact of Retinal Degeneration on Current Steering Strategies

In Chapter 4, we investigated the cortical response differences between stimulation of healthy and degenerate retinæ. In particular, we found that ERFs from stimulation of a degenerate retina were significantly larger than those in a normal retina, with a trend towards increased ERF size with more extensive retinal degeneration. As discussed in Chapter 4, increased ERF sizes may affect the applicability of current steering strategies, particularly when strategies require discrete ERF peaks between electrodes. This would likely be the case for the current steering strategy suggested in Chapter 5. As noted in Chapter 5, the number of dimensions over which the generator signal subspace can be reliably varied is dependent on the rank of the linear filter matrix, rather than the dimensionality of the subspace itself. Since in that experiment many nearby cortical recording locations were mapped to a small region of the retina, the linear filter matrix was rank degenerate. We found ERFs were larger for degenerate retinæ, meaning that each cortical channel is affected by a larger number of electrodes. This smearing of the ERFs may result in a decrease in the effective rank of the linear filter matrix.

All else being equal, a reduction of the linear filter matrix effective rank would result in a reduction in the number of basis vectors accounting for 95% of the subspace variance

(this can also be considered as a ‘stretching’ of the subspace along the axes of the strongest eigenvectors relative to weaker eigenvectors). This would reduce the number of dimensions along which the response could be reliably varied in a subject with retinal degeneration compared to a normally sighted subject. Despite this limitation, the current steering strategy described here would still be preferable to strategies that don’t take into account response characteristics like electrical receptive fields, such as that suggested by Dumm et al. (2014). In the study by Dumm et al. (2014), cortical response centres were shifted by varying the ratio of current applied to two simultaneously stimulated electrodes. Given our findings, it is likely that cortical responses to the protocol used by Dumm et al. (2014) would be different in the presence of retinal degeneration. However, without laboriously trialling various combinations of electrodes, it would be difficult to ascertain the extent of those differences and whether the strategy could still be of use for improving the efficacy of retinal implants. In contrast, the strategy presented here would provide an understanding of the underlying response characteristics in the degenerate retina, as well as an avenue to harness electrode interactions.

6.2.4 Effect of Multi-Electrode Stimulation on Tissue and Electrode Safety

Each electrode material has a limit for charge injection, stimulation above which results in damage to the retinal tissue and stimulating electrode (Cogan, 2008). This damage can include a change in the pH, delamination of the electrode, generation of hydrogen and oxygen resulting in formation of bubbles and corrosion of the electrode (Cogan, 2008). Therefore, it is critical to the success of retinal prostheses that stimulation strategies are able to elicit perceptions within safe charge limits. The electrodes used in the studies presented in this thesis were made of platinum and stimulation phase widths were 1 ms. For this phase duration, the maximum non-gassing charge injection limit for platinum electrodes is 300-350 $\mu\text{C}/\text{cm}^2$ (Brummer and Turner, 1977). The maximum current amplitude used during any experiment herein was 750 μA , which, with the above listed electrode and waveform characteristics, gives a maximum charge injection of approximately 265 $\mu\text{C}/\text{cm}^2$. This is within the theoretical limit of 300-350 $\mu\text{C}/\text{cm}^2$ and charge densities similar to those used here have been shown to be safe for acute stimulation of the retina with single electrodes (Nakauchi et al., 2007). However, studies investigating chronic stimulation of retinal prostheses have rarely trialled charge injection levels this high. For example, using a retinal prosthesis implanted in the suprachoroidal space, with the same electrode size and material as is used here, Nayagam et al. (2014) showed that stimulation with charge densities

of up to $77 \mu\text{C}/\text{cm}^2$ did not result in tissue or electrode damage. However, since levels above this were not tested, no conclusions can be drawn about the safety of higher charge injection levels. Therefore, the paradigm presented here needs to be assessed chronically under clinically relevant conditions before multi-electrode stimulation can be applied in patients. This is suggested for future studies.

An additional consideration in the application of the stimulation strategies presented here is the capability of retinal implants to deliver the required power. Several aspects of a stimulation paradigm contribute to power requirements, including current amplitude, pulse duration, pulse rate, electrode impedance and stimulation pattern. Stimulating multiple electrodes at once with independent current sources requires a high degree of flexibility to be designed into the device, resulting in greater power consumption. Increased power consumption without accompanying efficiency gains leads to more heat dissipation from electronics and shorter battery life, both of which are undesirable in retinal prostheses. The present stimulation strategy is possible with power provided via a wired connection to an external battery source and external current stimulator. However, clinical application would first require an investigation into the capability of existing implants to deliver the required power.

6.3 Final Remarks

This thesis has shown that, rather than being a liability to the success of retinal prostheses, electrode interactions occurring between simultaneously stimulated electrodes can be harnessed to increase the range of achievable responses. While further work is required before the strategies presented herein can be applied clinically, it is the author's hope that the present work will provide an avenue for further improving the efficacy of retinal prostheses and, in some way, benefit those living with blindness.

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Appendix A

Supporting Material for Chapter 5

Inverting the Linear-Nonlinear model to find a suitable stimulus $\mathbf{s}_{\text{new}}$ given a target pattern of neural response, $\mathbf{r}_{\text{new}}$, is complicated by the non-monotone non-linearity in the model. This introduces a high degree of multiplicity of potential solutions stemming. After approximating that the linear filters $\mathbf{V}_P$ and $\mathbf{V}_N$ are sufficiently similar, and therefore that $\mathbf{V}_P = \mathbf{V}_N = \mathbf{V}$, the model states that

$$\mathbf{r} = g^P(\mathbf{V}\mathbf{s}) + g^N(\mathbf{V}\mathbf{s}). \quad (\text{A.1})$$

Where $\mathbf{s}$ is a stimulus vector ($M \times 1$), $\mathbf{V}$ is the matrix with electrical receptive fields (ERFs) as its rows ($K \times M$; see Eq. 5.5), g^P and g^N are the sigmoidal non-linearities and $\hat{\mathbf{r}}$ is the vector of neural responses ($K \times 1$). The generator signal, $\mathbf{x} = \mathbf{V}\mathbf{s}$, is also a K dimensional vector, and the non-linearities act on each element of $\mathbf{x}$ individually. Alternatively, the non-linearities can be considered together as a “double-sided” non-linearity $g(\mathbf{x})$, where

$$g(\mathbf{x}) = \begin{cases} g^P & \forall x > 0 \\ g^N & \forall x < 0 \end{cases}. \quad (\text{A.2})$$

This reflects the fact that both positive and negative generator signals lead to elevated responses compared to a generator signal of zero. Therefore, for a single channel i , where $i \leq K$, A.1 can be written

$$r_i = g_i(\mathbf{v}_i\mathbf{s}). \quad (\text{A.3})$$

Thus for any given target response on a cortical channel, r_i , there are typically two choices (positive or negative) or the corresponding generator potential; over all K channels there

are 2^K choices. Comparing all of these options to determine optimality is extremely computationally challenging for even modestly large K . Instead we arbitrarily make one choice, that x_i is positive for all $i \leq K$. This choice then makes the problem tractable. Assume a target pattern of activity, r_{new} , that lies within the dynamic range of the positive orthant, i.e.,

$$r_{\min,i} \leq r \leq r_{\max,i}. \quad (\text{A.4})$$

Here, $r_{\min,i}$ is the minimum value of $g_i(x_i)$ such that $x_i > 0$, and $r_{\max,i}$ is the maximum value of $g_i(x_i)$ such that $x_i > 0$. These typically occur when $x_i = 0$ and x_i is very large, respectively. Then the non-linearity on the positive orthant can be inverted to get

$$x_{\text{new},i} = g_i^{-1}(r_{\text{new},i}). \quad (\text{A.5})$$

In principle the stimulus required to generate the activity pattern r_{new} can be found by applying the pseudo inverse of $\mathbf{V}$,

$$\mathbf{s}_{\text{new}} = \mathbf{V}^+ x_{\text{new},i} = \mathbf{V}^+ g_i^{-1}(r_{\text{new},i}). \quad (\text{A.6})$$

However in practice many choices of r_{new} may not be attainable because the matrix $\mathbf{V}$ is effectively degenerate, having neither full row or column rank. This means that the pseudo inverse of $\mathbf{V}$ is ill-conditioned. To make the range of possible values of $r_{\text{new},i}$ more explicit we define an operating point $\bar{x}_i > 0$, about which we will explore to create stimuli. In order to maximise the dynamic range about this operating point, $\bar{x}_i$ is chosen to be the inflection point (i.e. the threshold) of the sigmoidal non-linearity g_i which is positive:

$$x_i : g_i''(\bar{x}_i) = 0, \quad \bar{x}_i > 0. \quad (\text{A.7})$$

A corresponding stimulus $\bar{\mathbf{s}}$ is defined such that $\bar{x}_i = \mathbf{v}_i \bar{\mathbf{s}}$. The perturbation Δx_i about the operating point $\bar{x}_i$ is then

$$\Delta x_i = x_i - \bar{x}_i \equiv \mathbf{v}_i \Delta \mathbf{s}. \quad (\text{A.8})$$

To explore the range of Δx in a full and unbiased fashion, we assume $\Delta \mathbf{s}$ is distributed according to Gaussian white noise. The covariance matrix for Δx is then given by

$$\mathbf{C} = \Delta \mathbf{X} \Delta \mathbf{X}^{\text{tr}} = \mathbf{V} \mathbf{V}^{\text{tr}} \quad (\text{A.9})$$

As per Equations 5.9 and 5.10, we perform singular value decomposition of this matrix, to find the directions in generator signal space of maximum variance, above some designated threshold (such as those that account for 95% of the variance, as used in Chapter 5). These are retained in the matrix of eigenvectors

$$\hat{\mathbf{P}} = [\hat{\mathbf{p}}_1 \cdots \hat{\mathbf{p}}_L] \quad (\text{A.10})$$

For some number $L \leq K$.