

Preclinical Investigation of Electrical Field Shaping Strategies for Retinal Prostheses

Thomas Charles Spencer

December 2018

Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

Department of Medical Bionics
Faculty of Medicine, Dentistry & Health Sciences
University of Melbourne

Dedicated to the memory of my grandad, Rowland,
who was a constant source of inspiration to me

and

also to the memory of my friend, Jeremy Cheang,
a young scientist who never got the opportunities I did.

Declaration

I declare that:

1. This thesis comprises only my original work towards the degree of Doctor of Philosophy except where indicated in the preface.
2. Due acknowledgement has been made in the text to all other material used.
3. This thesis is fewer than the maximum word limit in length, exclusive of tables, maps, bibliographies and appendices.

Thomas Charles Spencer

Abstract

Retinal prostheses are a promising technology aimed at restoring vision in people suffering severe retinal degenerative conditions such as retinitis pigmentosa (RP). Present-generation devices achieve this by electrically stimulating the residual neuronal population in the retina following degeneration in order to elicit the perception of light. At present, patients implanted with these devices are able to perceive multiple localised flashes of light, termed phosphenes, which are used to build up an artificial image of the patient's surroundings. However, present-generation retinal implants lack the spatial resolution to provide a suitable replacement for everyday visual tasks. While adequate for rudimentary tasks such as object recognition, motion detection, and pattern recognition, more complex tasks such as reading, facial recognition, and independent navigation are still not possible with modern prosthetic vision devices. Two major issues that affect retinal prostheses are: the large spread of electrical potential in the retina resulting in widespread activation of neurons, undesirable electrical field interaction and the elicitation of large phosphenes that patients find difficult to discriminate between; and that many devices are not able to elicit enough phosphenes to convey complex visual information to patients.

The studies presented in this thesis investigated the effectiveness of two multichannel electrical field shaping techniques: focused multipolar (FMP) and virtual electrode (VE) current steering. These techniques have shown considerable promise in studies conducted with one-dimensional neural prosthetic devices, such as cochlear and deep brain implants, as ways to restrict and 'steer' electrical fields. In an effort to find new ways of improving spatial resolution, I have investigated whether these techniques can be adapted for use in a 2D retinal prosthesis. Using a normally-sighted cat model I have demonstrated that FMP stimulation is capable of restricting current spread in two dimensions and eliciting retinal and cortical response patterns with reduced spread compared with responses to the more conventional monopolar (MP) means of stimulation. I have also demonstrated that VE current steering between up to six electrodes can produce cortical activation patterns in predictable locations, with similar spread of neural activation as response patterns to physical electrode (PE) stimulation. By varying the proportions of charge applied to steering electrodes, it was also possible to shift the location of cortical activation in two dimensions in a predictable and intuitive fashion. To investigate the effectiveness of these techniques in a model more representative of patients, FMP stimulation and VE current steering were re-evaluated using a cat model of retinal degeneration. Unfortunately, many of the promising results from the normally-sighted cohort were

not maintained when applied to degenerate retinae. While FMP stimulation still activated a localised population of retinal neurons, it was not found to elicit cortical response patterns with reduced spread compared to MP stimulation. The location of cortical response patterns elicited by VE stimulation were also found to be unpredictable. These results also show evidence of compressed retinotopy and increased spatial selectivity in the degenerate visual system, which significantly altered neural responses to electrical stimulation. These findings demonstrate that FMP stimulation and VE current steering, in their present form, may not be as effective in focusing and steering neural activation when applied to degenerate retinae. These results also provide a greater understanding of the differences between the responses of healthy and degenerate visual systems to electrical stimulation, which I hope will inform the further development and optimisation of these stimulation strategies.

Preface

This thesis contains published works with multiple authors including Dr. Mohit Shivdasani, A/Prof James Fallon, and Mr. Patrick Thien. These journal articles were first-authored by myself and are included in this thesis as Chapters 3 and 4 respectively. Minor alterations have been made to content reflect recent developments in the field since publication and to standardise terminology and figure layout throughout the thesis. A subset of the results of Chapter 5 have also been published as full conference paper.

My responsibilities and contributions to this work can be categorised into three distinct areas. During the experimental phase of my work I was responsible for experimental design and planning; the development of software and hardware tools to aid in the delivery of multichannel electrical stimulation, performing transimpedance measurements, and in data collection; animal surgery and anaesthesia monitoring; the implantation and setup of electrophysiological recording apparatus; training overnight staff; the preparation of adenosine triphosphate (ATP) solutions; and aided in the assessment of retinal degeneration. During the analysis phase I developed tools and techniques to process the raw electrophysiological data; performed photomicrography of the retinal histological preparations; and designed image processing tools to aid in cell counting and analysis. As part of the write-up phase, I produced, with input from my supervisors and co-authors, two peer-reviewed journal articles and presentations international and local conferences. I also created a number of illustrations and diagrams displayed in these publications and in my literature review and methods chapters.

This work presented in this thesis was also carried out with valuable assistance from the following individuals:

Dr. Mohit Shivdasani contributed to the design of the studies presented, aided in carrying out electrophysiological experiments, provided intellectual input in to analysis of data and editing of papers.

A/Prof. James Fallon contributed to the design of the studies presented, and provided intellectual input in to analysis of data and editing of papers.

A/Prof. Chi Luu provided assistance by implanting the stimulating electrode arrays, performed intravitreal ATP injections, and aided in the assessment of retinal degeneration.

Dr. Carla Abbott and **Ms. Alice Brandli** provided assistance by performing OCT and fundus imaging of the eyes of unilaterally blinded cats, performing dark-adapted ERG measurements, and measuring and assessing the degree of retinal layer degeneration.

Dr. Penny Allen provided assistance by implanting the stimulating electrode arrays.

Ms. Ceara McGowen aided in animal care, provided assistance during electrophysiological experiments, and performed histological preparation of retinae presented in Chapter 5.

Ms. Stephanie Epp aided in animal care and provided assistance during blinding procedures and electrophysiological experiments.

Mr. Patrick Thien aided in animal care, provided assistance during electrophysiological experiments, and developed software enabling automatic transimpedance recording.

Mr. Owen Burns, **Ms. Vanessa Maxim**, **Ms. Helen Feng**, and **Ms. Jenny Zhou** aided in the fabrication of electrode arrays used in experiments.

Mr. Ali Alamas, **Dr. Felix Aplin**, **Ms. Lisa Cardamone**, **Dr. Kerry Halupka**, **Ms. Faith Lamont**, **Ms. Michelle Mcphedran**, **Ms. Alison Neil**, **Ms. Alexia Saunders** and **Ms. Dimitra Stathopoulos** also aided in animal care and data collection.

Publications

Peer-reviewed journal articles

Spencer, T. C., Fallon, J. B. & Shivdasani, M. N. (2018). Creating virtual electrodes with 2D current steering. *Journal of Neural Engineering*. 15(3), 35002.

Spencer, T. C., Fallon, J. B., Thien, P. C. & Shivdasani, M. N. (2016). Spatial restriction of neural activation using focused multipolar stimulation with a retinal prosthesis. *Investigative Ophthalmology & Visual Science*, 57(7), 3181-3191.

Peer-reviewed conference articles

Spencer, T.C., Fallon, J.B., Abbott, C.J., Allen, P.J., Brandli, A, Luu, C.D., Epp, S., Shivdasani, M.N. (2018), Electrical field shaping techniques in a feline model of retinal degeneration. *40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Honolulu, HI, USA*

Selected conference abstracts

Spencer, T. C., Fallon, J. B., Abbott, C. J., Allen, P. J., Brandli, A., Luu, C. D. & Shivdasani, M. N. (2017). Reducing spread of neural activation using focused multipolar stimulation in sighted and blinded cats. Annual Meeting of the Association for Research in Vision and Ophthalmology, Baltimore, MD, USA. (Poster presentation)

Spencer, T. C., Fallon, J. B., Abbott, C. J., Allen, P. J., Brandli, A., Luu, C. D. & Shivdasani, M. N. (2017). Restricting neural activation using focused multipolar electrical stimulation in a cat model of retinal degeneration. 4th International Conference on Medical Bionics, Brisbane, Australia. (Oral presentation)

Spencer, T. C., Fallon, J. B., Thien, P. C. & Shivdasani, M. N. (2015). Restricting spread of neural activation in the retina using focused multipolar stimulation. International Symposium on Visual Prosthetics, Aachen, Germany. (Oral presentation)

Spencer, T. C., Fallon, J. B., Thien, P. C. & Shivdasani, M. N. (2015). Can focused multipolar stimulation restrict the spread of neural activation elicited by retinal prostheses? Australian Biomedical Engineering Conference, Melbourne, Australia. (Oral presentation)

Acknowledgements

I would like to express my greatest thanks to my two supervisors: Dr. Mohit Shivdasani and A/Prof James Fallon for their continuous support and mentorship throughout my PhD studies. I would also like to thank the members of my advisory panel: E/Prof. Dexter Irvine and Dr. Hamish Meffin, for their help and advice.

I would like to thank the late Mr. Bart Reardon and the Harold Mitchell Foundation for bestowing me with the financial means to carry out my studies and attend international conferences.

I would also like to thank my fellow PhD prisoners: Joy, Sam, Madi, Shefin, Patrick, Shivy, Kerry, Nick, and Tim, for their support and solidarity. And thanks also to the other friends I've made along the way at the Bionics Institute, especially Ella, Jelena, Caitlin, Angus, and Emma.

Finally, I would like to thank my family, for their unwavering support in all I do.

Contents

List of Figures	xii
List of Abbreviations	xvi
1 Review of Literature	2
1.1 Overview of the visual system	3
1.1.1 Anatomy of the eye	3
1.1.2 Phototransduction	4
1.1.3 Visual pathway	6
1.2 Retinal degeneration	8
1.2.1 Preclinical models of retinal degeneration	10
1.3 Visual prostheses	11
1.3.1 History of visual prostheses	11
1.3.2 Progress in retinal prostheses	15
1.3.3 Resolution of artificial vision	16
1.4 Electrical field shaping	19
1.4.1 Contemporary stimulation strategies	19
1.4.2 Multipolar stimulation strategies	21
1.4.3 Field shaping with simultaneous stimulation	22
1.5 Research objectives	29
1.5.1 Hypotheses	29
1.5.2 Thesis structure	30
2 General Methods	31
2.1 Blinding procedure	32
2.2 Anaesthetic protocols	33
2.2.1 Anaesthetic protocol for normally-sighted cohort	33
2.2.2 Anaesthetic protocol for unilaterally-blinded cohort	33
2.3 Surgical procedures	34
2.3.1 Suprachoroidal implantation	34

2.3.2	Cortical implantation	35
2.4	Electrophysiological Analysis	36
2.4.1	Retinal stimulation	36
2.4.2	Adapted FMP methodology	37
2.4.3	Cortical recording	38
2.5	Histology	39
3	Spatial restriction of neural activation using focused multipolar stimulation	42
3.1	Introduction	43
3.2	Methods	46
3.2.1	Anaesthesia and surgery	46
3.2.2	Experimental protocols	47
3.2.3	Retinal voltage spread	49
3.2.4	Cortical data analysis	51
3.2.5	Retinal and cortical selectivity	52
3.3	Results	53
3.3.1	Retinal voltage spread	53
3.3.2	Cortical thresholds	54
3.3.3	Retinal selectivity	56
3.3.4	Cortical selectivity	56
3.4	Discussion	59
3.4.1	Retinal voltage spread	59
3.4.2	Cortical thresholds	62
3.4.3	Retinal and cortical selectivity	63
3.4.4	Future studies	65
3.4.5	Conclusion	66
3.5	Acknowledgements	66
4	Creating virtual electrodes with two-dimensional current steering	67
4.1	Introduction	68
4.2	Methods	70
4.2.1	Anaesthesia and surgery	70
4.2.2	Experimental protocols	72
4.2.3	Data analysis	74
4.3	Results	78
4.3.1	Differences between anaesthetic protocols	78
4.3.2	Reproducing responses to physical electrodes	78

4.3.3	Predicting location of intermediate electrodes	82
4.4	Discussion	85
4.4.1	Reproducing responses to physical electrodes	85
4.4.2	Predicting location of intermediate electrodes	86
4.4.3	Implications and future directions	87
4.4.4	Limitations	89
4.4.5	Conclusion	90
4.5	Acknowledgements	91
5	Electrical field shaping techniques in a model of retinal degeneration	92
5.1	Introduction	93
5.2	Methods	95
5.2.1	Blinding procedure	97
5.2.2	Anaesthesia and surgery	97
5.2.3	Electrical stimulation	99
5.2.4	FMP stimulation and analysis	100
5.2.5	Virtual electrode stimulation and analysis	101
5.2.6	Histological analysis	102
5.3	Results	103
5.3.1	Retinal degeneration and remodelling	103
5.3.2	Responses to focused multipolar stimulation	117
5.3.3	Responses to virtual electrode current steering	119
5.4	Discussion	126
5.4.1	Anatomical retinal changes following degeneration	126
5.4.2	Responses to electrical stimulation of the degenerate retinae .	127
5.4.3	Responses to FMP stimulation	128
5.4.4	Responses to VE current steering	130
5.4.5	Limitations	131
5.4.6	Implications for retinal prostheses	133
5.4.7	Conclusion	134
6	General discussion	136
6.1	Future directions	137
6.1.1	Alterations to FMP stimulation	138
6.1.2	VE current steering	141
6.1.3	Implications of retinal degeneration	143
6.1.4	Device design	144

6.2	Final conclusion	146
7	Bibliography	147
	Appendices	169
A	Transimpedance measurements	170

List of Figures

1.1	Diagram showing a medial transection of the optic globe, showing the main structural elements of the eye. This is an original illustration using Purves et al. (2004) as a reference.	4
1.2	Diagram showing the different cell types and layers of the retina and their arrangement. This is an original illustration using Kolb (2003) as a reference point.	5
1.3	Schematic diagram of the visual pathway from the eyes to the primary visual cortex. Orange fibres relate to the right visual field, turquoise fibres relate to the left visual field. This is an original illustration using Purves et al. (2004) as reference points.	7
1.4	Immuohistological images of healthy and degenerated human retinae.	9
1.5	Images of various visual prostheses that have been used by patients .	12
1.6	World map showing the locations of the most relevant visual prostheses research teams currently operating.	14
1.7	Patient drawn phosphene shapes	17
1.8	Schematic of current focusing strategies	24
1.9	Schematic of current steering strategies	26
1.10	Preliminary psychophysics current steering data	27
2.1	Photograph of a preclinical 44-channel suprachoroidal electrode array	34
2.2	Images illustrating positioning of stimulating electrode array	35
2.3	Diagram of stimulation hardware	36
2.4	Images showing application of dye to the sclera	39
2.5	Histological section of the full wall of the eye showing how the coloured dye applied to the sclera is used to indicate electrode location.	40
3.1	Illustration of electrode stimuli weights for MP, hexapolar (HP), and FMP stimulation techniques.	47
3.2	Examples of electrically evoked multiunit activity on a single cortical recording channel.	50

3.3	Example of voltages recorded on the suprachoroidal array in response to MP and FMP stimulation	51
3.4	Average voltage of electrodes directly adjacent to each primary stimulating electrode (PSE) during MP and FMP stimulation. Average surrounding voltages were significantly lower for FMP stimulation when compared with MP stimulation (paired t-test $p < 0.001$). Error bars show standard error of the mean.	54
3.5	Input-output functions and average cortical thresholds for MP, HP and FMP stimulation	55
3.6	Examples of retinal selectivity maps for a single cortical channel for MP, HP and FMP stimulation	57
3.7	Retinal selectivity plots for MP, HP and FMP stimulation.	58
3.8	Examples of cortical selectivity maps for a single retinal electrode for MP, HP and FMP stimulation	60
3.9	Cortical selectivity plots for MP, HP and FMP stimulation.	61
4.1	Illustration of different combinations of electrode shapes and steering configurations.	73
4.2	Diagram illustrating our method of predicting the direction of centroid shift.	76
4.3	Input-output functions of a single cortical channel in response to different steering configurations	79
4.4	Examples of cortical spatial maps constructed in response to each steering configuration.	80
4.5	Bar chart showing the average centroid shifts for each steering configuration.	81
4.6	Polar probability density histogram showing error from predicted centroid shift angle between central physical electrode (CPE)-outer physical electrode (OPE) stimulation.	82
4.7	Example of centroid shift in the cortex and the methodology of directional prediction.	83
4.8	Polar probability density histograms showing error from predicted centroid shift angle between central virtual electrode (CVE)-intermediate virtual electrode (IVE) stimulation.	84
5.1	Schematic of current focusing strategies	95
5.2	Schematic of VE current steering modes	96

5.3	Electroretinograms (ERGs) of each subject before and after retinal degeneration	104
5.4	Example fundus images of degenerate retinae	105
5.5	Example OCT images of control and degenerated retinae	106
5.6	Example histology of the degenerate retina of one cat	107
5.7	Bar chart showing % reduction of health indicators of the degenerate retinae.	108
5.8	Example infrared images of implanted control and degenerate retinae	109
5.9	Scatter plots showing the relationship between ONL and INL cell densities at each electrode site	111
5.10	Scatter plots showing the relationship between ONL and INL cell densities and neural threshold	112
5.11	Bar chart of average cortical thresholds for MP and FMP stimulation of the control and degenerate retinae.	113
5.12	Scatter plots showing the relationship between ONL and INL cell densities and neural threshold	114
5.13	Retinal selectivity plots for MP and FMP stimulation of the control and degenerate retinae	115
5.14	Cortical selectivity plots for MP and FMP stimulation of the control and degenerate retinae	116
5.15	Retinal and cortical selectivity plots for MP stimulation of the control and degenerate retinae	118
5.16	Bar chart of the average P50 charge levels for for PE and VE stimulation of the control and degenerate retinae.	120
5.17	Bar chart of the average cortical selectivity values ($1/\tau$) for for PE and VE stimulation of the control and degenerate retinae.	121
5.18	Example cortical spatial maps of P90 responses to CPE, hexagonal CVE, and OPE stimulation of the same electrode shape.	123
5.19	Bar graphs showing the average distance between the centroid of activation calculated for stimulation of the CPE, and centroids calculated for CPE, CVE, IVE and OPE stimulation.	124
5.20	Bar graph showing the average distance between centroids calculated for responses to independently stimulated adjacent electrodes in the control and degenerate retinae.	125
5.21	Bar graph showing the average distance between centroids calculated for responses to CVE stimulation, and centroids calculated for stimulation of the OPE with the highest and lowest P50 thresholds. . . .	125

A.1	Example of a transimpedance matrix that produced usable weights. .	171
A.2	Example of a transimpedance matrix that did not produce usable weights.	172
A.3	Example of how a transimpedance matrix could be modified to pro- duce usable weights	173
A.4	Example of a transimpedance matrix that did not produce usable weights, even with modification.	174

List of Abbreviations

d' discrimination index.

AMD age-related macular degeneration.

ANOVA analysis of variance.

ATP adenosine triphosphate.

BCE best cortical electrode.

BP bipolar.

BRE best retinal electrode.

BVA Bionic Vision Australia.

CERA Centre for Eye Research Australia.

CPE central physical electrode.

CVE central virtual electrode.

DBS deep brain stimulation.

EP evoked potential.

ERG electroretinogram.

FMP focused multipolar.

GABA gamma-aminobutyric acid.

GCL ganglion cell layer.

GLMM generalised linear mixed model.

H&E hematoxylin and eosin.

HP hexapolar.

i.m. intramuscular.

i.v. intravenous.

INL inner nuclear layer.

IO input-output.

IVE intermediate virtual electrode.

LGN lateral geniculate nucleus.

MP monopolar.

OCT optical coherence tomography.

ONL outer nuclear layer.

OPE outer physical electrode.

OR outer retinal.

P50 50% of the maximum saturating spike rate.

P90 90% of the maximum saturating spike rate.

PE physical electrode.

PSE primary stimulating electrode.

RGC retinal ganglion cell.

RMS root mean square.

RP retinitis pigmentosa.

RPE retinal pigment epithelium.

s.c. subcutaneous.

VC visual cortex.

VE virtual electrode.

Chapter 1

Review of Literature

Retinal prostheses are a promising early-stage technology aimed at restoring vision in people suffering from blindness caused by photoreceptor loss. Present-generation devices achieve this by electrically stimulating the remaining neuronal population in the retina to elicit the perception of localised flashes of light, termed phosphenes, which are used to build up an artificial image of the patient's surroundings. However, the spatial resolution attainable with the most advanced retinal implants are still severely limiting for implant recipients and are not yet able to provide a suitable replacement for everyday visual tasks. While adequate for rudimentary tasks such as object recognition, motion detection, and pattern recognition, more complex tasks such as reading, facial recognition, and independent navigation are still not possible with modern prosthetic vision devices.

Major limiting factors in improving resolution are the size and number of phosphenes that present-day implants are capable of eliciting. Clinically-used stimulation techniques result in a large spread of activation within the retina, resulting in large overlapping phosphenes that patients have difficulty discriminating between. Additionally, a single electrode can elicit a maximum of one phosphene, making the number of phosphenes directly proportional to the number of electrodes on the array, which is in turn constrained by surgical and engineering limitations.

This thesis presents a number of studies investigating two novel electrical field shaping techniques using simultaneous stimulation of multiple electrodes, focused multipolar (FMP) stimulation and virtual electrode (VE) current steering, in normally-sighted and unilaterally-blinded animal models. Cortical responses to these techniques were characterised and compared with traditional stimulation paradigms in order to determine their effectiveness in reducing the spread of neural activation and

eliciting additional phosphenes.

1.1 Overview of the visual system

1.1.1 Anatomy of the eye

The wall of the eye is made up of three distinct layers: the sclera, the main structural element which maintains the shape of the eye; the choroid, a highly vascularised layer comprised mainly of connective tissue; and the retina, the sensory portion of the eye where light is transduced to physiological signals (Purves et al. 2004). The front of the eye is host to a number of accessory structures that help direct and focus light on to the retina. Light enters through the pupil and is refracted through the cornea and lens before passing in to the vitreous cavity, which is filled with a transparent gel called vitreous humour (Purves et al. 2004). The pupil's diameter can be altered to control the amount of light that can enter the eye by contraction of the iris sphincter muscle and the iris dilator muscle, which constrict and dilate the pupil respectively (Purves et al. 2004). The shape of the lens can also be modified to be more or less convex in order to alter the focal distance. This process, known as accommodation, allows for sharper images of objects at varying distances. To achieve this, the lens' shape is dictated by the state of contraction of a surrounding ring of smooth muscle known as ciliary muscle, which is controlled by the autonomic nervous system (Purves et al. 2004). The location of these structures in relation to each other is shown in Figure 1.1.

The retina is a relatively thin piece of layered neural tissue that lines the inner surface of the eye. It contains the light-sensitive photoreceptor cells and also performs the first stage of visual processing (Kolb 2003). The structure of the retina is contrary to what one may expect, as the photoreceptors are located at the very back of the retina, requiring light to pass through the other layers before being received (Kolb 2003). This is required as the photoreceptors receive the molecule *retinal* from the pigment epithelial layer, which in turn requires contact with the choroid for nourishment (Kolb 2003). Photoreceptor cells are at their most dense in a small pit directly behind the lens, known as the fovea centralis. This region is where the light from the lens is primarily focused and is responsible for sharp central vision (Kolb 2003). Approximately half of the optic nerve fibres synapse in the fovea. The rest of the retina is less densely populated and is responsible for peripheral vision (Kolb 2003). The retina can be separated in to multiple layers, as shown in Figure 1.2.

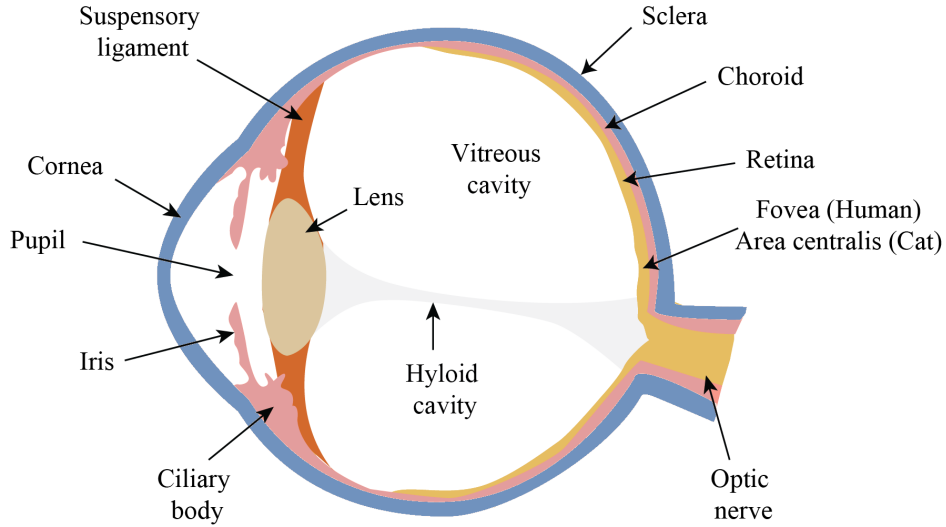


Figure 1.1: Diagram showing a medial transection of the optic globe, showing the main structural elements of the eye. This is an original illustration using Purves et al. (2004) as a reference.

1.1.2 Phototransduction

Phototransduction is the process of converting light energy into physiological signals (Purves et al. 2004, Kolb 2003). This begins in the rod and cone cells, which respectively contain the pigments *rhodopsin* and *photopsin*. These cells are important for the perception of light of different intensities. Rods are extremely light-sensitive and are responsible for low-light achromatic vision, whereas cone cells are specialised for normal or bright daylight vision. There are 3 types of cone cells which are more sensitive to long, middle, and short wavelengths of light in the visible spectrum (Wald 1964). An important constituent of both *rhodopsin* and *photopsin* is the molecule *retinal* (*1-cis-retinal*), or vitamin A. In the presence of light, *retinal* isomerizes and changes its conformation to become *retinol* (*all-trans-retinol*) (Kolb 2003). This new state activates their associated pigment and promotes their binding to the G-protein *transducin* which in turn activates a secondary cascade (Kolb 2003). In their inactive state, photoreceptors are depolarized and release the neurotransmitter glutamate. The light-induced cascade described above results in the closure of membrane ion channels, hyperpolarizing the cell and stopping the release of glutamate. The cessation of glutamate secretion by the photoreceptors causes depolarisation and activation of ON rod bipolar (BP) and ON cone BP cells and hyperpolarisation of OFF cone BP cells, which are the beginnings of the ON-OFF parallel pathways that continue to the higher visual structures (Wässle 2004).

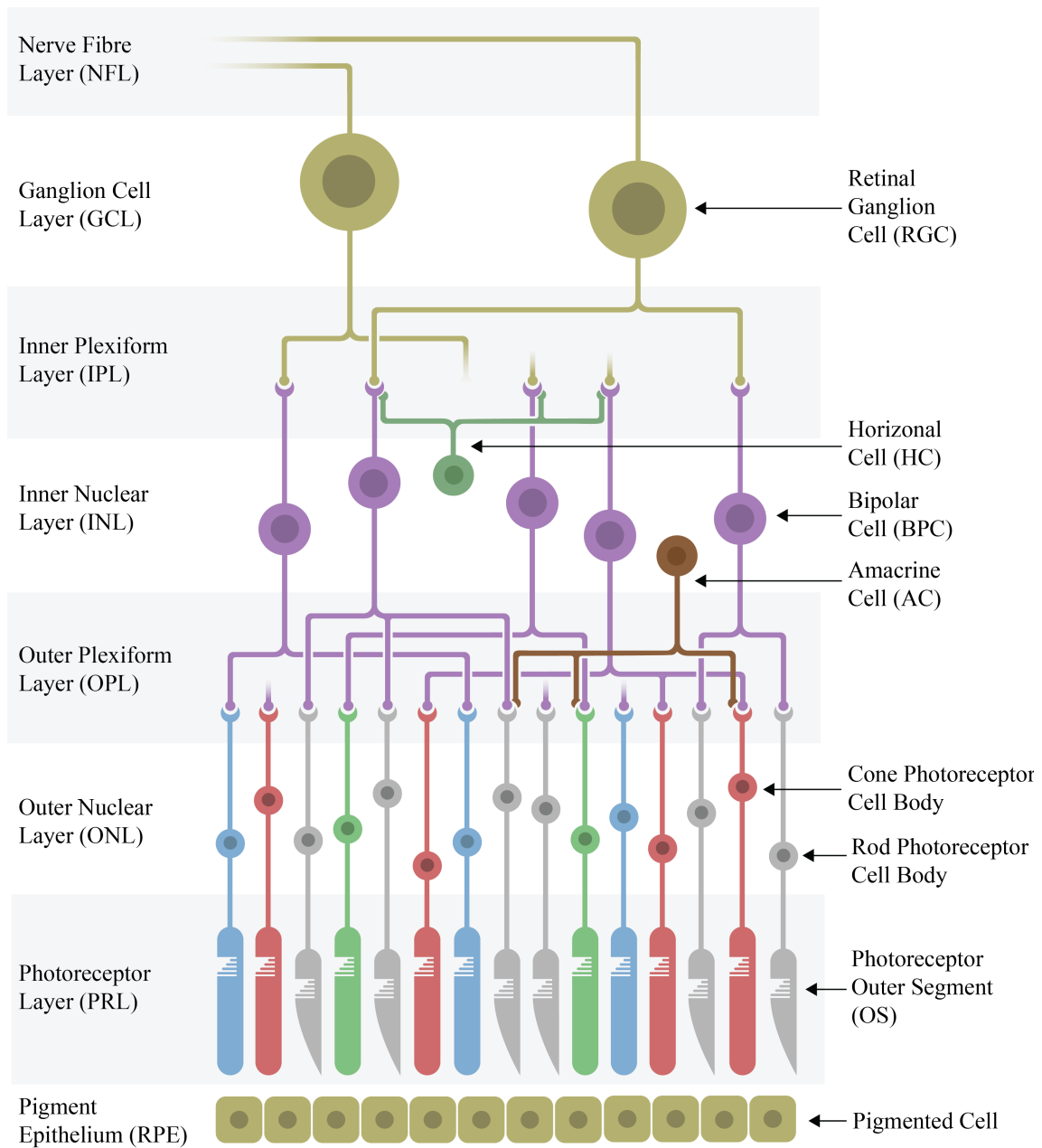


Figure 1.2: Diagram showing the different cell types and layers of the retina and their arrangement. This is an original illustration using Kolb (2003) as a reference point.

1.1.3 Visual pathway

Following phototransduction and the modulation of BP cell activity, the neural signal is communicated through the retinal circuitry before reaching the optic nerve and ultimately the visual centres of the brain (Purves et al. 2004, Kolb 2003). A single BP cell will receive input from multiple photoreceptors resulting in a large number of overlapping receptive fields. There are many types of BP cells that have different levels of sensitivity to fluctuations in photoreceptor signals and are specialised depending on whether they receive input from rod or cone photoreceptors. However, at the most basic level, they can be sorted in to ON rod BP, ON cone BP and OFF cone BP cells. ON-BP cells express inhibitory glutamate receptors, which keep the cell hyperpolarised when their associated photoreceptors are not reacting to light, whereas OFF-BP cells express excitatory glutamate receptors which activate the cell when no light is present in its receptive field. These receptors modulate the ON and OFF parallel visual pathways and present the visual system with two opposite contrast images (Wässle 2004). The ON pathway is how bright images are detected against dark backgrounds, and the OFF pathway is how dark images are interpreted, such as reading black letters on a white page (Wässle 2004). These pathways are propagated through to the retinal ganglion cells (RGCs) in the innermost nuclear layer, called the ganglion cell layer, and on to the central visual structures. The axons of these cells extend across the inner surface of retina where they coalesce and exit the eye as the optic nerve.

The earliest forms of processing in the retina occurs prior to the BP cells and are mediated by horizontal and amacrine cells present in the inner nuclear layer. Each horizontal cell synapses with a much larger number of cones and has larger receptive fields than the bipolar cells (Kolb 1974, Wässle et al. 1989). Horizontal cells send signals to both photoreceptors and the dendrites of bipolar cells, adding a surround signal of opposite luminance polarity. This form of lateral inhibition is important in further restricting the receptive fields of BP cells in a method known as centre-surround organisation (Kolb 1974, Boycott et al. 1987, Wässle et al. 1989). It also plays an important role in modulating cone sensitivity under different light intensities (Boycott et al. 1987, Schnapf et al. 1987). Amacrine cells play a large role in vertical communication between retinal layers. Over twenty-two different types of amacrine cells have been identified so far, and can be equally divided by those that utilise glycine or gamma-aminobutyric acid (GABA) neurotransmitters (Frumkes et al. 1981, MacNeil MA 1998). Glycinergic amacrine cells receive input from bipolar cells, transmit information to RGCs and mediate connections between the ON and

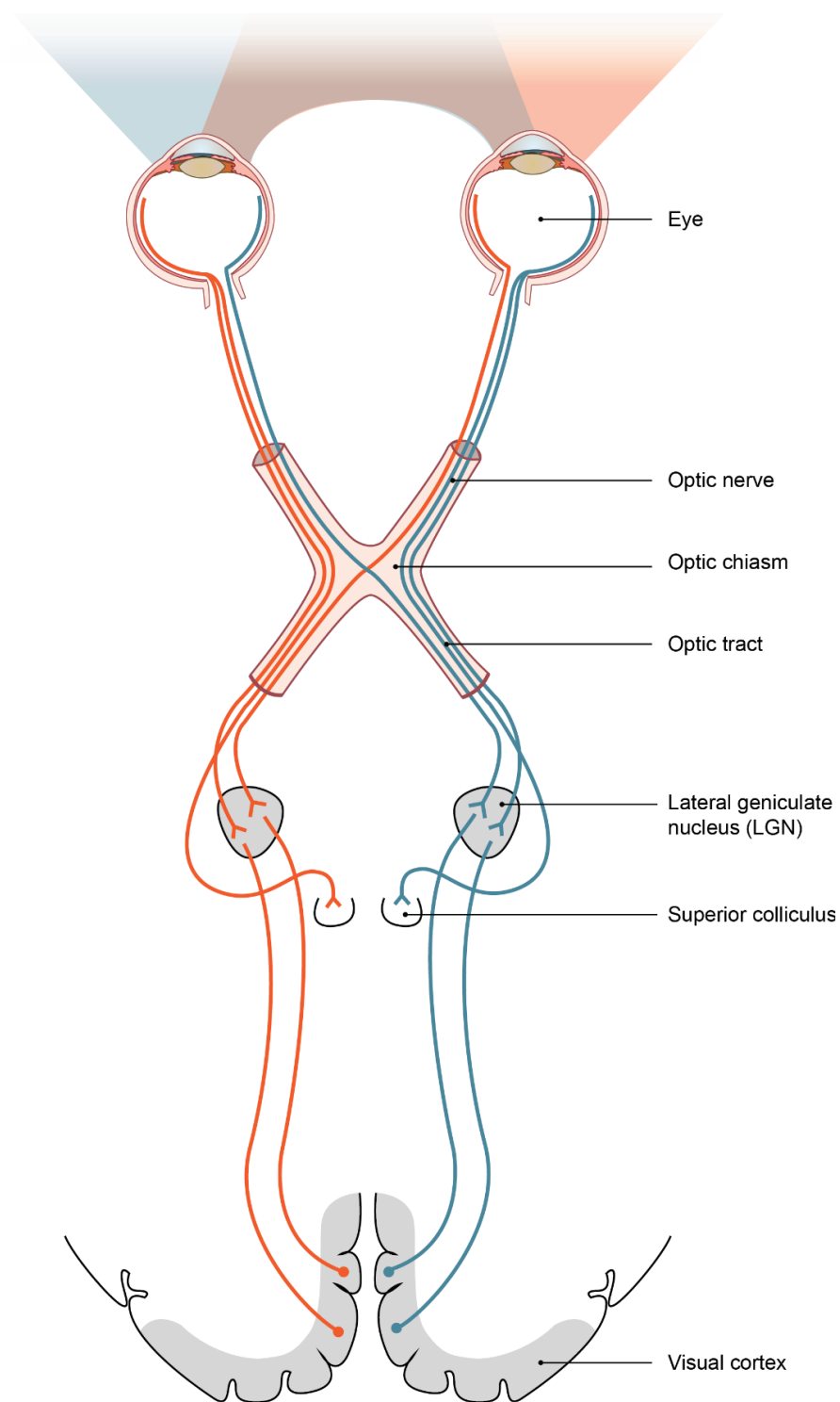


Figure 1.3: Schematic diagram of the visual pathway from the eyes to the primary visual cortex. Orange fibres relate to the right visual field, turquoise fibres relate to the left visual field. This is an original illustration using Purves et al. (2004) as reference points.

OFF pathways (Wässle 2004). These cells are often referred to as "small field" as they generally span relatively short distances horizontally, in contrast to GABA-ergic amacrine cells, which can spread hundreds of microns and interact with hundreds of BP cells and RGCs (Kolb 2003). These cells have been found to play a role in the detection of directional motion (Kolb 2003).

After exiting the eye, visual information is relayed and processed in a number of central structures as shown in Figure 1.3. The optic nerves of each eye meet at the optic chiasm on the inferior surface of the brain beneath the hypothalamus (Guillery 1982, Godement et al. 1990). At this point projections from the nasal retinae of both eyes cross over to the opposite hemisphere as visual signals from each side of the visual field are processed contralaterally (Guillery 1982). These reorganised nerves enter the brain, becoming the optic tracts, and terminate in the lateral geniculate nucleus (LGN) in the thalamus (Hoffmann et al. 1972, Sherman & Koch 1986). Each LGN acts as a sensory relay and sends axons to its ipsilateral primary visual cortex (V1) located caudally within and on each side of the calcarine sulcus (Hoffmann et al. 1972, Sherman & Koch 1986). Information from each V1 is fed forward on to the other regions of the visual cortex such as V2, V3, V4, V5, and V6 for further processing (Zeki 1978, Tootell et al. 1993).

An important characteristic to note about the visual system up to this point is its maintenance of retinotopicity along the pathway (Schwartz 1977, Horton & Hoyt 1991, Chen et al. 1999). All structures up to and including V1 are organised according to the spatial distribution of the cells in the retina (Tusa 1978, Horton & Hoyt 1991, Chen et al. 1999). This is extremely helpful for electrophysiological studies as it is possible to observe patterns of activation in the central structures that correspond with patterns of activation in the retina for the same point in visual space (Tusa 1978, Horton & Hoyt 1991).

1.2 Retinal degeneration

Retinitis pigmentosa (RP) is the name given to a set of hereditary diseases featuring degeneration of photoreceptor cells (Hartong et al. 2006). RP is highly variable and is not consistent in progression of symptoms (Hartong et al. 2006). It is characterised by a gradual decline in visual acuity beginning anywhere between infancy and the age of sixty. The majority of RP sufferers are legally blind by the age of forty. Over 1 million people worldwide are affected by RP, making it one of the most common forms of inherited retinal dystrophies. With the notable exceptions of the recently

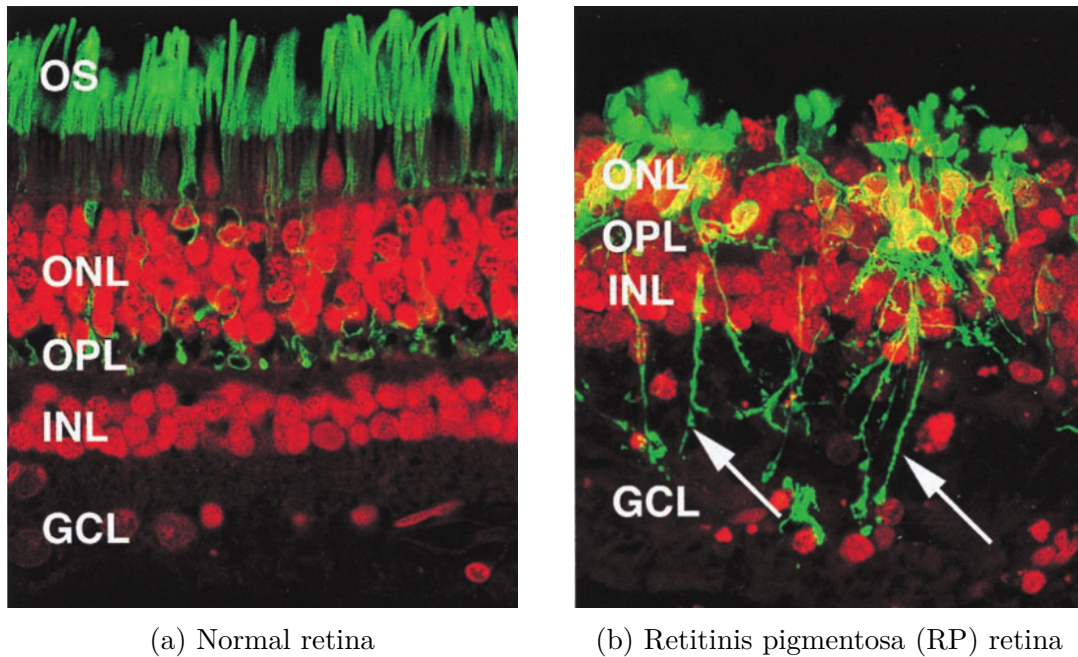


Figure 1.4: Image adapted from Farris et al, 2003. Immunolabeling of rod photoreceptors with anti-opsin (green) in (a) normal and (b) RP human retinæ. Nuclei in the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL) are stained red with propidium iodide. Opsin immunolabeling (green) is strongest in the outer segments of the photoreceptors. The surviving rods are opsin-positive but few retain outer segments. Arrows show rod neurites projecting past the INL, with some terminating in the GCL. This comparison highlights that, while inner retinal layers are not directly affected by RP, significant structural changes occur in response to photoreceptor death.

FDA approved gene replacement therapy ‘Luxturna’ for RPE65 mutation-associated RP, and three rare forms of RP that can be treated with dietary changes, there are no treatments available to halt or reverse the effects (Smalley 2017, Grant & Berson 2001).

The mode of inheritance is also highly variable, with 30-40% of cases inherited in an autosomal-dominant fashion, 50-60% as an autosomal-recessive trait, and 5-15% X-linked (Dryja et al. 1990, Hartong et al. 2006). Over 45 genes have been currently identified as causes for RP, however this only accounts for approximately only 60% of patients (Hartong et al. 2006, Dryja et al. 1990). Due to the multiple genetic components involved in RP there is no single mechanism by which degeneration occurs, however, the results are the same. Rod and cone photoreceptor cells gradually degenerate over the course of years, resulting in visual impairment and eventually blindness (Portera-Cailliau et al. 1994, Hartong et al. 2006). Typically, patients first present with night blindness during adolescence due to the higher rate of rod photoreceptor degeneration (Hartong et al. 2006). Throughout adulthood patients gradually lose peripheral vision, resulting in severe tunnelling of the visual field and eventually complete loss of central vision (Hartong et al. 2006). For patients with end-stage RP, bare light perception is often all that remains (Hartong et al. 2006). Other components of the neural retina in the higher layers, such as RGCs, BP cells, and horizontal cell are not directly affected by RP, however, the decrease in photoreceptor neurotransmitter output often results in long-term maladaptive plastic changes in the retinal circuitry (as shown in Figure 1.4) (Santos et al. 1997, Humayun et al. 1999, Jones et al. 2003, Jones & Marc 2005).

1.2.1 Preclinical models of retinal degeneration

In order to study retinal degenerative diseases and investigate treatments, work has been undertaken to develop animal models of photoreceptor degeneration for preclinical use. Some companion animal breeds, such as Abyssinian cats, Briard dogs, and Irish Settler dogs, exhibit high rates of hereditary progressive retinal atrophic diseases that bear close resemblance to the human RP pathology (Pardue et al. 2001, Musarella & Macdonald 2011, Bertschinger et al. 2008, Dryja et al. 1990, Kondo et al. 2009). Transgenic models have also been developed, introducing mutations in genes influencing the synthesis of rhodopsin in pigs, sheep, and rabbits (Li et al. 1998, Weitz et al. 2013, Portera-Cailliau et al. 1994, Jones et al. 2003). However, limited availability, and a long time course before symptoms manifest, make studies using these animals extremely costly. Inducing retinal degeneration pharmacolog-

ically, such as by intravitreal injection of sodium iodide, N-methyl-N-nitrosourea (MNU), or adenosine triphosphate (ATP), is an alternative approach that has the advantage of faster rates of retinal cell death, the ability to induce degeneration in a single eye, and is theoretically applicable to wide range of animal species (Nakajima et al. 1996, Yoshizawa et al. 2000, Vessey et al. 2014, Aplin et al. 2014).

In collaboration with the Centre for Eye Research Australia and the University of Melbourne, the Bionics Institute developed an ATP-induced model of retinal degeneration in cats (Aplin et al. 2014, 2016a,b). Intravitreal injection of ATP solution has been shown to result in rapid degeneration of the ONL without significant direct effects on inner retinal layers (Aplin et al. 2016b). The mechanism behind this action is not well understood. It is likely that degeneration is mediated by the binding of ATP to purinergic receptors, particularly the receptor P2X7, which are exclusively expressed by both rod and cone photoreceptors in the retina (Vessey et al. 2014, Puthussery et al. 2006). Prior work with this model has shown significant levels of inner retinal remodelling and gliosis (Aplin et al. 2016b).

1.3 Visual prostheses

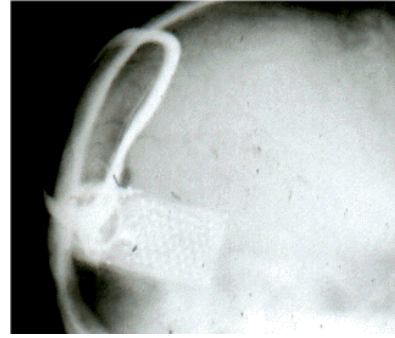
1.3.1 History of visual prostheses

Visual prostheses are devices designed to provide compensatory artificial input to the visual pathway in cases where there has been degeneration or damage to visual structures, in an effort to provide a replacement for natural vision (Shepherd et al. 2013). The majority of these devices attempt this by electrically stimulating neural tissue in the visual pathway in a way that elicits, as closely as possible, the normal neural response to visual stimuli (Shepherd et al. 2013). The most prevalent neural targets are the retina and the visual cortex, with some research groups also attempting to place electrodes in the LGN and around or within the optic nerve (Veraart et al. 1998, Pezaris & Reid 2007).

Otfried Foerster, a German neurologist and neurosurgeon, was the first to demonstrate that exposing and electrically stimulating the visual cortex could elicit the perception of a discrete spot of light in 1929 (Foerster 1929). A continuation of this work conducted by Krause & Schum in 1932 found that this perception of light, or “phosphene”, could also be elicited in a patient who had been rendered blind over eight years prior by gunshot wound (Krause & Schum 1931). Inspired by this finding that the visual cortex does not wholly lose its function following loss of sight,



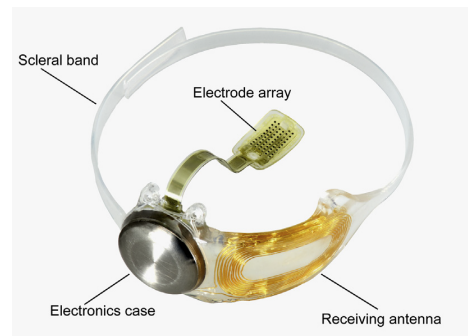
(a) Images of glasses-mounted camera worn by a patient implanted with the Dobelle cortical prosthesis (Image adapted from Dobelle, 2000)



(b) X-ray of the head of a patient implanted with the Dobelle cortical prosthesis. (Image adapted from Dobelle, 2000)



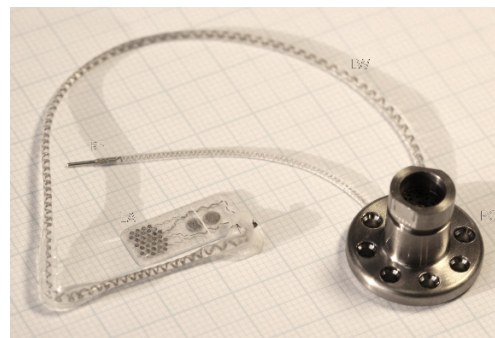
(c) Images of glasses-mounted camera and wireless transmitter worn by a patient implanted with Second Sight's Argus II. (image adapted from de Cruz et al, 2016)



(d) Implantable components of the epiretinal Second Sight Argus II Retinal Prosthesis System. (image adapted from de Cruz et al, 2016)



(e) Image of a patient implanted with a suprachoroidal prototype retinal implant participating in the Bionic Vision Australia clinical trial. (image provided by The Bionics Institute)



(f) Implantable components of the suprachoroidal prototype device used during the Bionic Vision Australia clinical trial. (image provided by The Bionics Institute)

Figure 1.5: Images of various visual prostheses that have been used by patients

in 1968 Brindley & Lewin implanted a prototype visual prosthesis in to a fifty-two year old blind patient (Brindley & Lewin 1968). By wirelessly transmitting power to eighty-one intracranial electrodes in contact with the occipital pole of the right hemisphere, they were able to elicit multiple phosphenes (Brindley & Lewin 1968). They were also able to deduce that the location of the phosphenes in the patient’s visual field agreed with the contemporary understanding of the topographical mapping of the human visual cortex (Brindley & Lewin 1968). Following Brindley & Lewin’s first attempt at a visual prosthesis, a number of other cortical implants were developed and tested over the decades with varying degrees of success (Dobelle et al. 1974, Talalla et al. 1974, Pollen 1975, Dobelle et al. 1976). Ongoing improvements in the miniaturisation of electrical hardware and advances in computer technology ultimately enabled William Dobelle to develop an implantable cortical device coupled with an externally worn camera and portable image processor, affording one patient limited object recognition and mobility aid (Dobelle 2000). However, there were significant downsides to the approach taken. The high charge required by the surface-based electrodes to reach perceptual thresholds increased the risk of seizures in patients (Naumann 2012). Electrodes were directly connected to the stimulator by cabling exiting the patients’ heads via a transcutaneous connector, resulting in recurrent infections (Naumann 2012).

Cortical visual prostheses continue to be developed, taking advantage of modern developments in materials and wireless communication (Lowery et al. 2015, Lewis et al. 2015). However, much of the development in visual prostheses has shifted to targeting other parts of the visual system. Inspired by the success of the cochlear implant in bypassing damaged cochlear hair cells and directly activating sensory fibres with electrical stimulation, efforts have been made to develop devices that take advantage of the residual neurons that remain in the retina following photoreceptor degeneration in conditions such as RP and age-related macular degeneration (AMD) (Shepherd et al. 2013, Weiland et al. 2005). Stimulating early in the visual pathway is believed to result in more biologically analogous signals as it bypasses fewer visual processing structures (Shepherd et al. 2013, Zrenner 2002). However, retinal stimulation would have little benefit for people who have had extensive damage to their retinas or eyes, or who suffer from pathologies that affect higher visual processing centres.

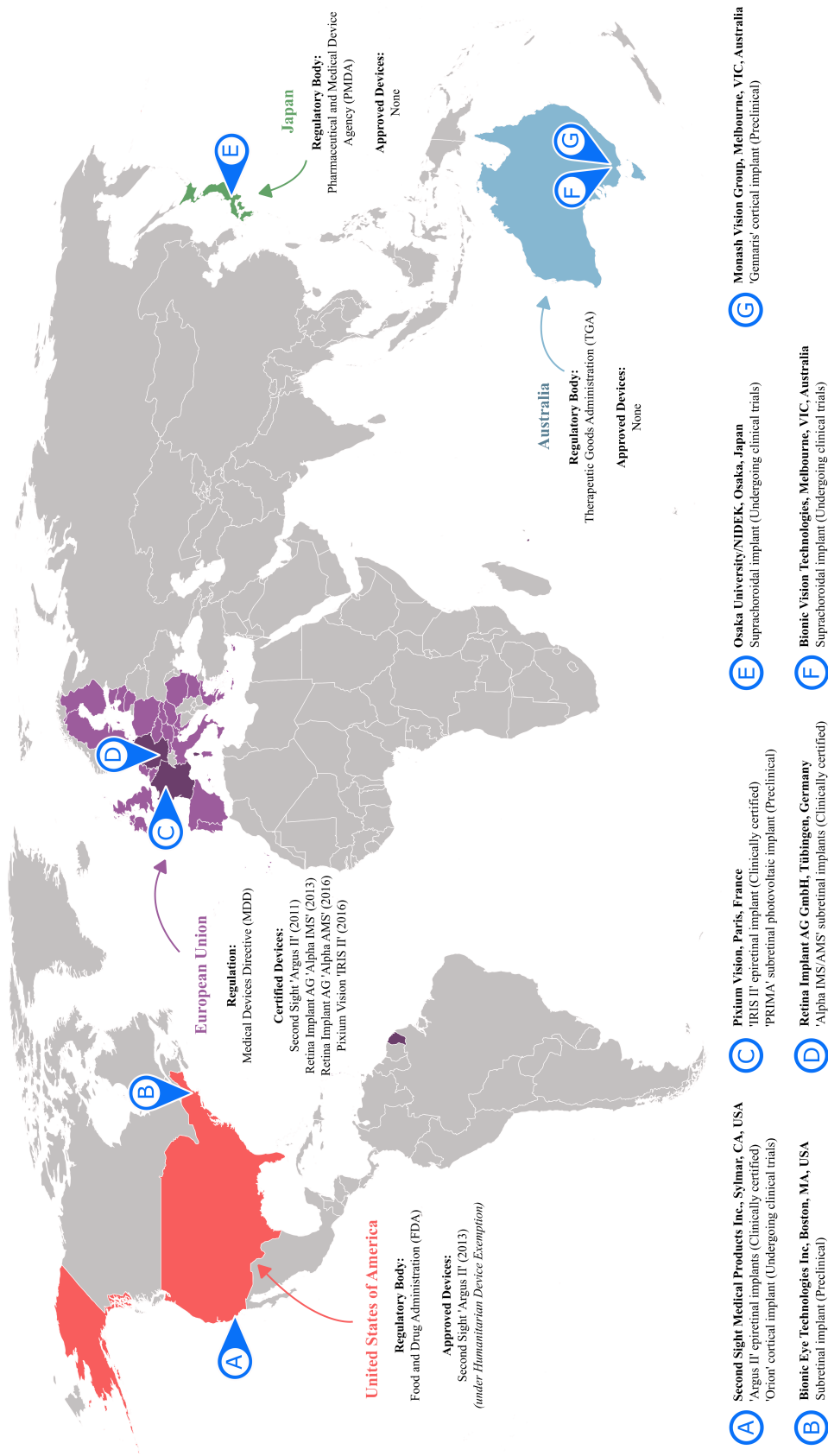


Figure 1.6: World map showing the locations of the most relevant visual prostheses research teams currently operating.

1.3.2 Progress in retinal prostheses

The concept of a retinal prosthesis was first outlined in 1952 by Melbourne-based inventor Graham Tassicker (Tassicker 1956). He described that implanting a light-sensitive selenium cell behind the retina briefly restored a patient’s perception of light (Tassicker 1956). Despite this early finding, visual prosthetic research was mainly confined to cortical devices for the majority of the 20th Century. It wasn’t until the 1980s that many researchers began revisiting this approach. Promising psychophysics studies conducted in the 1990s led to the first clinical trials of implantable retinal prostheses in the early 2000s by multiple groups around the world. (Michelson 1986, Humayun et al. 1996, 1999, Humayun 2001, Zrenner 2002, Humayun et al. 2003). Today, retinal implants targeting residual BP cells and RGCs are the only neural prosthetic devices with regulatory approval for use in patients with RP. In 2011, the Argus II, developed by Second Sight Medical Products (Sylmar, CA, USA), became the first device approved for the treatment of RP in the European Union, followed by FDA approval in the United States in 2013 (da Cruz et al. 2016, Dagnelie et al. 2017, Humayun et al. 2012). Soon after, Retina Implant AG GmbH’s (Tübingen, Germany) Alpha IMS and AMS devices (Stingl et al. 2015, Edwards et al. 2018a), along with the Pixium Vision (Paris, France) IRIS II, gained regulatory approval for clinical use in the European Union (Hornig et al. 2017). Many other devices are at various stages of clinical trials and commercialisation, such as Bionic Vision Technology’s 44-channel Bionic Eye (Ayton et al. 2014), Pixium Vision’s ‘PRIMA Bionic Vision System’, and Osaka University/NIDEK’s Visual Prosthesis Project (Illustrated in 1.6)

Much like with the development of the cochlear implant for hearing restoration, surgical placement of the electrode array in the eye has been a challenge and has yielded a number of competing approaches. Epiretinal and subretinal devices are placed inside the globe of the eye and are aimed to be in direct contact with the neural retina. Epiretinal devices are attached to the inner surface of the retina, whilst subretinal devices are implanted between the retina and choroid. The primary advantage of these approaches is that the implanted electrodes are relatively close to the target neurons, thereby reducing the required stimulating charge to activate retinal tissue and allowing for very small electrodes to be used (da Cruz et al. 2016, Zrenner et al. 2010, Shepherd et al. 2013). However, these methods are surgically complex and their positioning poses high risks of further damage to the retina or other structures in the eye (Majji et al. 2015). Subretinal implantation has been shown to impede nutrient flow between the choroid and the retina (Montezuma et al. 2006). As such,

these implants must be highly porous in order to facilitate nutrient diffusion (Julien et al. 2011, Wang et al. 2012). Close proximity to target neurons and low activation thresholds have allowed subretinal devices to be fabricated that employ photodiodes, rather than electrodes driven by an external stimulator. Retina Implant AG’s Alpha AMS device utilises a photodiode array, with a connected amplifier, that responds to natural light entering through the pupil (Daschner et al. 2018). However, despite hardware and software developments since its predecessor, the subretinal Alpha AMS device also suffers from a short clinical lifespan, with manufacturer estimates of five years before failure of its implantable light-sensitive CMOS chip (Daschner et al. 2018). Pixium’s PRIMA system uses a modified approach, whereby an image recorded by an externally worn camera is ‘projected’ through the pupil using a higher energy near-infrared light, directly activating the subretinally implanted photodiodes (Palanker et al. 2005, Wang et al. 2012).

Some devices are implanted in the suprachoroidal space of the eye; between the choroid and sclera of the optic globe. To achieve this, the posterior surface of the eye is exposed and a small incision is made in the sclera (Sakaguchi et al. 2004, Kanda et al. 2004, Villalobos et al. 2012). The electrode array is then placed in a pocket made between the sclera and choroid, without the need for intra-ocular surgery or contact with the retina (Villalobos et al. 2012). One disadvantage of this technique is that, due to greater distance from the retina, higher levels of stimulation are required to reach the neurons’ activation thresholds compared to epiretinal and subretinal approaches (Yamauchi et al. 2005, Shivdasani et al. 2014). Electrodes on these arrays must have a greater surface area in contact with the tissue in order to maintain safe charge levels. This compromise limits the number of electrodes that can be safely implanted and consequently the number of percepts that the device is capable of eliciting, sacrificing spatial resolution for safety and surgical ease. As part of the Bionic Vision Australia (BVA) research group, the Bionics Institute developed a suprachoroidal retinal implant for a clinical trial with three RP patients conducted from 2012 to 2014 (Ayton et al. 2014). Following the successful trial, a fully-implantable 44-channel suprachoroidal retinal prosthesis is currently undergoing patient testing (Abbott et al. 2018).

1.3.3 Resolution of artificial vision

When individual electrodes of a retinal implant are stimulated, most patients are able to perceive discrete flashes of light. These flashes, termed phosphenes, are used

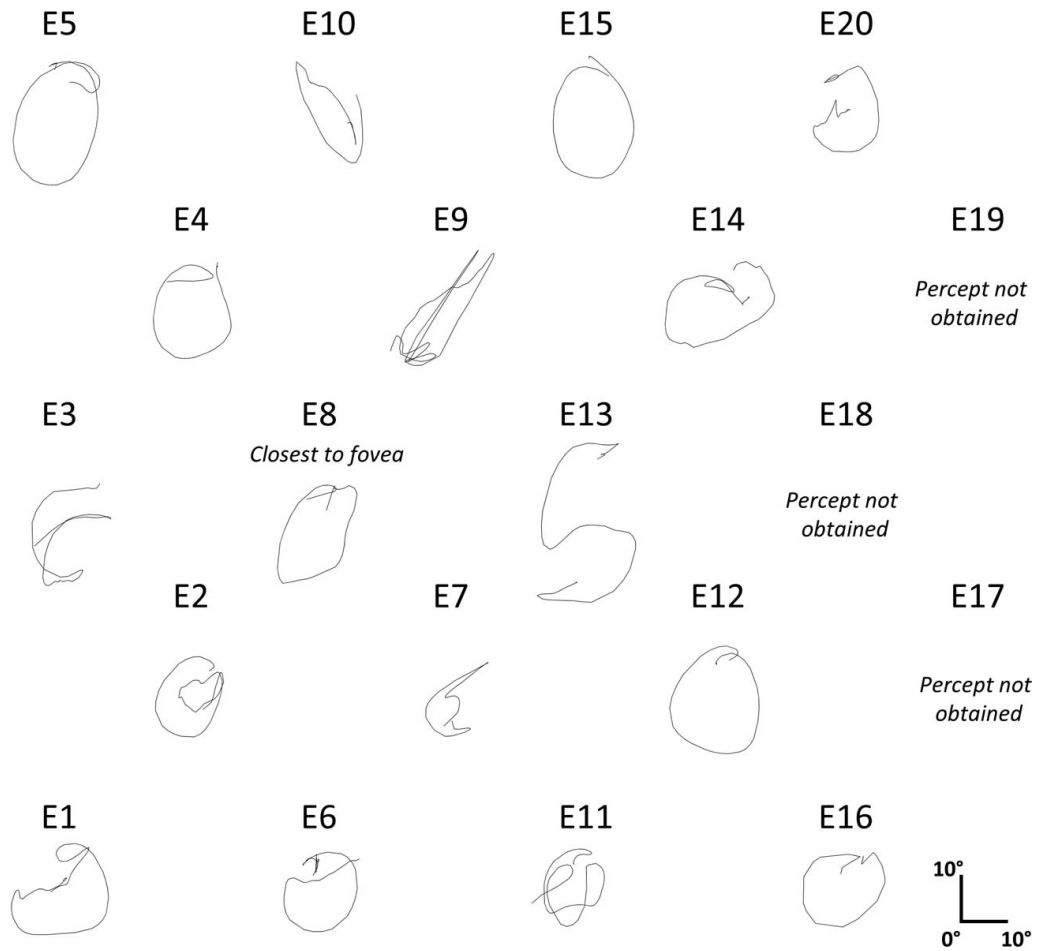


Figure 1.7: Phosphenes drawn by a patient implanted with a suprachoroidal retinal prosthesis. Drawings are of the appearance of the initial flash of each phosphene elicited at 4 dB above perceptual threshold. Each drawing is to scale shown by the axes in the lower right corner. Image adapted from Sinclair et al. (2016)

as building blocks to generate an artificial image. Under present stimulus paradigms, each electrode elicits a single phosphene, making the number of individual elicitable phosphenes directly proportional to the number of implanted electrodes. Visual acuity attainable with the most advanced retinal implants is still severely limiting for implant recipients and is not yet able to provide a suitable replacement for everyday visual tasks. Results from trials with Second Sight’s Argus II retinal prosthesis showed that 60 electrodes were able to provide enough functional phosphenes to improve performance in blind patients for rudimentary tasks such as object recognition, motion detection, pattern recognition, and basic navigation, as well as provide a significant long-term increase in the patient’s self-reported quality of life (Ahuja et al. 2011, Humayun et al. 2012, Dagnelie et al. 2017, Duncan et al. 2017). However, the best visual acuity recorded in a patient implanted with an Argus II is 1.8 logMAR, still poorer than the World Health Organisation’s threshold for legal blindness at 1 logMAR (Humayun et al. 2012). More complex tasks such as reading, facial recognition, and independent navigation are generally not possible with modern prosthetic vision devices. Simulation studies suggest that with a high enough resolution, complex tasks such as navigation, reading and even facial recognition is possible with prosthetic vision (Chen et al. 2009, Hayes et al. 2003, Thompson et al. 2003). However, these resolutions are much higher than the resolutions currently attainable, estimating that a minimum of 100 discriminable phosphenes would be required for independent navigation, and over 1000 for comprehending text and faces (Chen et al. 2009).

The Alpha IMS and AMS subretinal implants use 1500 and 1600 photodiodes respectively, and while patient trials have shown considerable improvements in object recognition and reading, and a record best logMAR of 1.4 for optotype acuity, patients have not attained the levels indicated by simulation studies (Zrenner et al. 2011, Wilke et al. 2011a, Daschner et al. 2018, Edwards et al. 2018b). This is in part due to large regions of neural activation caused by uncontrolled current spread as photodiodes in these devices are not individually controlled. Clinically-used stimulation strategies can result in an undesirably large spread of neural activation in the retina, which consequently elicits large phosphenes that overlap and are difficult for patients to discriminate between (Zrenner et al. 2011). Simply increasing the number of electrodes on the array is one way to increase the number of perceivable phosphenes, however being able to discriminate between them is also an important factor in comprehension of the artificial image (Chen et al. 2009, Wilke et al. 2011a). The number of electrodes that can be safely implanted is constrained by a number of factors. In order to maintain safe charge density levels in the tissue, the size of

each electrode can only be reduced finitely. The size of the array must also be small enough to safely implant and remain stable in the eye over a long period of time.

Present methods of stimulation are indiscriminate in their activation of different neuron types in the retina. In addition to the complications of activating multiple cell types at different points in the same pathway, stimulation can also result in activation of RGC axons of passage travelling toward the optic disc from remote retinal regions. In psychophysical experiments with suprachoroidal retinal stimulation for example, phosphenes vary greatly in appearance, as shown in Figure 1.7 (Fujikado et al. 2007, Sinclair et al. 2016, Rizzo et al. 2014, Nanduri et al. 2008, 2012). Electrodes close to the fovea yielded large complex shaped phosphenes with regions of varying brightness, whereas more eccentric electrodes elicited smaller, more uniformly bright, phosphenes (Blamey et al. 2013, Sinclair et al. 2016). Phosphenes with this level of variability are difficult to use as components of a larger image

1.4 Electrical field shaping

In order to convey complex information to patients about their visual scene, visual prosthetics need to be able to elicit the perception of many more discriminable phosphenes than present devices are capable of. Multipolar stimulation has been shown to result in electrical field interactions. Unaccounted for, these interactions can cause undesirable spatial and temporal effects on the resultant percept, making it difficult to elicit multiple percepts simultaneously (Horsager et al. 2010, 2011, Wilke et al. 2011b). As such, stimuli are commonly presented with a phase shift that is long enough to reduce field interactions but short enough to be perceived by the visual system as a combined and persistent percept. Using this strategy, each electrode can elicit a maximum of one phosphenes each. This limits the spatial resolution of the device to the number of implanted electrodes, which is constrained by engineering and surgical limitations. While electrical field interactions are typically considered undesirable, incorporation of these interactions in to the stimulation strategy may provide a way of increasing the number of unique percepts that can be elicited using the device without significant alteration. (George et al. 2014, Dumm et al. 2014, Valente et al. 2012, Senn et al. 2018, Spencer et al. 2016).

1.4.1 Contemporary stimulation strategies

With the exception of photodiode-based devices such as the Alpha IMS, clinical stimulation of the retina has mostly been limited to sequential monopolar (MP)

biphasic constant current pulses. MP stimulation is a technique whereby an “active” electrode is stimulated near the target cells against a remote “return” electrode placed somewhere else on the subjects body (Lilly et al. 1955). The return electrode serves to complete the circuit and disperse charge in order to prevent thermal damage to underlying tissue (Lilly et al. 1955). The term biphasic pulse refers to the shape of the current waveform, whereby the active electrode is stimulated using two sequential phases of opposing polarity. Biphasic stimuli been shown to be significantly safer than monophasic stimuli as they maintain charge balance in the tissue and prevent damage (Lilly et al. 1955, Shepherd et al. 1991, 1999). As such, biphasic pulses are a widely used form of electrical stimulation in medical devices (Shepherd et al. 1999). Stimuli are often presented sequentially as it has been reported in neural stimulation devices that simultaneous stimulation results in channel interaction, causing undesirable percepts and neural activation patterns. Stimuli are instead presented together on multiple electrodes with a short time shift which, provided the frequency is above the flicker fusion limit (40 Hz to 50 Hz), are perceived as a single persistent percept (Brindley & Lewin 1968, Humayun et al. 1999). In cochlear implants, channel interaction can result in spatial smearing and perceptual effects such as uncontrollable loudness growth (Bierer & Middlebrooks 2004, Bierer 2007, Stickney et al. 2006). Both sequential and simultaneous stimulation strategies have been investigated in psychophysical studies in blind patients implanted with retinal implants. These studies have shown that patients are able to distinguish between the percepts elicited by each type of stimulation (Horsager et al. 2010, Wilke et al. 2011a, Klauke et al. 2011).

Even as far back as the first visual prosthesis, it has been observed that simultaneous stimulation of neighbouring electrodes altered perception compared to single electrodes by having variously facilitative and suppressive effects on brightness (Brindley & Lewin 1968, Horsager et al. 2010). Wilke et al. (2011b) also showed that it was possible to elicit recognisable patterned percepts with simultaneous stimulation. Though less effective than sequential stimulation of the same electrodes, it was conceded that a limitation of the study was an inability to produce varied currents according to the activation threshold of each electrode (Wilke et al. 2011b). A cortical *in vivo* study with a suprachoroidal implant concluded that simultaneous stimulation yielded lower activation thresholds (Shivdasani et al. 2012). It is thus clear that simultaneous stimulation of nearby electrodes results in electrical crosstalk and alterations in perception. While channel interaction is often considered to be an unwanted side effect of multichannel stimulation and something to be avoided, it may be possible to use channel interaction to gain better control over the electrical

field generated by the electrodes.

1.4.2 Multipolar stimulation strategies

A consequence of MP stimulation in the retina is a large uncontrolled spread of neural activation. In these circumstances, smaller, higher density electrodes increase the number of perceivable phosphenes, but they may be too large to discriminate between as evidenced by patient performance with the Alpha IMS and Argus II devices (Zrenner et al. 2011, Wilke et al. 2011a,b). Current focusing methods have been proposed with the aim of restricting the spread of current in the hope that this will produce more discrete phosphenes (Wilke et al. 2011b, Cicione et al. 2012, Matteucci et al. 2013). Cochlear implant studies have shown in the past that the electrode return configuration used during stimulation, such as BP and tripolar returns, can have significant effects on the spatial distribution of neural activation. As such, similar approaches have been attempted in retinal implants. Different return configurations for stimulation have been investigated in preclinical studies, such as BP, common ground, hexapolar (HP), and quasimonopolar configurations (Cicione et al. 2012, Matteucci et al. 2013).

HP stimulation is where the six adjacent electrodes are used as a return pathway, each receiving approximately one sixth of the total return current, and theoretically restricts charge from spreading further. *In vitro* and *in vivo* electrophysiological recordings show that HP stimulation results in more restricted activation of retinal tissue, albeit at the expense of higher activation thresholds (Habib et al. 2013, Matteucci et al. 2013, Shivdasani et al. 2012, Fan et al. 2017). However, whether this translates to more restricted cortical activation patterns or smaller phosphenes has shown conflicting results in the literature. Investigation of cortical responses to MP and HP stimulation of the retina showed that there was no significant difference in activation spread (Cicione et al. 2012). However, a similar study found that HP stimulation was able to reduce cortical activation spread (Matteucci et al. 2013). In order to account for differences in activation threshold, both of these studies compared cortical activity along the same point along the dynamic range for each response (Cicione et al. 2012, Matteucci et al. 2013). Findings from studies in cochlear implants (George et al. 2014, 2015), have shown that, due to increased activation thresholds, focused stimulation at even the highest safe charge injection limit commonly fails to result in saturation of neural activity. Without a reliable estimation of the upper dynamic range, it is uncertain whether these charge levels would have elicited comparable level of activity. As such, it is unclear whether HP

stimulation can effectively reduce the spread of cortical activation, and whether this translates to the perception of suitably discrete phosphenes.

Increased activation thresholds for these focusing configurations indicate that these techniques may not be ideal for safe clinical use (Cicione et al. 2012, Shivdasani et al. 2014). A quasimonopolar paradigm has been suggested as a mid-point between MP and HP stimulation. This method uses two current drives, one connected in a MP return configuration and the other using a HP return configuration (Matteucci et al. 2013). Stimulating on the same electrode and controlling the proportion of current returned via either the remote return or the surrounding electrodes, it is possible to bring thresholds closer to those observed during MP stimulation alone, akin to the partial tripolar stimulation for cochlear implants (Matteucci et al. 2013, Wu & Luo 2013). However, this is achieved at the cost of increased technical complexity by adding an additional current drive and, depending on the ratio of MP to HP stimulation, does not yield the same level of spatial selectivity as unaugmented HP stimulation.

It is also possible to steer current by utilising adjacent electrodes as current returns. By stimulating opposite polarity pulses, current spread can be shifted in the direction of the flanking electrode stimulated at the lower amplitude (Bonham & Litvak 2008, Wu & Luo 2013, Butson & McIntyre 2008, Chaturvedi et al. 2012). Studies conducted assessing the effectiveness of BP and partial-tripolar steering with cochlear implants and deep brain stimulation (DBS) implants have shown that the spread of current can be shifted away from the main stimulating electrode (Bonham & Litvak 2008, Wu & Luo 2013, Butson & McIntyre 2008, Chaturvedi et al. 2012).

1.4.3 Field shaping with simultaneous stimulation

In an effort to better understand the effects of electrical crosstalk, studies have been conducted to model neural responses to simultaneous multichannel stimulation. Jepson et al. (2014) demonstrated, using a piecewise linear model, that it was possible to accurately predict which among a set of fixed stimulation ratios were capable of maximising activation of a single retinal cell while minimising activation of a neighbouring cell. Using a linear-nonlinear model, RGC spiking activity from *in vitro* preparations of retina could be accurately predicted in response to arbitrary patterns of electrical stimulation (Maturana et al. 2016). An adaptation of this model was later applied to show that multi-unit visual cortex responses to white noise retinal stimuli could also be predicted, and that the interactions between simul-

taneously stimulated electrodes are predominantly linear (?). These studies show that neural responses to simultaneous multichannel stimulation are reproducible and predictable, and that it may be possible to exploit these interactions to improve the selectivity of neural activation or produce distinct percepts that would not be elicitable using traditional methods of stimulation.

While incredibly useful in developing a greater understanding of neural responses to multichannel stimulation, the ability to model neural responses and produce accurate predictions of responses is dependent on extensive sampling of retinal or cortical activity. The technical challenge of ensuring long-term high-resolution acquisition of neural activity in implanted patients limits the potential of these models for clinical use. A number of techniques have been proposed and investigated in other neural prosthetic devices, providing more intuitive ways to focus or steer the electrical field in neural tissue such as FMP and VE current steering.

FMP stimulation

FMP stimulation, also referred to as phased array stimulation, is a method that was developed for cochlear implants (van den Honert & Kelsall 2007, van den Honert et al. 2007). The technique employs simultaneous multichannel stimulation of all electrodes on the array to manipulate the shape of the electric field in the cochlea and produce more discrete regions of neural activation. To produce a summated restricted electrical field around a particular electrode, all other electrodes on the array are simultaneously stimulated according to predefined positive and negative weights calculated to nullify voltage changes at each surrounding electrode site (illustrated on a linear electrode array in Figure 1.8). These surrounding weights, termed the “phased array”, are different for each electrode and are calculated prior to stimulation by measuring the transimpedance between each electrode. When each electrode on the array is stimulated, a voltage change can be measured at any other electrode. The transimpedance between two electrodes is then defined as voltage change recorded by one of the electrodes when the other electrode is stimulated, divided by the current applied to the stimulating electrode. The most significant difference between this technique and other current focusing techniques is that FMP stimulation employs active unequally weighted simultaneous stimulation of electrodes, as opposed to changing return configurations or splitting charge equally across multiple electrodes.

Prior to the studies outlined in this thesis, FMP stimulation had never been ap-

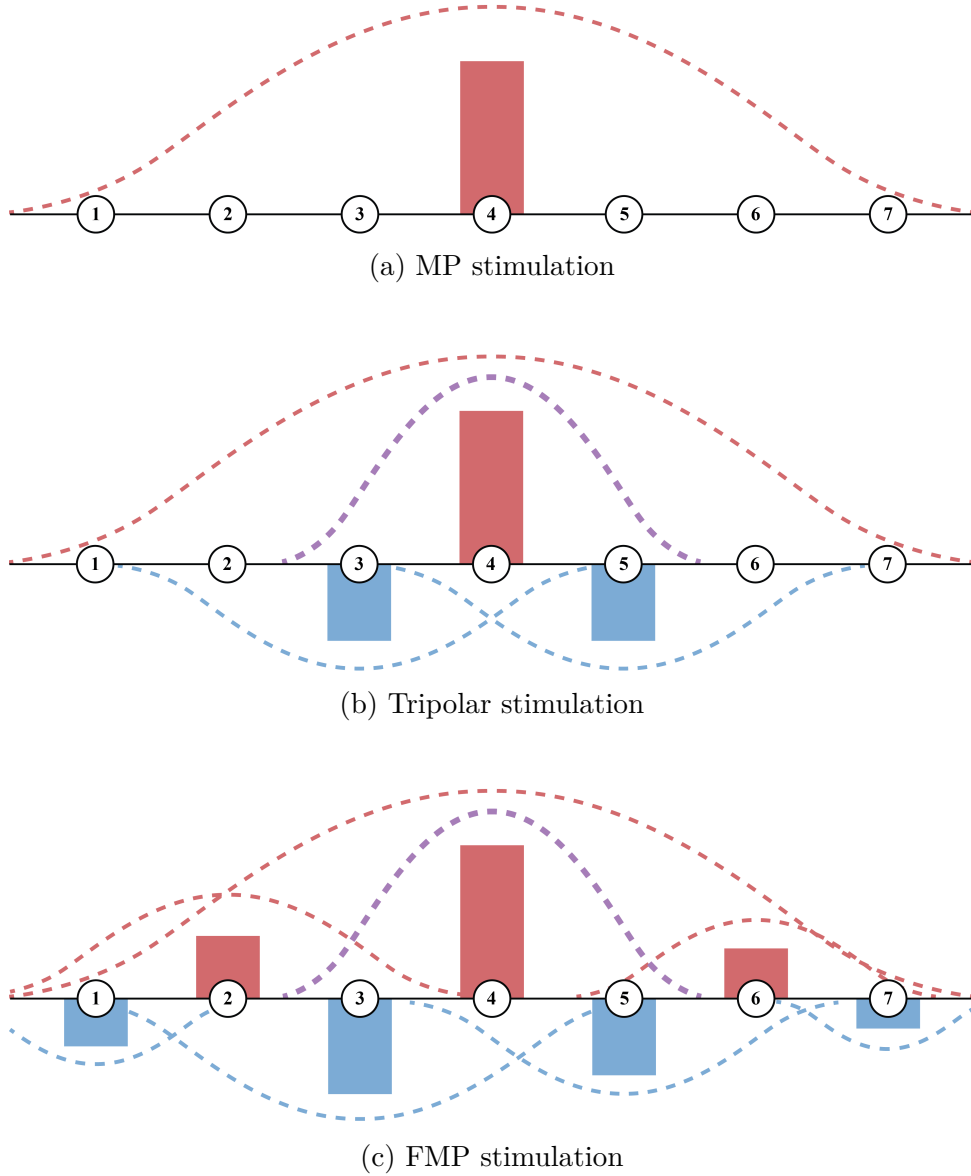


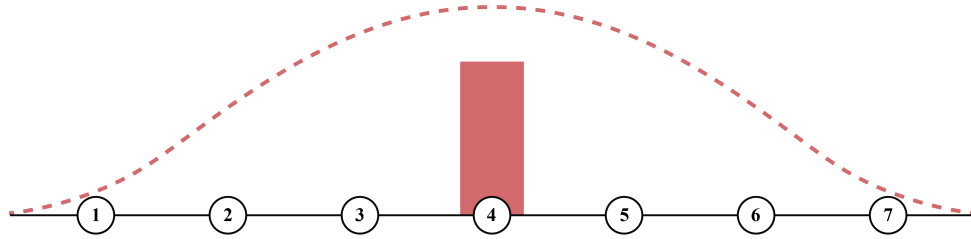
Figure 1.8: Schematic of electrical field interactions for (a) monopolar (MP), (b) tripolar (TP), and (c) focused multipolar (FMP) stimulation on a linear electrode array. Electrode sites are numbered, and the size of the bars represent the relative intensity of stimulation at each site. Red and blue dotted lines represent the shape of the individual electrical fields generated by each electrode, and the purple dotted lines represent the summated electrical field. This shows the theory behind the present implementation of FMP strategy in cochlear implants. Translation to a retinal array requires extension to a second dimension.

plied to a 2D electrode array and had not been investigated for use in the retina. However, psychophysical studies in cochlear implant patients have shown that FMP significantly improves patients' ability to discriminate spectral features and detect fine changes in sound stimuli compared with tripolar stimulation (Smith et al. 2013, van den Honert & Kelsall 2007, Marozeau et al. 2015). When applied to a computational model of the cochlear environment, FMP stimulation was shown to result in more localised regions of current spread compared to MP stimulation (Frijns et al. 2011). This study also explored the possibility of using the technique to create multiple discrete regions of activation simultaneously. Recordings from the inferior colliculus of cochlear-implanted short-term and long-term deafened cats also showed more restricted regions of activation in response to FMP stimulation over MP (George et al. 2014, 2015).

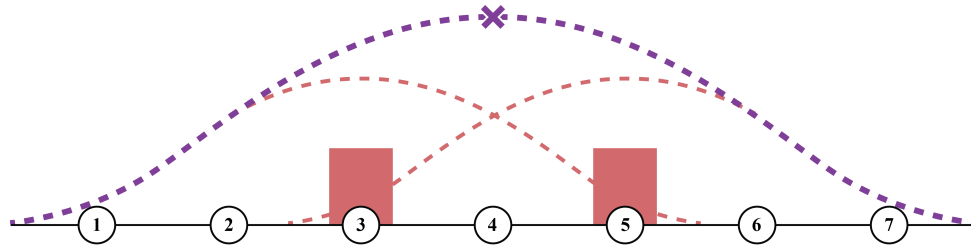
Prior to these studies, it was uncertain whether the FMP methodology could be successfully adapted for use on a 2D retinal stimulating array. The methodology behind calculation of weights required alteration to account for the added dimension, it was also unclear what effects differences in electrode geometry, size, and arrangement from cochlear implants would have on the interaction of electrical fields. In addition, like tripolar and HP stimulation in previous studies, neural thresholds were found to be significantly higher for cochlear FMP stimulation. As our device is implanted in to the suprachoroidal space, activation thresholds are already significantly higher than competing approaches (Shivdasani et al. 2014, Fujikado et al. 2011).

VE current steering

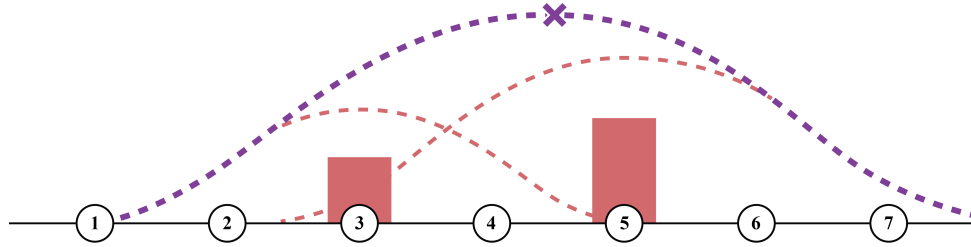
One promising form of current steering uses simultaneous stimulation of pairs of electrodes against a common return to elicit neural activation in the region between the pair. This technique has been explored in cochlear implants and deep brain stimulation devices as a way to specify the desired region of stimulation (Firszt et al. 2007, Koch et al. 2007, Brendel et al. 2009, Saoji & Litvak 2010). Interaction between the electric fields generated by stimulation of two neighbouring electrodes results in a single summated electrical field. Assuming similar impedances and that the charge is split equally, the peak of the resultant field lies midway between the two electrodes. This has been termed a VE, as the summated electric field is intended to mimic the neural response of a hypothetical physical electrode placed directly between the steering electrode pair (as illustrated in Figure 1.9). The VE can then be moved between physical electrodes by modulating the ratio of current applied to each. This



(a) Physical electrode (PE) stimulation

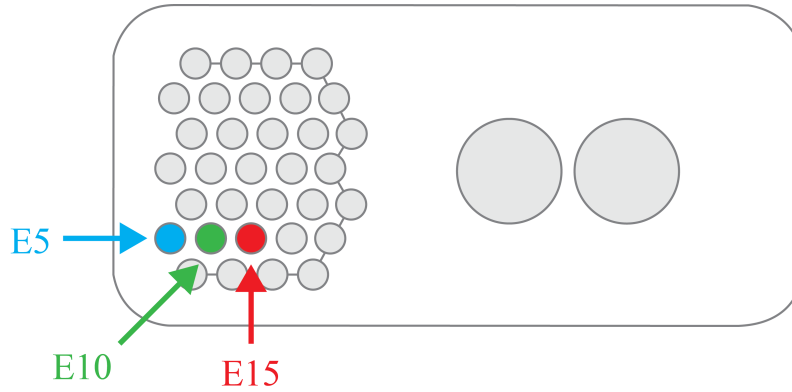


(b) Equally-weighted VE stimulation

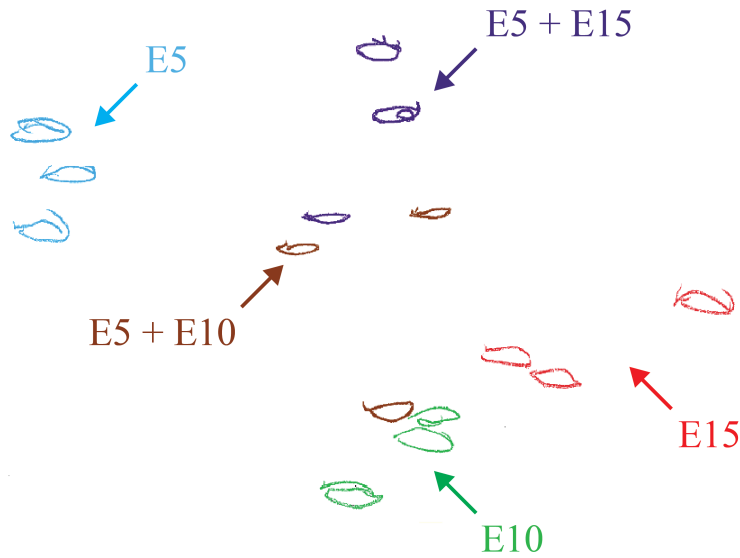


(c) Unequally-weighted VE stimulation

Figure 1.9: Representation of electrical field interactions for one-dimensional VE current steering using a linear electrode array. Electrode sites are numbered, and the size of the bars represent the relative intensity of stimulation at each site. Red dotted lines represent the shape of the individual electrical fields generated by each electrode, and the purple dotted lines represent the summed electrical field. In (b), equally weighted stimulation of electrodes 3 and 5 produce a summed electrical field with similar properties to that produced by stimulation of electrode 4 shown in (a). (c) demonstrates how the peak of the summed field can be shifted toward the electrode with the greater proportion of the charge.



(a) Diagram of human stimulating array



(b) Location of phosphenes in patients vision

Figure 1.10: Psychophysical data showing preliminary evidence of phosphenes evoked through current steering. This data is from a patient implanted with a suprachoroidal 33 -electrode device as part of a clinical trial carried out by Bionic Vision Australia between 2012 and 2014. The patient was asked to point to the location of phosphenes on a drawing board placed in front of them with the operator marking each pointed location. Phosphenes elicited by stimulation of physical electrodes E5, E10 and E15 stimulated are shown in blue, green and red respectively. Drawings of phosphenes elicited by steered stimulation between E5 and E10 are shown in brown, and those for steered stimulation between E5 and E15 are shown in purple. (Shivdasani et al, Unpublished data).

technique has been investigated using cochlear implants. A psychophysical study of a large number of cochlear implant recipients using VE current steering between electrode pairs demonstrated that an average of sixty-three discriminable auditory percepts could be elicited using only twelve electrodes (Firszt et al. 2007). This manner of current steering is also used clinically as part of Advanced Bionics' HiRes cochlear implant products. However, continued patient outcome testing has found that, despite increased spectral resolution and subjective comfort, little improvement is measured for speech understanding or music appreciation (Firszt et al. 2009, Buechner et al. 2008, Berenstein et al. 2008).

VE current steering has also been applied to retinal prostheses. A cortical study using a cat model also showed that the location of the centroid of cortical activation could be manipulated by varying the ratio of presented current between electrode pairs (Dumm et al. 2014). These findings showed that unique cortical patterns could be elicited using current steering and that the centroids of these patterns could be shifted as a function of the ratio of charge apportioned to each electrode (Dumm et al. 2014). These data provide strong evidence that current steering between electrodes is possible in at least one dimension in the retina. A pilot experiment was also conducted as part of the BVA suprachoroidal retinal implant clinical trials to investigate the perception of VEs in the eye. The patient was asked to point out the location of perceived phosphenes on a board in front of them while different combinations of electrodes were stimulated (three trials for each electrode). The marks made by the researcher in the areas that the patient pointed are shown in Figure 1.10. Individual stimulation of electrodes 5, 10 and 15 showed distinct regions of phosphene perception. Simultaneous stimulation of electrodes 5 and 10, and of electrodes 5 and 15, both elicited single phosphenes perceived in between the percepts elicited by the single electrodes. These findings indicate that current steering has the potential to increase the number of phosphenes that can be elicited for retinal prostheses recipients without increasing the number of physical electrodes. Using current steering, VEs could complement or replace phosphenes elicited via MP stimulation. Further investigation of this technique and optimisation for a 2D retinal array could provide a useful tool in conveying more complex visual information to patients.

Presently, the reach and limitations of VE current steering have not been fully explored. Current steering on a 1D array has been shown to be an effective way to produce VEs, however there are many opportunities to be investigated using a two-dimensional array. Deep brain stimulation linear arrays have shown that

more than two electrodes can contribute to the distribution of charge, but the more complex electrode arrangements that two-dimensional arrays afford could allow far greater control of the location of VEs. Cortical activation patterns to multi-electrode stimulation in the retina has been shown to yield repeatable and predictable results (Halupka et al. 2014, 2016). VEs created by electrode pairs can be shifted between neighbouring physical electrodes, however it is unclear whether the same can be achieved using 2D current steering, and whether this can be achieved in a similar predictable manner. The differences between cortical responses to physical and virtual electrode stimulation have also not been fully explored in past studies. It is uncertain that virtual electrode stimulation would effectively complement physical electrode stimulation in conveying complex visual information to patients (Spencer et al. 2016). Significant changes have been observed between cortical responses to stimulation of healthy and degenerate retinæ (Aplin et al. 2016b, Halupka et al. 2017). As with FMP stimulation, it is also uncertain how severe degeneration of the retina will impact the effectiveness of VE current steering given that inner retinal neurons are also activated by VE steering and they undergo significant remodelling in response to photoreceptor degeneration.

1.5 Research objectives

The work presented in this thesis investigates the effectiveness of two electrical field shaping techniques: FMP stimulation and 2D VE current steering. These experiments were conducted in normally-sighted and unilaterally-blinded cats. The following studies were designed with the aim of improving the spatial resolution of neural prosthetic devices, specifically retinal prostheses, constrained by surgical and engineering limitations.

1.5.1 Hypotheses

The hypotheses under investigation in the following studies are as follows:

1. FMP stimulation of the retina will result in more spatially discrete patterns of retinal and cortical activation than MP and HP stimulation.
2. VE current steering will produce activation patterns with similar spatial properties elicited by physical electrodes, by steering between multiple adjacent electrodes in a 2D arrangement.
3. VEs can be shifted in a predictable direction by altering the proportions of charge allocated to electrodes in a two-dimensional arrangement.

4. The advantages of FMP stimulation and 2D VE current steering will be maintained in a model of retinal degeneration.

1.5.2 Thesis structure

This thesis presents and discusses findings from work conducted over the course of my doctoral studies, investigating a number of electrical field shaping techniques in a preclinical setting. Generalised methodology for these studies are presented in the following chapter. Subsequent chapters present results from three different studies, two of which have been published in their entirety in academic journals (Chapters 3 and 4), and a third published as a full conference paper (Chapter 5).

The first two studies, presented in Chapter 3 and 4, detail an investigation in to the use of current focusing and current steering techniques respectively, in a normally-sighted cat model. Chapter 3 investigates whether FMP stimulation can be adapted for use on a two-dimensional array and evaluate its effectiveness in reducing the spread of neural activation compared with MP and HP stimulation. Previous studies investigating the effectiveness of HP stimulation as a method of improving the spatial selectivity of neural activation have shown conflicting results and have not been applied to suprachoroidal clinical electrode dimensions. This study aimed to reassess the use of HP stimulation in the retina, and attempt to translate the FMP methodology from a cochlear stimulation technique to a two-dimensional electrode array for retinal stimulation. Chapter 4 builds upon previous work in current steering research, and aimed to determine whether VEs can be produced in the retina using two-dimensional electrode arrangements.

Following promising results from the preceding chapters, Chapter 5 details the effectiveness of these same techniques in a cat model of retinal degeneration. Recipients of retinal implants have a severely degenerated visual system. The loss of photoreceptors and afferent input leads to extensive remodelling in the retina and central visual structures. In order to effectively validate these techniques and inform translational endeavours, we reassessed these techniques in a model that closer resembles the pathological state of patients with retinal degeneration.

The final chapter summarises the findings from the work presented in this thesis, places them in the context of existing literature, and discusses the implications for future work and clinical practice.

Chapter 2

General Methods

This general methods chapter describes methodology from the studies presented in the following three chapters and bears close resemblance to their respective methods sections. This chapter has been included to provide an overall and more comprehensive overview of the methods used during my PhD work. However, as Chapters 3 and 4 have been published in their entirety, significant portions of this chapter have also been published in their respective peer-reviewed journal articles:

Spencer, T. C., Fallon, J. B., Thien, P. C. & Shivdasani, M. N. (2016). Spatial restriction of neural activation using focused multipolar stimulation with a retinal prosthesis. *Investigative Ophthalmology & Visual Science*, 57(7), 3181-3191.

Spencer, T. C., Fallon, J. B. & Shivdasani, M. N. (2018). Creating virtual electrodes with 2D current steering. *Journal of Neural Engineering*, 15(3), 35002.

The following chapters present data from three distinct studies. All studies involved acute *in vivo* experiments where multichannel neural spiking responses were measured from the visual cortex of domestic cats in response to different forms of retinal electrical stimulation. The first study was an investigation of the effectiveness of focused multipolar (FMP) stimulation in eliciting more discrete activation of retinal and cortical neural populations than the more widely used monopolar (MP) stimulation technique. The second study investigated whether virtual electrode (VE) current steering can be adapted for use in two dimensions to create VEs that elicit cortical responses with similar characteristics to those elicited by physical electrodes (PEs) in predictable locations. The third study reevaluated these experiments in a

feline model of retinal degeneration in order to gain a better understanding of the clinical implications of these field shaping techniques, and to investigate the differing responses of a healthy and degenerate visual system to electrical stimulation.

All procedures were approved by the Bionics Institute Animal Research Ethics Committee (Project #14/304AB), were in accordance with the Australian Code of Practice for the Care Use of Animals for Scientific Purposes (8th edition) and with the National Institute of Health, USA guidelines regarding the care and use of animals for experimental procedures. Twelve domestic cats were used in these studies.

2.1 Blinding procedure

The blinding procedure used was adapted from work carried out in a collaboration between the Bionics Institute and the Centre for Eye Research Australia (CERA) as detailed in Aplin, Luu, Vessey, Guymer, Shepherd & Fletcher (2014). Intravitreal introduction of adenosine triphosphate (ATP) has been shown to result in apoptosis of photoreceptors within ~ 36 hours along with significant retinal remodeling features seen after 12 weeks post injection. This technique was chosen in order to approximate the retinal health of patients with retinitis pigmentosa (RP).

Prior to intravitreal injection, cats were anaesthetised with ketamine (intramuscular [i.m.], 20 mg kg^{-1}) and xylazil (subcutaneous [s.c.], 2 mg kg^{-1}). Baseline retinal health was assessed using optical coherence tomography (OCT), dark-adapted electroretinogram (ERG), and fundus imaging. A paracentesis was performed to prevent dangerous increase in intravitreal pressure after injection of ATP solution. A $200 \mu\text{l}$ solution of three parts sterile saline ($0.9\% \text{ NaCl}$) and one-part Dexamethasone (4 mg ml^{-1}), containing approximately 200 mM to 217 mM ATP disodium salt (Sigma-Aldrich Corp., St Louis, MO, USA) was injected into the intravitreal space of one randomly chosen eye. Dosages were determined prior to the procedure in collaboration with clinical ophthalmologists. Two weeks following recovery, retinal health was reassessed under anaesthesia in order to determine whether the ATP solution had successfully resulted in photoreceptor death. We considered a minimum 50% reduction in a-wave amplitude of a dark-adapted ERG from baseline to be our threshold for significant global photoreceptor loss. For more localised assessment, outer retinal thickness obtained from OCT and pigment deposits in fundus imaging in the area centralis region of the retina was assessed by a clinical ophthalmologist. If it was determined that there was not a sufficient level of degeneration, an additional dose of ATP solution was injected into the eye. The acute electrophysiological

experiments were carried out at least 14 weeks following observation of significant degeneration in order to allow time for retinal remodelling to occur.

2.2 Anaesthetic protocols

During the experimental phase of these studies there was an increase in the regulation of pentobarbitone in Australia making it more difficult to acquire. As a result, two different anaesthetic protocols were used for terminal acute electrophysiological experiments. As shown in Chapter 4.3.1, we found there were no significant differences in electrically-evoked cortical responses between the two protocols.

2.2.1 Anaesthetic protocol for normally-sighted cohort

Normally sighted adult cats ($n=8$) were pre-medicated with ketamine (intramuscular [i.m.], 20 mg kg^{-1}) and xylazil (s.c., 2 mg kg^{-1}). Anaesthesia was maintained over the experimental period for up to three days with a continuous intravenous (intravenous (i.v.)) infusion of sodium pentobarbitone ($3 \text{ mg kg}^{-1} \text{ h}^{-1}$ to $8 \text{ mg kg}^{-1} \text{ h}^{-1}$). Respiration rate, end-tidal CO_2 , blood pressure and temperature were monitored consistently throughout the experiment and maintained at normal levels by adjusting the anaesthetic flow rate. A continuous i.v. infusion of Hartmann's solution (sodium lactate, $2.5 \text{ mg kg}^{-1} \text{ h}^{-1}$) and daily injections of dexamethasone (i.m., 0.1 mg kg^{-1}) and clavulox (s.c. 10 mg kg^{-1}) were also administered. Pupils were dilated by regular topical application of a mixture of phenylephrine hydrochloride (2.5%) and tropicamide (1%). Following the completion of the experiment all animals were euthanised via an overdose of pentobarbitone.

2.2.2 Anaesthetic protocol for unilaterally-blinded cohort

Unilaterally-blinded adult cats ($n=4$) were pre-medicated with ketamine (i.m., 8 mg kg^{-1}), medetomidine (i.m., 0.012 mg kg^{-1}), and methadone (i.m., 0.4 mg kg^{-1}). Anaesthesia was maintained with continuous i.v. infusion of Propofol (24 mg kg^{-1}). A continuous i.v. infusion of methadone in Hartmann's solution (0.5 ml of 10 mg ml^{-1} Methadone in 250 ml compound sodium lactate, $0.05 \text{ mg kg}^{-1} \text{ h}^{-1}$) was also administered throughout the period of anaesthesia. Due to the greater depressive effect of Propofol on the respiratory system compared with pentobarbitone, tracheostomies were performed and the animals ventilated on 100% oxygen (20 to 25 breaths/min) (model 6025; Ugo Basile, Monvalle, VA, Italy). Respiration rate, heart rate, end-

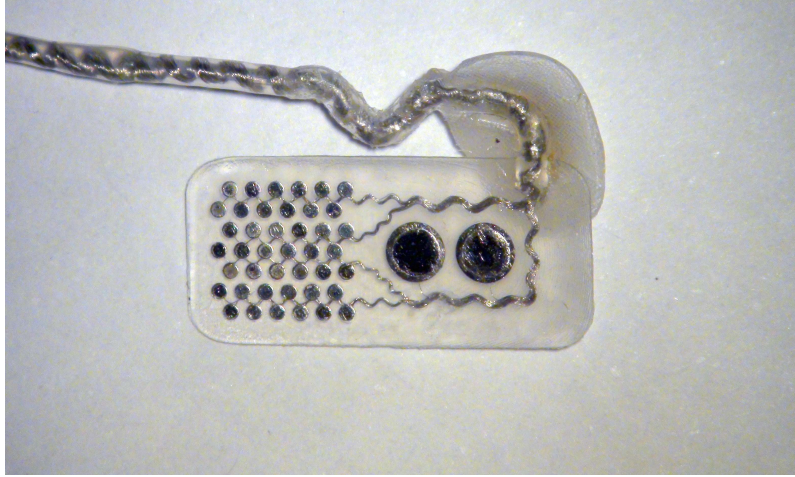


Figure 2.1: Photograph of a preclinical 44-channel suprachoroidal electrode array. Stimulating electrodes are $600\text{ }\mu\text{m}$ in diameter and 1 mm centre-to-centre. The larger electrodes are used to return current.

tidal CO_2 , blood pressure and temperature were monitored consistently throughout the experiment and maintained at normal levels by adjusting the anaesthetic flow rate. Daily injections of dexamethasone (i.m.. 0.1 mg kg^{-1}) and clavulox (s.c. 10 mg kg^{-1}) were administered to all four cats. Pupils were dilated by regular topical application of a mixture of phenylephrine hydrochloride (2.5%) and tropicamide (1%). Following the completion of the experiment all animals were euthanised via an overdose of Propofol.

2.3 Surgical procedures

2.3.1 Suprachoroidal implantation

The suprachoroidal electrode array was similar to that which has been used in previous work (Ayton et al. 2014, Villalobos et al. 2013), fabricated on a biocompatible silicon substrate and consisting of 42 platinum electrodes of $600\text{ }\mu\text{m}$ diameter spaced 1 mm from centre to centre. The implantation procedure is detailed in our previous studies (Saunders et al. 2014, Villalobos et al. 2013). Briefly, following a lateral canthotomy, scleral incision, and dissection of a pocket between the sclera and choroid, the array was inserted $\sim 15\text{ mm}$ into the suprachoroidal space until the tip was beneath area centralis. Electrical connections to the electrodes on the array were tested using an automated impedance monitoring software developed in LabVIEW (National Instruments, Austin, TX, USA) used in previous studies (John et al. 2011). The animal was placed in a stereotaxic frame inside a darkened electrically shielded

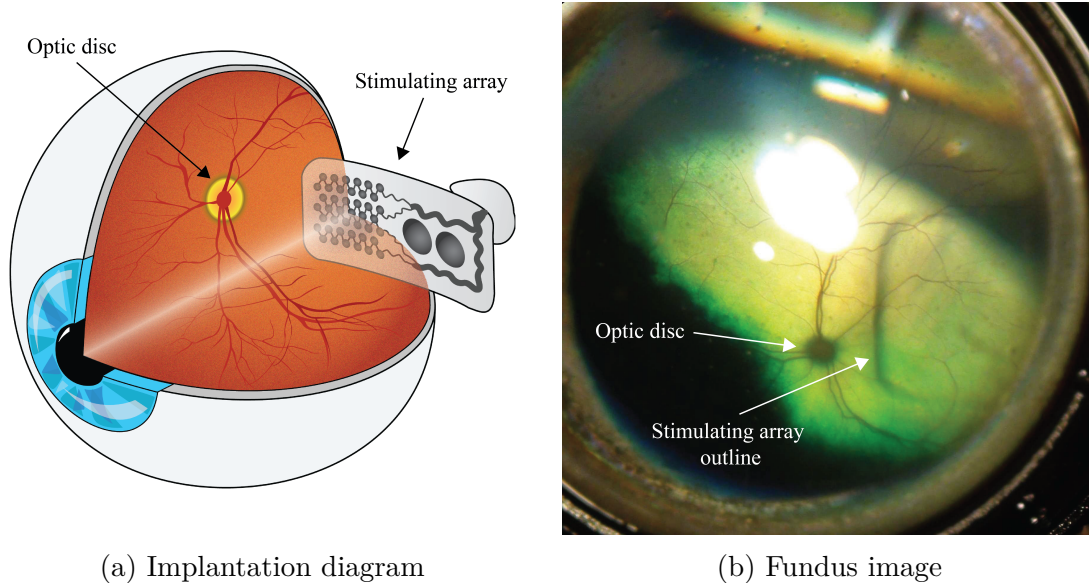


Figure 2.2: Images illustrating positioning of the stimulating electrode array in the eye. (a) Diagram showing the location of the array over the area centralis in a 3D eye cross section. (b) Fundus image of an implanted cat eye. the protrusion of the array in the suprachoroidal pocket can be seen on the inner surface of the retina.

room. Visual inspection of the eye and fundus photographs were taken to determine the health of the eye and positioning of the array. For the normally-sighted cohort, a single eye was chosen at random to be implanted. For the unilaterally-blinded cohort, the healthy eyes were implanted with a stimulating array along with the degenerate eyes to act as a control.

2.3.2 Cortical implantation

The visual cortex was chosen as our recording site due to its well-understood organisation and topography. Unlike other structures in the visual pathway, access to the cortex requires no significant damage to brain structures. Its relatively large surface area also allows for the implantation of multiple high-density multi-electrode recording arrays. Craniotomies were performed spanning 15 mm rostral and 5 mm caudal from the interaural line, and 7 mm lateral from the sagittal suture on the side contralateral to the implanted eye, exposing the visual cortex. For the unilaterally-blinded cohort, a larger craniotomy was performed that extended 7 mm each side of the sagittal suture, exposing both hemispheres of the brain and allowing the recording of contralateral evoked responses to electrical stimulation of both implanted eyes. The dura mater was carefully excised from the region. Using two parylene-C-based flexible platinum micro-electrocorticography arrays (as described in Fallon et al. (2016)), electrically evoked potentials (EPs) in response to cathodic-leading

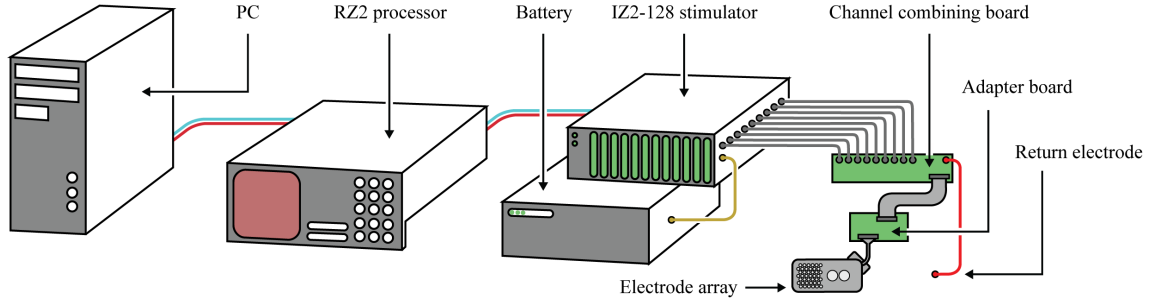


Figure 2.3: Overview of the stimulation hardware. A digital waveform and list of current amplitudes are sent from the PC to the RZ2 processor. The waveform is converted to analogue and sent to the IZ2 stimulator. The 128-channel output is condensed to 42 channels and connected to the implantable electrode array. Current is returned through a platinum electrode placed in the conjunctiva of the implanted eye.

biphasic charge-balanced current pulses ($0\text{--}750\text{ }\mu\text{A}$, 1 ms per phase) applied to the suprachoroidal retinal electrodes were mapped along the surface of the visual cortex to determine the cortical region with the lowest EP thresholds. Up to two planar “Utah” 60- (6×10) or 36- (6×6) channel penetrating microelectrode arrays (Blackrock Microsystems, Foxborough, MA, USA) were inserted in the regions of the visual cortex (VC) with the lowest EP thresholds to a depth of approximately 1 mm . The recording electrodes were separated by a distance of $400\text{ }\mu\text{m}$ and sampled $\sim 7.2\text{ mm}^2$ and $\sim 4\text{ mm}^2$ of the cortex for the 60- and 36-channel arrays respectively. The size and number of electrode arrays used for each animal was determined by the prevalence and location of large blood vessels, as care was taken to minimise damage these structures during insertion.

2.4 Electrophysiological Analysis

2.4.1 Retinal stimulation

Electrical stimuli were generated with a 128-channel IZ2 stimulator (Tucker-Davis Technologies, Alachua, FL, USA). Due to the low maximum current output per channel ($300\text{ }\mu\text{A}$), each group of three channels was combined using a custom-built circuit board bringing the maximum functional number of combined stimulating channels to 42 and the maximum current output to $900\text{ }\mu\text{A}$ per combined channel. Each combined channel was connected via a second breakout board to an electrode on the suprachoroidal array enabling independent simultaneous stimulation of all 42 electrodes. Digital current waveforms were produced using custom-written MATLAB scripts and were, using an ActiveX API as an interface, converted to analogue

waveforms using an RZ2 processor and an IZ2 isolated stimulator (Tucker-Davis Technologies, Alachua, FL, USA). Pulses were presented at randomly varied currents ranging from 0 μA to 750 μA in 50 μA steps at a repetition rate of 1 Hz. Each current step was repeated 10 times. Stimulus pulses were cathodic-first and symmetrically biphasic with a 1000 μs phase width and 25 μs interphase gap. Either all the charge from each pulse was presented to a single electrode or the total charge was split across groups of electrodes for steered stimulation according to predefined charge ratios. Thus the maximum charge delivered to the retina was capped to 750 nC corresponding to a maximum charge density of 265 μCcm^{-2} (for single electrode stimulation) which is below the safe limit for gassing when using platinum electrodes (Leung et al. 2015). Platinum needle electrodes were placed in the conjunctiva to provide an extraocular return path for MP, FMP, and VE stimulation.

2.4.2 Adapted FMP methodology

Unlike hexapolar (HP) stimulation, FMP stimulation actively stimulates surrounding electrodes according to predefined weights. FMP weight calculations were initially for use on a linear intracochlear electrode array by van den Honert & Kelsall (2007) and, to our knowledge, had not previously been adapted for use in two dimensions. Their publication describes a method for producing focused electrical stimulation at a single electrode site by simultaneously delivering positive and negative current ratios to all other electrodes calculated to nullify voltage changes. In these studies, the electrode site where we desire to produce a focused electrical field is referred to as the primary stimulating electrode (PSE) and the surrounding stimulation weights are referred to as the ‘phased array’. Phased array values are calculated using previously obtained transimpedance measurements between each electrode of the implanted array. This was achieved by connecting the implanted stimulating electrode array to a 512 cross-point switch matrix (PXI 2523, National Instruments, Austin, TX, USA). Channels of the switch matrix were also connected to an isolated constant current stimulator and a digital multimeter (PXI 4072, National Instruments, Austin, TX, USA), allowing stimulation and voltage recording at any electrode site. Voltage waveforms were collected from every electrode in response to MP stimulation of every other electrode. Each trial was repeated five times and the waveforms were averaged. Transimpedance was calculated for each stimulating/recording electrode combination by dividing the voltage measured at the end of the first phase by the current. These values are then arranged into a matrix where the columns corresponded to stimulating electrode, and the rows to recording electrode as in the following example:

$$Z_m = \begin{bmatrix} Z_{E1,E1} & Z_{E2,E1} & \dots & Z_{E42,E1} \\ Z_{E1,E2} & Z_{E2,E2} & \dots & Z_{E42,E2} \\ \vdots & \vdots & \ddots & \vdots \\ Z_{E1,E42} & Z_{E2,E42} & \dots & Z_{E42,E42} \end{bmatrix}$$

Where $Z_{x,y}$ is the transimpedance between stimulating electrode x and recording electrode y . Transimpedance measurements where the stimulating and recording electrodes are the same (on the diagonal of the matrix) can not be accurately measured due to the effects of polarization on the electrode carrying the current pulse. Therefore van den Honert & Kelsall (2007) devised a method of estimating these values using linear extrapolation from surrounding adjacent electrode pairs in the matrix according to the following expression:

$$Z_{j,j} = \max(2Z_{j+1,j} - Z_{j+2,j}, 2Z_{j-1,j} - Z_{j-2,j}, 2Z_{j,j+1} - Z_{j,j+2}, 2Z_{j,j-1} - Z_{j,j-2})$$

As a retinal array is two dimensional, we repeated this technique in up to six directions, yielding, depending on electrode positioning, up to twelve extrapolated values. The maximum extrapolated value was chosen as the transimpedance of each stimulating electrode on the diagonal. Transimpedance values between each electrode pair should ideally be identical irrespective of which electrode acted as the stimulating electrode, as such it is assumed that any deviation is attributed to noise. In order to improve the signal-to-noise ratio, the $Z_{x,y}$ is averaged with its transpose ($Z_{x,y}^T$), producing a new diagonally symmetric transimpedance matrix. The matrix was then inverted ($Y = Z^{-1}$), yielding a matrix of transadmittance values. Each column (j) of transadmittances was then normalised to the admittance values on the diagonal of the matrix, to produce a unique phased array for each PSE.

2.4.3 Cortical recording

Cortical recordings were analysed using custom scripts written in Igor Pro (Wave-metrics, Lake Oswego, OR, USA) and MATLAB (Mathworks, Natick, MA, USA). Signal artefacts were removed using techniques described by Heffer and Fallon (Heffer & Fallon 2008). The signal was bandpass filtered after artefact removal (Butterworth filter 0.3-5 kHz; order 3) and spikes were timestamped when the signal exceeded 4 times the root mean square value. Spikes detected within a 3- 20 ms window were included in the analysis, as it is hypothesised to corresponding to

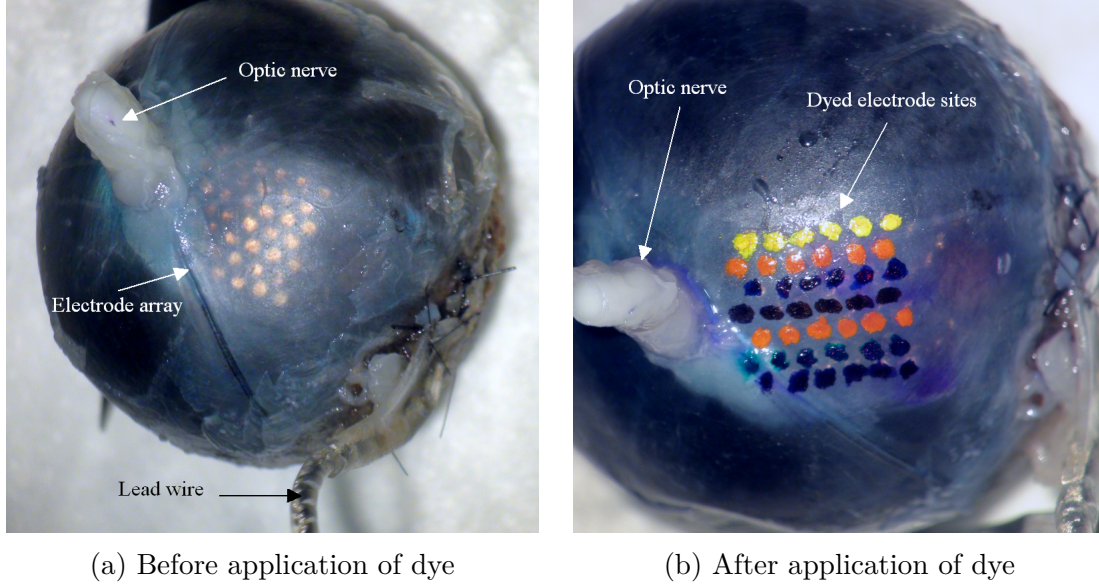


Figure 2.4: Images showing the application of dye to the sclera of an enucleated eye. Note that the electrodes are clearly visible through the sclera due to their reflective nature. Coloured dye is applied at each electrode site to provide a reference for analysis of histological sections.

both direct activation of retinal ganglion cells and indirect activation of retinal circuitry (Boinagrov et al. 2014), and to remain consistent with earlier studies (Shivdasani et al. 2010, Wong et al. 2008, Spencer et al. 2016, Dumm et al. 2014, Cicione et al. 2012). For each stimulus trial, the number of spikes were also counted 3-20 ms prior to the stimulus in order to estimate the spontaneous firing rate. The average spontaneous firing rate over the ten stimulus repeats was subtracted from spike rates recorded during the evoked response period. Following cortical implantation, evoked potentials were recorded in response to full-field flashes (0.001 cdm^2 to 3 cdm^2) to determine whether the implanted region can be driven by light stimulus.

2.5 Histology

Following euthanasia, without removing the stimulating arrays, both eyes of each unilaterally-blinded animal were enucleated and placed in Davidson’s fixative for 18h to 36h at room temperature. The eyes were then transferred to a 50% ethanol solution for 6h to 8h before being stored in 70% ethanol at 4%. As described in Nayagam et al. (2013), coloured dye was applied to the outer sclera to indicate the location of each electrode before the array was carefully removed from the supra-choroidal space (as shown in Figure 2.4). Full thickness strips of retinal, choroidal, and scleral tissue were cut from the implanted regions and embedded in agar before

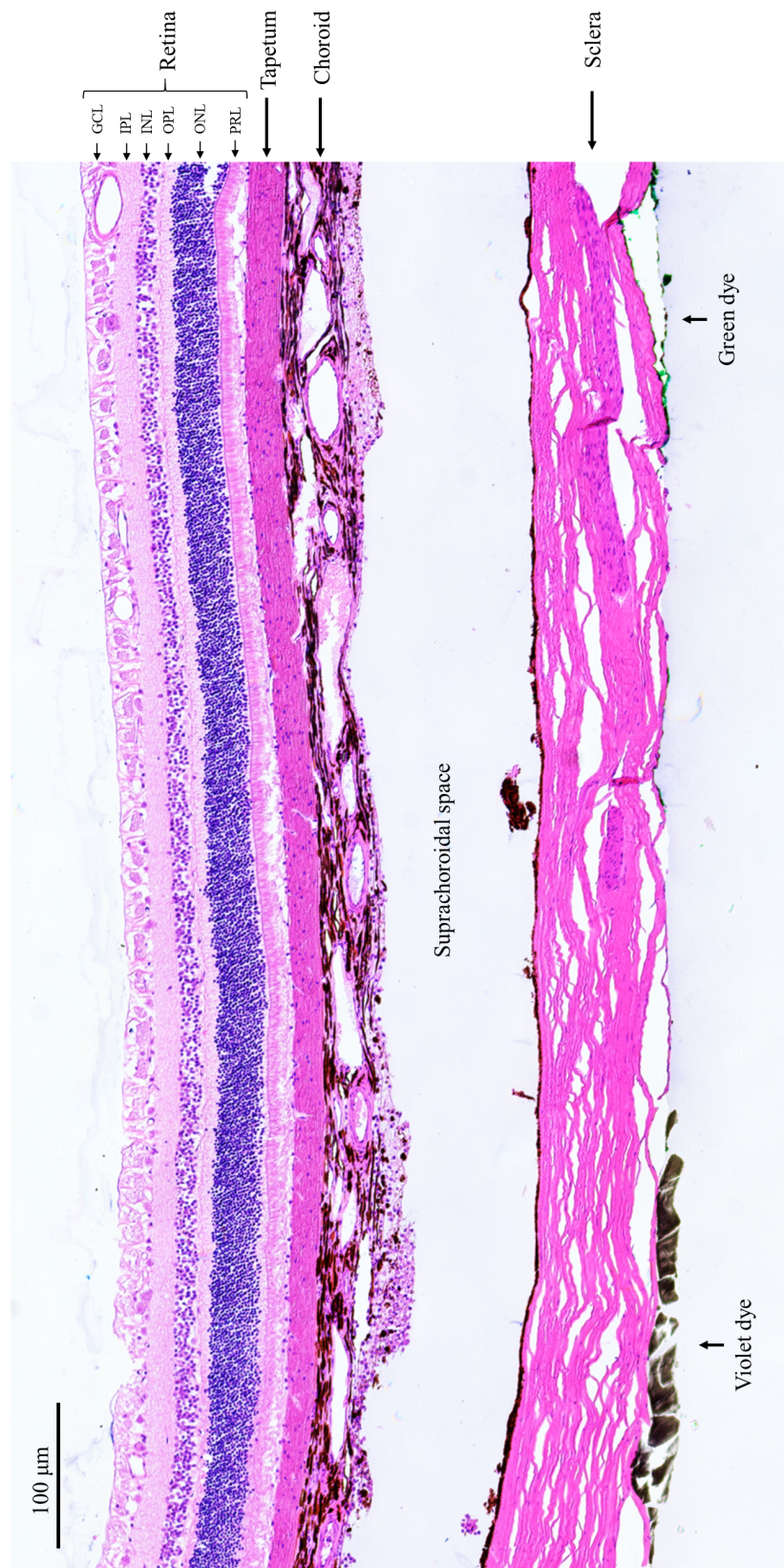


Figure 2.5: Histological section of the full wall of the eye showing how the coloured dye applied to the sclera is used to indicate electrode location.

being transferred to a 10% neutral-buffered formalin solution for 1 h to 3 h. The tissue was then embedded in paraffin in a standard automated cycle, cutting 5 μm thick vertical sections with a microtome and staining with hematoxylin and eosin (H&E). Using the scleral dye as reference, as shown in Figure 2.5, 800 μm -wide micrographs near each electrode site were taken with an Axio Imager 2 upright microscope (Carl Zeiss AG, Oberkochen, Germany) and Axiovision V4.8.2 software. Dehydration of the tissue due to the use of paraffin embedding resulted in a distortion of retinal layer thicknesses. As such, inner nuclear layer (INL) cell counts were obtained through manual counting. outer nuclear layer (ONL) cell counts were obtained through the use of custom scripts written in ImageJ (National Institutes of Health, Bethesda, MD, USA) and MATLAB. In many regions of the degenerate retinae there was severe breakdown of outer photoreceptor segments and the nuclear layers leading to cell migration and distortion of the retina's laminar structure. INL cells were defined in this study as any cell body present between the outer and inner plexiform layers. ONL cells were defined as any cell body present between the outer plexiform layer and the inner limiting membrane. Cell bodies that had migrated to the plexiform layers or in to the space left vacant by absent outer segments were not counted. In the few cases of extreme degeneration and remodelling, counting was undertaken with advice from a histologist.

Chapter 3

Spatial restriction of neural activation using focused multipolar stimulation

This chapter has been published in its entirety as the following peer-reviewed journal article:

Spencer, T. C., Fallon, J. B., Thien, P. C. & Shivdasani, M. N. (2016). Spatial restriction of neural activation using focused multipolar stimulation with a retinal prosthesis. *Investigative Ophthalmology & Visual Science*, 57(7), 3181-3191.

Objective: The resolution provided by present state-of-the-art retinal prostheses is severely limiting for recipients, partly owing to the broad spread of activation in the retina in response to monopolar (MP) electrical stimulation. Focused multipolar (FMP) stimulation has been shown to restrict neural activation in the cochlea compared with MP stimulation. In this study we extended the FMP stimulation technique to a two-dimensional electrode array and compared its efficacy to MP and hexapolar (HP) stimulation in the retina.

Approach: Normally-sighted cats (n=6) were implanted with a suprachoroidal electrode array containing 42 electrodes. Multichannel multiunit spiking activity was recorded from the visual cortex (VC) in response to MP, HP and FMP retinal stimulation.

Results: When inferring retinal spread using voltage recordings off the stimulating array, FMP stimulation showed significantly reduced voltages in regions surround-

ing the primary stimulating electrode. When measuring the retinal and cortical selectivity of neural responses, FMP and HP stimulation showed significantly higher selectivity compared to MP stimulation (separate two way ANOVAs, $p < 0.05$). However, the lowest cortical thresholds for each stimulating electrode were found to be higher for FMP and HP compared to MP stimulation (one-way analysis of variance (ANOVA), $p < 0.001$). No significant differences were observed between FMP and HP stimulation in any measures.

Conclusions: FMP and HP stimulation using a two-dimensional array are promising techniques to reduce the spread of activation for a retinal prosthesis. Clinical application would be expected to result in smaller phosphenes, thus reducing phosphene overlap between electrodes and increasing the resolution at the expense of higher thresholds for activation.

3.1 Introduction

Retinitis pigmentosa (RP) is one of the most common forms of visual impairment, with over one million people worldwide affected (Hartong et al. 2006). It is characterised by a progressive loss of photoreceptors in the retina resulting in the majority of RP sufferers becoming legally blind by the age of 40. There is no treatment currently available to halt or reverse the degenerative effects (Hartong et al. 2006). As a result, prosthetic devices have emerged as a way to restore function by providing artificial input to the visual system (Fujikado et al. 2011, Humayun et al. 2012, Ayton et al. 2014, Zrenner et al. 2011, Shepherd et al. 2013). These devices work by electrically stimulating residual neurons in the visual pathway (Shepherd et al. 2013). The retina has become a favoured region for prosthetic intervention due to a well-understood topographic map of visual space (Tusa 1978), its early placement in the visual processing pathway, the relative ease of surgical access, and reasonable preservation of non-photoreceptor neural circuitry (Santos et al. 1997, Jones et al. 2003). When individual electrodes on a retinal prosthesis are stimulated, patients are able to perceive discrete flashes of light (Ahuja et al. 2011, Shivdasani et al. 2014). These flashes, termed phosphenes, are used as building blocks to generate an artificial image, much like pixels on a computer monitor. A number of groups have undertaken human clinical trials that utilise either the epiretinal (Humayun et al. 2012, De Balthasar et al. 2008, Klauke et al. 2011) subretinal (Wilke et al. 2011a, Rizzo et al. 2003, Stingl et al. 2013) or suprachoroidal (Fujikado et al. 2011, Ayton et al. 2014) locations for implantation of electrode arrays.

Of the three commercially available devices currently available today, Second Sight’s Argus II and the Pixium IRIS utilise epiretinal placement and Retina Implant AG’s Alpha AMS is implanted subretinally. Suprachoroidal placement, on the other hand, has the advantage of a relatively non-invasive implantation procedure (Villalobos et al. 2013, Saunders et al. 2014) albeit with higher levels of stimulation required to reach activation thresholds (Ayton et al. 2014, Yamauchi et al. 2005, Fujikado et al. 2007). Higher stimulation levels have a detrimental effect on charge containment, impeding the formation of discrete phosphenes and limiting the visual resolution afforded to the patient. Being able to discriminate between phosphenes is an important factor in comprehending the image presented, which may not be possible using conventional methods of stimulation (Zrenner et al. 2011, Chen et al. 2009). Another factor that may limit resolution is the interactions between overlapping electric fields generated by simultaneous stimulation of electrodes (Wilke et al. 2011b, Horsager et al. 2010). Channel interaction can result in activation of neural regions outside of the individual range of each electrode due to the effects of the summed current fields. This is an established phenomenon in cochlear (George et al. 2015, Boex et al. 2003, Bierer 2007) and retinal implants (Wilke et al. 2011b, Horsager et al. 2010, Gerhardt et al. 2010, Lovell et al. 2005, Horsager et al. 2011), resulting in unpredictable spectral or spatial smearing of the percept. While this is typically circumvented using sequential stimulation presented within the flicker fusion rate of the visual system (~ 50 Hz), spatiotemporal interactions between neural populations will become more prominent as timing is compressed to accommodate for an increasing number of stimulating electrodes (Horsager et al. 2010, 2011).

Current focusing techniques have been suggested to restrict the spread of current with the aim of reducing channel interactions and producing more discrete phosphenes (Wilke et al. 2011b, Cicione et al. 2012, Matteucci et al. 2013). Cochlear implant studies show that the electrode return mode used during stimulation, such as bipolar and tripolar stimulation, can significantly reduce the spread of neural activation in the cochlea and can also reduce the extent of auditory brain activation (George et al. 2014, 2015, Berenstein et al. 2008, Pfingst et al. 1997). Similar approaches have been attempted in the retina such as the use of bipolar stimulation (Cicione et al. 2012, Wong et al. 2009), common ground returns (Cicione et al. 2012), hexapolar (HP) returns (Cicione et al. 2012, Matteucci et al. 2013, Wong et al. 2009, Shivdasani et al. 2012, Matteucci et al. 2016), and quasimonopolar modes (Matteucci et al. 2013) with varying levels of success. HP stimulation, where the six electrodes surrounding each stimulating electrode are connected to each other and used a return or ‘guard’, has been shown in previous studies to increase the

specificity of activation in the retina and reduce electrical cross-talk (Cicione et al. 2014, Matteucci et al. 2016, Habib et al. 2013). However, this technique also results in a significant increase in stimulation threshold to elicit a cortical response (Cicione et al. 2014, Matteucci et al. 2013, Wong et al. 2009, Habib et al. 2013), which is consistent with studies showing increased perceptual thresholds for cochlear implant patients when receiving bipolar and tripolar stimulation (Bierer 2007, George et al. 2015, Wu & Luo 2013). While, at matched current levels, cortical activation has shown to be reduced with HP stimulation compared to monopolar (MP) stimulation (Wong et al. 2009); when responses have been compared at the same point along the dynamic range of each stimulation mode, no significant change in cortical spread has been found (Cicione et al. 2012). Also, reports from patients when asked to compare the appearance of phosphenes generated by MP and HP stimulation have anecdotally suggested no evidence of size reduction with HP stimulation (Sinclair et al. 2016). These results indicate that while HP stimulation can increase retinal specificity, cortical specificity and beneficial perceptual effects have not yet been achieved.

Focused multipolar (FMP) is a technique pioneered in cochlear implants that utilizes simultaneous stimulation of multiple electrodes according to positive and negative weights in order to restrict the electric field and reduce the spread of neural activation (van den Honert & Kelsall 2007). Current weights are calculated by measuring the impedances between electrodes on the array and inferring the currents required to nullify voltage changes in the region surrounding a specific electrode (van den Honert & Kelsall 2007). This form of stimulation has been validated using cochlear implants in clinical and animal studies showing reduced intracochlear voltages⁴³, increased spectral resolution in patients (Smith et al. 2013), and reduced spread of neural activation in the inferior colliculus of cats (George et al. 2014). However, the benefits of FMP stimulation have been restricted to a one-dimensional cochlear implant electrode array and this technique has not previously been assessed in the retina, or adapted for use on a two-dimensional electrode array. It is possible that the FMP technique may reduce the spread of neural activity over that found with HP stimulation. The HP return mode uses symmetrical negatively weighted flanking electrodes, while, as detailed in this study, FMP weights often show significant asymmetry. Failure to account for individual electrode impedances and the local tissue environment may in part explain why HP stimulation has not been shown to achieve reductions in spread of cortical activity.

In this study we investigated the efficacy of FMP stimulation in reducing current

spread and neural activation through recordings in the visual cortex (VC). Comparisons made between recordings taken from the VC of implanted cats in response to MP, HP and FMP stimulation were used to evaluate the relative effectiveness of these stimulation modes in producing focused activation.

3.2 Methods

3.2.1 Anaesthesia and surgery

All procedures were approved by the Bionics Institute Animal Research Ethics Committee (Project #14/304AB), complied with the ARVO statement for use of animals in ophthalmic research, and were in accordance with the Australian Code of Practice for the Care Use of Animals for scientific purposes and with the National Institutes of Health, USA guidelines regarding the care and use of animals for experimental procedures. Normally sighted adult cats ($n=6$) were anaesthetised with ketamine (intramuscular [i.m.], 20 mg kg^{-1}) and xylazil (subcutaneous [s.c.], 2 mg kg^{-1}). Anaesthesia was maintained over the experimental period for up to three days with a continuous intravenous [i.v.] infusion of sodium pentobarbitone ($3 \text{ mg kg}^{-1} \text{ h}^{-1}$ to $8 \text{ mg kg}^{-1} \text{ h}^{-1}$). Respiration rate, end-tidal CO_2 , blood pressure and temperature were monitored consistently throughout the experiment and maintained at normal levels by adjusting the anaesthetic flow rate. A continuous i.v. infusion of Hartmann's solution (sodium lactate, $2.5 \text{ mg kg}^{-1} \text{ h}^{-1}$) and daily injections of dexamethasone (i.m. 0.1 mg kg^{-1}) and clavulox (s.c. 10 mg kg^{-1}) were also administered. Pupils were dilated by regular topical application of a mixture of phenylephrine hydrochloride (2.5%) and tropicamide (1%).

The suprachoroidal electrode array was similar to that which has been used in our previous work (Ayton et al. 2014, Villalobos et al. 2013), fabricated on a biocompatible silicon substrate and consisting of 42 platinum electrodes of $600 \mu\text{m}$ diameter spaced 1 mm from centre to centre. The implantation procedure is detailed in our previous studies (Saunders et al. 2014, Villalobos et al. 2013). Briefly, following a lateral canthotomy, scleral incision, and dissection of a pocket between the sclera and choroid, the array was inserted $\sim 15 \text{ mm}$ into the suprachoroidal space until the tip was beneath area centralis. Electrical connections to the electrodes on the array were tested using an automated impedance monitoring software developed in LabVIEW (National Instruments, Austin, TX, USA) used in previous studies (John et al. 2011). The animal was placed in a stereotaxic frame inside a darkened electrically shielded room. Visual inspection of the eye and fundus photographs were taken

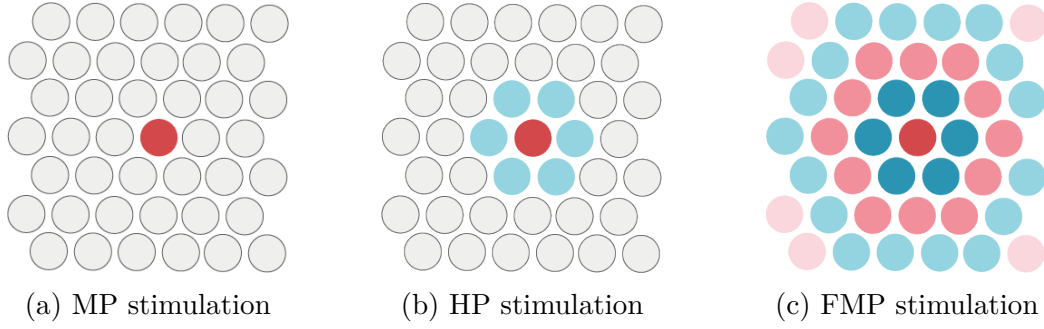


Figure 3.1: Illustration of electrode stimuli weights for MP, HP, and FMP stimulation techniques. MP stimulation is represented as a single stimulating electrode. HP stimulation is shown to have negative weights on the six surrounding electrodes as these are used as return electrodes. FMP weights often presented as concentric rings of alternating positive and negative currents radiating from a central electrode. Each ring aims to cancel out the spread of current produced by the former.

to determine the health of the eye and positioning of the array. A craniotomy was then performed spanning 15 mm rostral and 5 mm caudal from the interaural line, and 7 mm lateral from the sagittal suture on the side contralateral to the implanted eye, exposing the VC. The dura mater was carefully excised from the region. Using two parylene-based flexible platinum electrode arrays, electrically evoked potential (EP) in response to cathodic-leading biphasic charge-balanced current pulses (0 μA to 750 μA , 1 ms per phase) were mapped along the surface of the VC to determine the cortical region with the lowest evoked potential thresholds. Up to two planar “Utah” 36- (6x6) or 60- (6x10) channel penetrating microelectrode arrays (Blackrock Microsystems, Foxborough, MA, USA) were inserted in the regions of the VC with the lowest evoked potential thresholds to a depth of approximately 1 mm. The recording electrodes were separated by a distance of 400 μm and sampled $\sim 7.2 \text{ mm}^2$ and $\sim 4 \text{ mm}^2$ of the cortex for the 60- and 36- channel arrays respectively. The size and number of electrode arrays used for each animal was determined by the prevalence and location of large blood vessels, as care was taken to minimise damage these structures during insertion.

3.2.2 Experimental protocols

Electrical stimuli were generated with a 128-channel IZ2 stimulator (Tucker-Davis Technologies, Alachua, FL, USA). Due to the low maximum current output per channel (300 μA), each group of three channels was combined using a custom-built circuit board bringing the maximum functional number of combined stimulating channels to 42 and a maximum current output to 900 μA per combined channel. Each combined channel was connected directly to each electrode on the suprachoroidal array

enabling independent simultaneous stimulation of all 42 electrodes. Three different modes of stimulation were trialled: (a) MP stimulation, whereby a single electrode was stimulated against an extraocular platinum return electrode inserted in to the conjunctiva of the implanted eye; (b) HP stimulation, where a single electrode was stimulated against the immediately surrounding ‘hexagon’ of electrodes shorted together as a return; and (c) FMP stimulation, where all electrodes on the array were stimulated simultaneously against the extraocular platinum return electrode using positive and negative weights, with one of these electrodes chosen as the primary stimulating electrode (PSE) with a weight equal to 1. Pulses were presented at randomly varied currents ranging from 0 μA to 750 μA in 50 μA steps at a repetition rate of 1 Hz. Each current step was repeated 10 times. Stimulus pulses were symmetrically biphasic with a 1000 μs phase width and 25 μs interphase gap. Thus the maximum charge on each electrode was capped to 750 nC corresponding to the maximum charge density of 265 μCcm^{-1} which is below the safe limit for gassing when using platinum electrodes (Leung et al. 2015). MP, HP, and positively weighted FMP stimulation pulses were presented cathodic-first, whereas negatively weighted FMP pulses were presented anodic first. For electrodes on the edge of the array without a full complement of six flanking electrodes, the HP mode only consisted of a partial ring of electrodes as a return directly adjacent to the stimulating electrode.

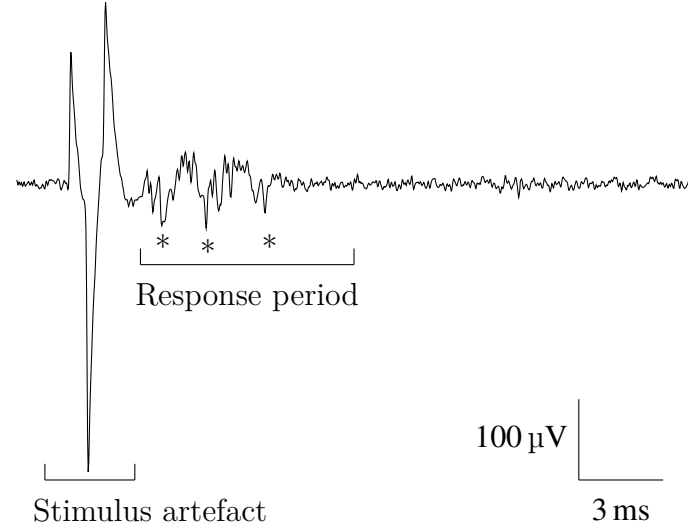
FMP weights were constructed using methods adapted from van den Honert & Kellsall (2007) for the cochlear implant. A transimpedance matrix was constructed by stimulating each electrode in turn while recording voltages on every other electrode. To measure these voltages, the electrode array was connected to a 512 cross-point switch matrix (PXI 2523, National Instruments, Austin, TX, USA). Channels of the switch matrix were also connected to an isolated constant current stimulator and a digital multimeter (PXI 4072, National Instruments, Austin, TX, USA), allowing stimulation and voltage recording at any electrode site. Voltage waveforms were collected from every electrode in response to MP stimulation of every other electrode. Each trial was repeated 5 times and the waveforms were averaged. Transimpedance was calculated by dividing the voltage at the end of the first phase by the current, and arranged in a matrix where the columns corresponded to stimulating electrode, and the rows to recording electrode as follows:

$$Z_m = \begin{bmatrix} Z_{E1,E1} & Z_{E2,E1} & \dots & Z_{E42,E1} \\ Z_{E1,E2} & Z_{E2,E2} & \dots & Z_{E42,E2} \\ \vdots & \vdots & \ddots & \vdots \\ Z_{E1,E42} & Z_{E2,E42} & \dots & Z_{E42,E42} \end{bmatrix}$$

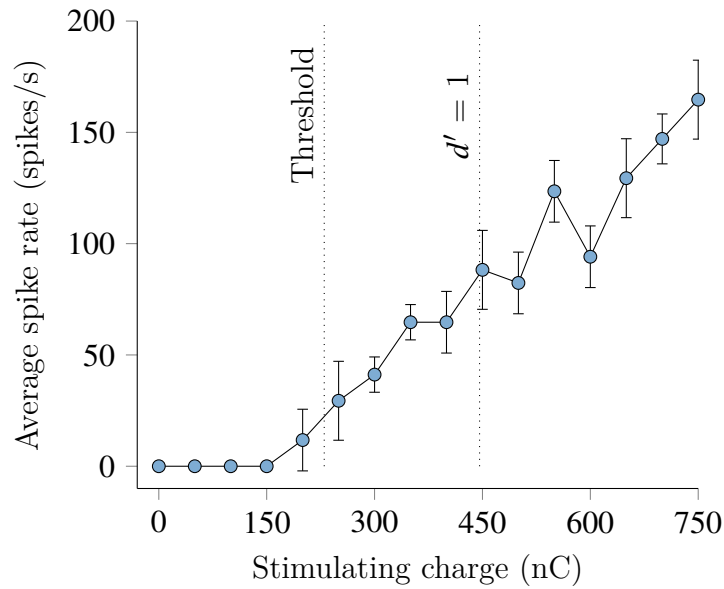
Where $Z_{x,y}$ is the transimpedance between stimulating electrode x and recording electrode y . Transimpedance measurements where the stimulating and recording electrodes are the same (on the diagonal of the matrix) could not be accurately measured due to the effects of polarization on the electrode carrying the current pulse. Therefore these values were estimated using a linear extrapolation method adapted from van den Honert & Kelsalls (van den Honert & Kelsall 2007). For a linear electrode array, such as the one used in a cochlear implant, values are estimated by taking the maximum among four values extrapolated from adjacent electrode pairs (van den Honert & Kelsall 2007). As the retinal array is two-dimensional, this was repeated in up to six directions, yielding, depending on electrode positioning, up to twelve extrapolated values. The maximum extrapolated value was chosen as the transimpedance of each stimulating electrode on the diagonal. The transimpedance matrix was averaged with its own transpose ($Z = Z_m + Z_{Tm}$) to decrease measurement noise and then inverted ($Y = Z^{-1}$), yielding a matrix of transadmittance values. Each column (j) of transadmittances were then normalised to the admittance value of the PSE ($Y_{j,j}$ on the diagonal of the matrix), to give the current weights to nullify the voltage at every electrode on the array except the PSE. These weights have been referred to as the phased array (van den Honert & Kelsall 2007). FMP weights typically presented as concentric circles of alternating positive and negative weights progressively approaching zero with greater distance from the PSE (see Figure 3.1).

3.2.3 Retinal voltage spread

In order to validate whether FMP stimulation was able to nullify voltages on surrounding electrodes, recordings from the retinal electrodes were used to assess the spread of voltage in the retina. For these recordings, single biphasic pulses at the maximum safe charge level of 750 nC, using MP or FMP stimulation, were applied to each electrode on the array and recordings were made from the electrodes directly surrounding. Due to hardware constraints, we were only able to record from a single electrode at a time. Therefore, a single pulse was applied to each electrode multiple times, but each time a recording was made from a different electrode. To eliminate the effects of electrode polarisation during FMP stimulation, for each trial, the recording electrode was not included in the transimpedance calculations



(a) Bandpass filtered signal (without artefact removal)



(b) Input-output function from a single cortical channel

Figure 3.2: Examples of electrically evoked multiunit activity on a single cortical recording channel. (a) Recording of multiunit activity in response to stimulation of a single retinal electrode. Stimulation artefact and the recorded response are highlighted. Asterisks denote detected spikes. (b) Input-output function of a single cortical channel in response to monopolar stimulation from a retinal electrode. The average number of recorded spikes is plotted against the stimulation charge. The leftmost dotted vertical line denotes the threshold for that channel. The rightmost dotted vertical line denotes the charge required on this channel to reach a discrimination index (d') of 1 above threshold.

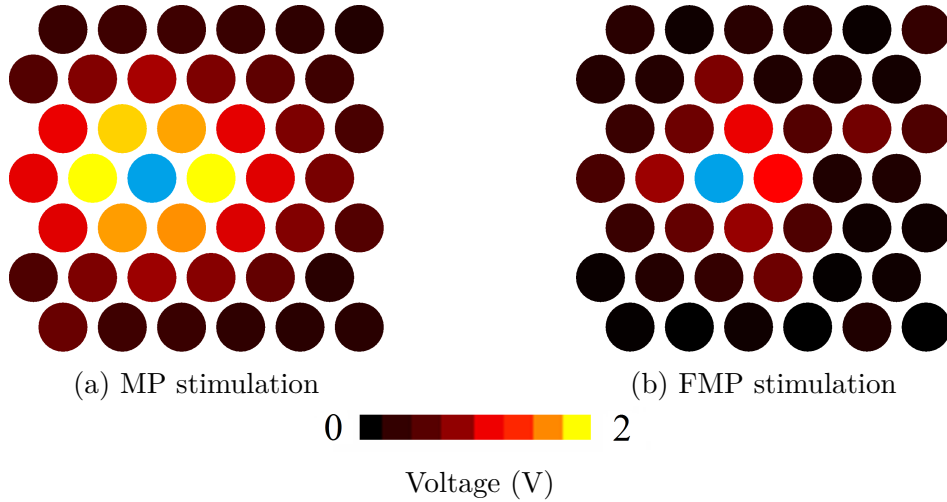


Figure 3.3: Example of voltages recorded on the suprachoroidal array in response to MP and FMP stimulation applied to the same electrode (highlighted blue). Each circle represents an electrode on the array. The colour of the dot represents voltage as indicated by the colour bar on the left. Voltages of nearby electrodes appear to be higher for MP stimulation than for FMP stimulation. Note, for FMP stimulation when recording a voltage on a given electrode, all other electrodes were stimulated using their corresponding current weights.

and was not stimulated as part of the phased array. While this technique did not employ true FMP stimulation, as the phased array was not complete without the recording electrode, it provided a reasonable estimate of the voltage reduction capabilities of FMP stimulation. To quantify the difference between stimulation modes, the average of the summed voltage values of electrodes directly surrounding each PSE were compared between FMP and MP stimulation with a paired t-test. Due to time constraints, we did not record the voltage spread in response to HP stimulation. Simulations in previous studies have shown that HP stimulation results in a more discrete electric field than MP stimulation (Wilke et al. 2011a, Matteucci et al. 2013).

3.2.4 Cortical data analysis

Data recorded during the experiment was analysed offline using custom scripts written in Igor Pro (Wavemetrics, Lake Oswego, OR, USA) and MATLAB (Mathworks, Natick, MA, USA). Signal artefacts from electrical stimulation were first removed using techniques described by Heffer & Fallon (2008). The signal was bandpass filtered (Butterworth filter, 0.3 Hz to 5 Hz, order: 3) and background activity was estimated by calculating the root mean square (RMS) value every 60 seconds. Spikes on each cortical channel were timestamped if the signal exceeded negative four times

the RMS value. Only spikes detected within a 3 ms to 20 ms window from stimulus onset were included in analyses. On some channels, there was a secondary response following the 3 ms to 20 ms window but we chose to only include the early response, as the secondary response is hypothesized to result from synaptically-mediated activation of retinal ganglion cell (RGC) via bipolar (BP) cell activation as opposed to direct activation of RGCs, and in order to remain consistent with previous studies (Cicione et al. 2012, Shivdasani et al. 2010, Wong et al. 2009). For each stimulating electrode, threshold was defined as the lowest current required to exhibit spike rates greater than the baseline spontaneous activity (typically near zero, See Figure 3.2a) while showing a monotonic increase in spiking activity at all higher current levels (Figure 3.2b) on any cortical channel. This differed from our previous studies where threshold has been defined as the current required to elicit 50% of the maximum spike rate observed on a channel (Cicione et al. 2012, Fallon et al. 2009, Shivdasani et al. 2012). An alternative threshold definition was used in this study as we found that on many cortical channels, FMP and HP stimulation (and sometimes MP stimulation as shown in Figure 3.2b) were not able to produce saturated responses even at the maximum safe charge used (750 nC), making it difficult to estimate dynamic range. As the previous threshold techniques relied on estimating the dynamic ranges in response to all stimulation modes to be known, we could not use this technique.

3.2.5 Retinal and cortical selectivity

Selectivity was measured in both the retina and cortex using the data recorded in the cortex from stimulation of the retinal electrodes. Prior studies have shown that different return modes can result in varying thresholds, indicating that cortical spread should not be compared at matched stimulus intensity, but rather at matched ‘brightness’ levels. Our previous studies have used % of maximum spike rate as a way of comparing responses at the same point along the dynamic range. However, as many recording channels failed to reach saturation of spiking activity, in this study we quantified the growth of neural response by calculating the discrimination index (d') of spike rates at each current level compared with spiking activity at threshold. This was achieved using methodology derived from signal detection theory (Green & Swets 1966). A receiver operator curve was constructed for each channel using the spike rates for threshold and supra-threshold stimulus intensities. The area underneath the curve was converted to a standard deviate and multiplied by $\sqrt{2}$ to calculate d' . The current level at which a channel reached d' above threshold was derived through linear interpolation. When estimating retinal selectivity, for

each cortical channel, a best retinal electrode (BRE) was defined as the stimulating electrode that required the least current to elicit a spike rate on that cortical channel that yielded d' above threshold. The d' values elicited on that cortical channel in response to the other retinal electrodes at that same current were also calculated and placed in 1 mm width bins (corresponding to the distance between adjacent retinal electrodes), then plotted as a function of distance from the BRE. In order to represent the worst spread, in each distance bin the maximum d' value was used for further analyses. These maximum binned values were then averaged across all cortical channels and compared across stimulation modes.

Cortical selectivity was estimated using a similar approach. A best cortical electrode (BCE) was determined for each stimulating retinal electrode. The BCE was defined as the cortical channel that required the smallest current to elicit a spike rate that yielded d' above threshold. The d' values of the other cortical channels elicited by that same current were then calculated as a function of distance from BCE (in mm). These d' values were then placed in 0.4 mm wide (corresponding to the distance between adjacent cortical electrodes) bins and the maximum values of each bin were averaged over the plots for all retinal electrodes and compared between stimulation modes. Statistical analyses were performed using SigmaPlot (Systat Software, San Jose, CA, USA). Comparison of retinal electrode voltage spread between MP and FMP stimulation was performed using a paired t-test. A one-way analysis of variance (ANOVA) was used to compare threshold differences between the three stimulation modes and post-hoc differences were calculated using the Tukey test. Differences in cortical and retinal selectivity were determined with two-way ANOVAs, using binned distance and stimulus mode as factors, with post-hoc differences calculated using the Tukey test.

3.3 Results

3.3.1 Retinal voltage spread

When using FMP stimulation, the calculated stimulation weights of electrodes immediately surrounding the PSE were found to be greatly asymmetrical unlike HP stimulation. Excluding electrodes that were not surrounded by a full ring of six electrodes, the average FMP weight applied to immediately adjacent electrodes was -0.237 ± 0.017 ($\pm$ SEM), which is significantly higher (paired t-test $p < 0.001$) than the average weight for HP stimulation at -0.167 , assuming current was distributed evenly across the return electrodes during HP stimulation.

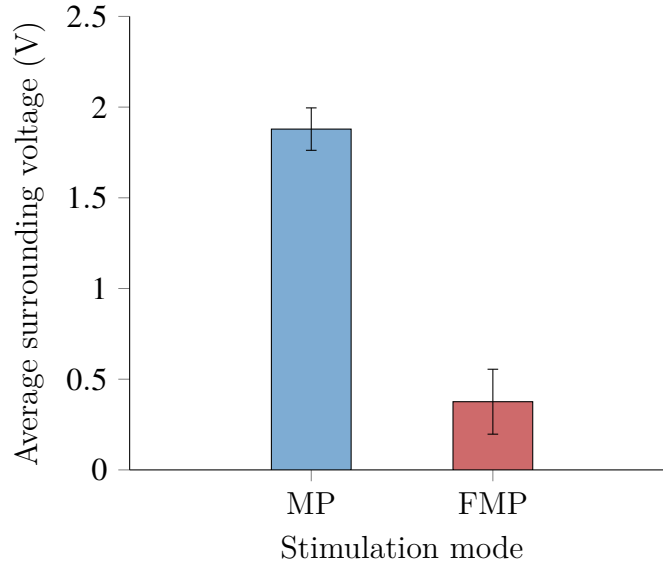
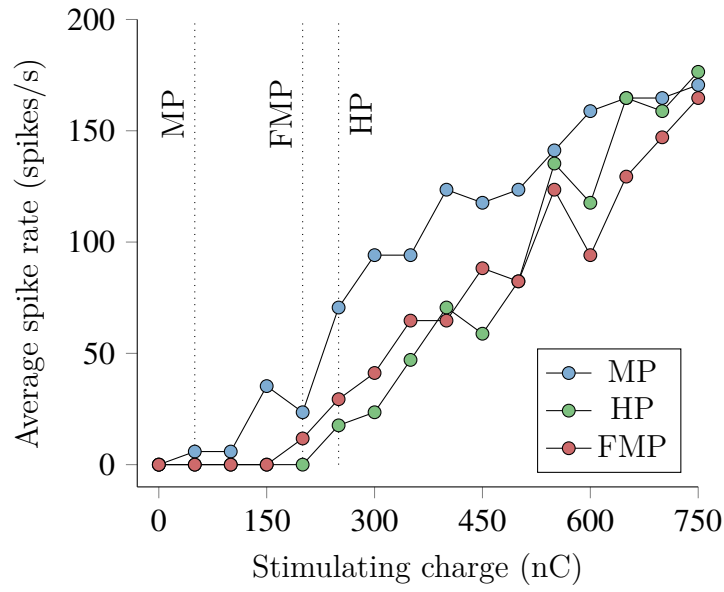


Figure 3.4: Average voltage of electrodes directly adjacent to each PSE during MP and FMP stimulation. Average surrounding voltages were significantly lower for FMP stimulation when compared with MP stimulation (paired t-test $p < 0.001$). Error bars show standard error of the mean.

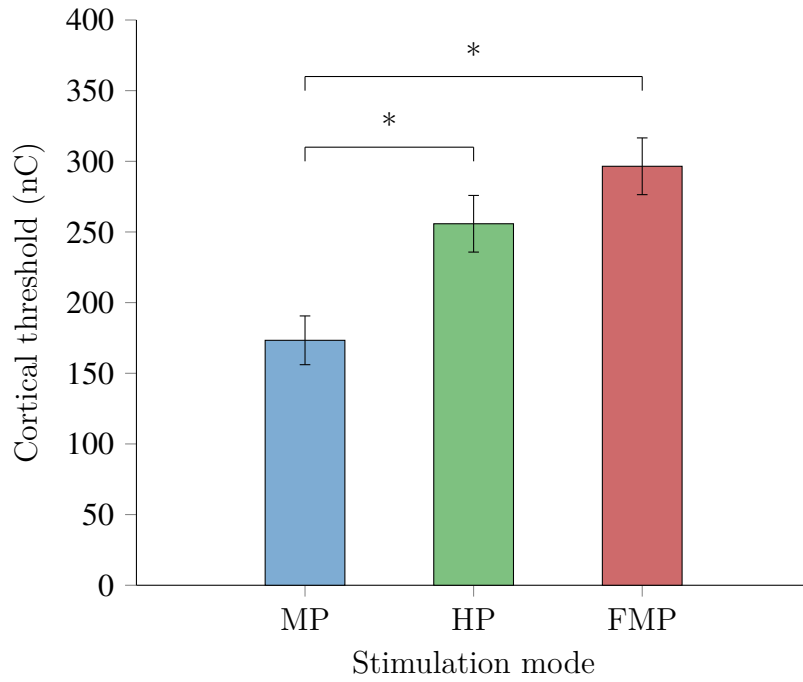
Voltage recordings were obtained from 224 surrounding electrodes in response to 39 stimulating electrodes. Voltages recorded at these sites were significantly reduced when FMP stimulation was utilised (paired t-test $p < 0.001$), with a drop from 1.88 ± 0.12 V (mean $\pm$ SEM) for MP stimulation to 0.38 ± 0.18 V for FMP stimulation (shown in Figure 3.3 and 3.4), representing an average 80% drop in voltage. This results suggest that the adaptation of FMP stimulation from a one-dimensional cochlear array to a two-dimensional retinal array was successful and that FMP stimulation successfully restricted the electric field in the retina.

3.3.2 Cortical thresholds

Cortical responses were collected in response to stimulation of 150 retinal electrodes using the three modes (MP, HP and FMP) across all six animals. It was not possible during the experiments to stimulate all 42 electrodes on each array due to time limitations. The number of retinal electrodes that elicited a response on at least one cortical channel reaching d' above threshold was 111 for MP stimulation, 93 for HP stimulation, and 90 for FMP stimulation. Only the 73 retinal electrodes that elicited at least one cortical channel to reach $d'=1$ above threshold using all stimulation modes were included in analysis. Out of a total of 12,516 recording channels in the VC, 8173 channels recorded activity reaching d' above threshold for at least one stimulating electrode (4296 sites for MP, 1547 sites for HP, and 1330



(a) Input-output functions on a single cortical channel



(b) Average thresholds for different stimulation modes

Figure 3.5: (a) Input-output function on a single cortical channel in response to MP, HP and FMP stimulation showing the thresholds for each mode. (b) Average threshold values for MP, HP, and FMP stimulation ($n=150$). Threshold for a retinal electrode was determined as the lowest current required to elicit spiking activity above spontaneous activity on any cortical recording channel. There was a significant increase in thresholds for HP and FMP stimulation when compared to MP stimulation (*one-way ANOVA $p<0.001$), however there was no difference between HP and FMP stimulation. Error bars show standard error of the mean.

sites for FMP). A total of 195 channels recorded spike rates that reached d' above threshold in response to at least one retinal electrode for all three stimulation modes.

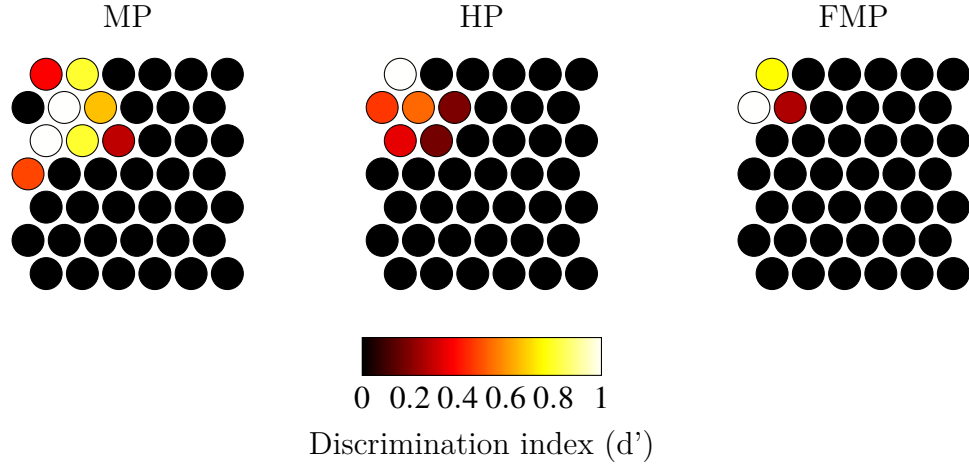
Significant differences in cortical activation thresholds were observed between the two focusing techniques and monopolar stimulation. Average thresholds for HP and FMP stimulation (255 ± 20 nC and 296 ± 20 nC respectively) were both significantly higher than MP stimulation (173 ± 17 nC) (one-way ANOVA $p < 0.001$) as shown in Figure 3.5. No difference was found between HP and FMP stimulation.

3.3.3 Retinal selectivity

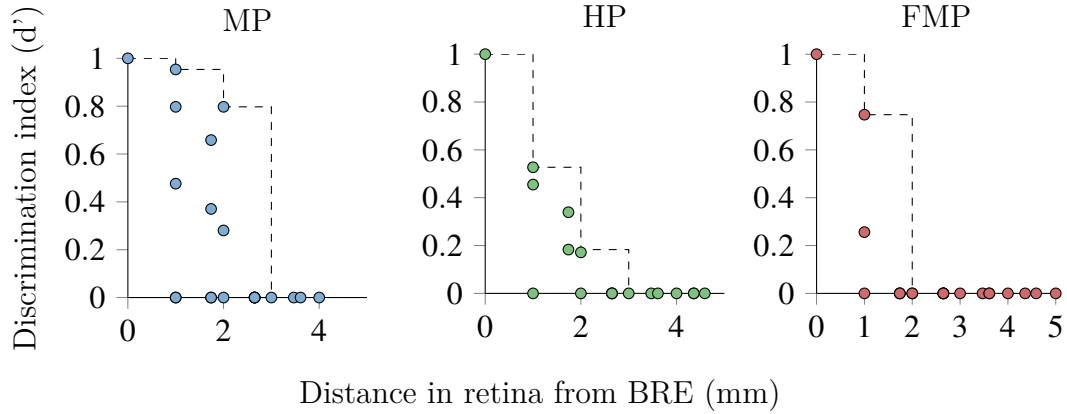
There was a significant reduction in retinal activation spread when HP and FMP stimulation were used compared with MP stimulation (two-way ANOVA $p < 0.001$, Tukey post-hoc test). Figure 3.6 shows an example of the d' values calculated on a single cortical channel when stimulation is applied to each electrode at the lowest current to reach d' above threshold on the BRE, using each stimulation mode. In this example, the responses elicited on the cortical channel by electrodes adjacent to the BRE were smaller when HP and FMP stimulation were applied than when MP stimulation was applied. When averaging across all data, there was a significant interaction between distance from BRE and stimulation mode (two-way ANOVA, $p < 0.001$). Figure 3.6 shows that activity elicited in the cortex by stimulating electrodes within 2 mm of the BRE was found to be significantly reduced (two way ANOVA; $p < 0.001$) with HP and FMP stimulation compared to MP stimulation, with no significant difference between the stimulation modes beyond 2 mm ($p > 0.05$). There was also no significant difference in activity elicited between HP and FMP stimulation ($p > 0.05$).

3.3.4 Cortical selectivity

At the current required to reach d' above threshold on the BCE, a significant reduction in cortical activation spread was observed for HP and FMP stimulation modes over MP stimulation. An example of activity recorded with a 6x10 planar recording array in response to the three stimulation modes applied to the same retinal electrode is shown in Figure 3.8a. The spread of activation in the cortex in response to MP stimulation was far broader than that elicited by HP and FMP stimulation in this example. Maximum d' values of channels surrounding the BCE were found to be lower for HP and FMP than MP stimulation in this example (Figure 3.8b). Average



(a) Retinal response maps for responses on a single cortical channel to MP, HP, and FMP stimulation



(b) Retinal selectivity plots for responses on a single cortical channel to MP, HP, and FMP stimulation

Figure 3.6: (a) Examples of retinal selectivity maps for a single cortical channel when MP, HP and FMP stimulation were used. Maps show d' above threshold values elicited on the same cortical channel when electrodes were stimulated at the current level required to reach d' above threshold on the BRE. MP stimulation elicited responses on the cortical channel to stimulation of a broader range of retinal electrodes than for HP and FMP stimulation. (b) Examples of selectivity plots for responses to MP, HP, and FMP constructed from the data shown in (a). The d' values are plotted against the distance of the stimulating electrode from the BRE. Electrodes that did not result in a response on the cortical channel at any current level are excluded. The dotted line shows the running maximum of each 1 mm wide bin.

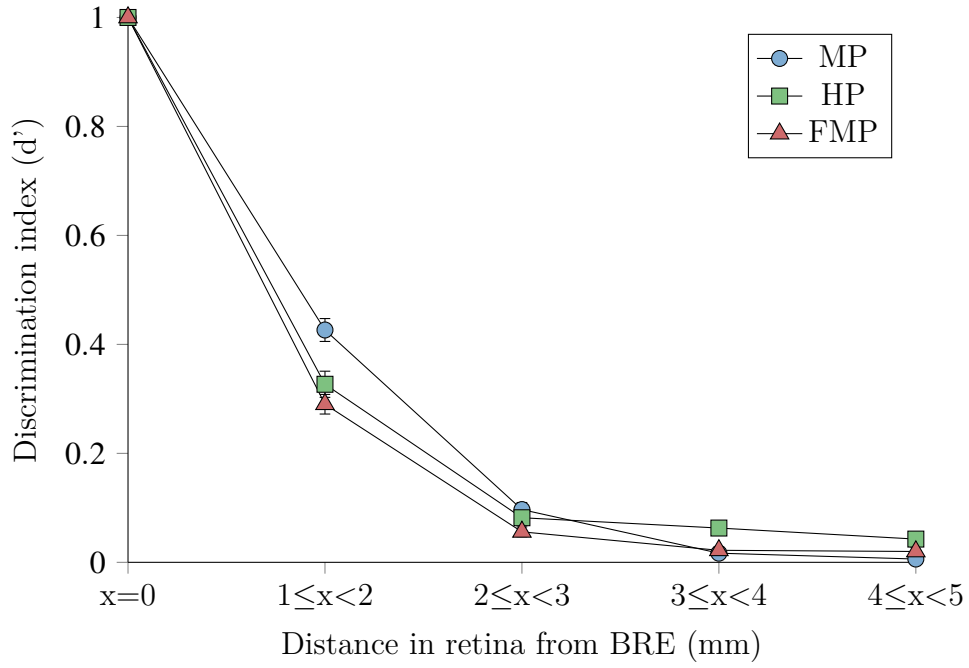


Figure 3.7: Average of the maximum d' values elicited on cortical channels by retinal electrodes, at the current required to reach d' above threshold on the best retinal electrode (BRE) for each stimulation mode. All stimulation modes showed a steady decline in discriminability above threshold with increasing distance from the BRE. HP and FMP stimulation showed a significantly steeper decline for the first bin when compared with MP stimulation (two-way ANOVA $p < 0.001$). No significant difference was found between HP and FMP stimulation (two-way ANOVA $p > 0.05$). Error bars show standard error of the mean.

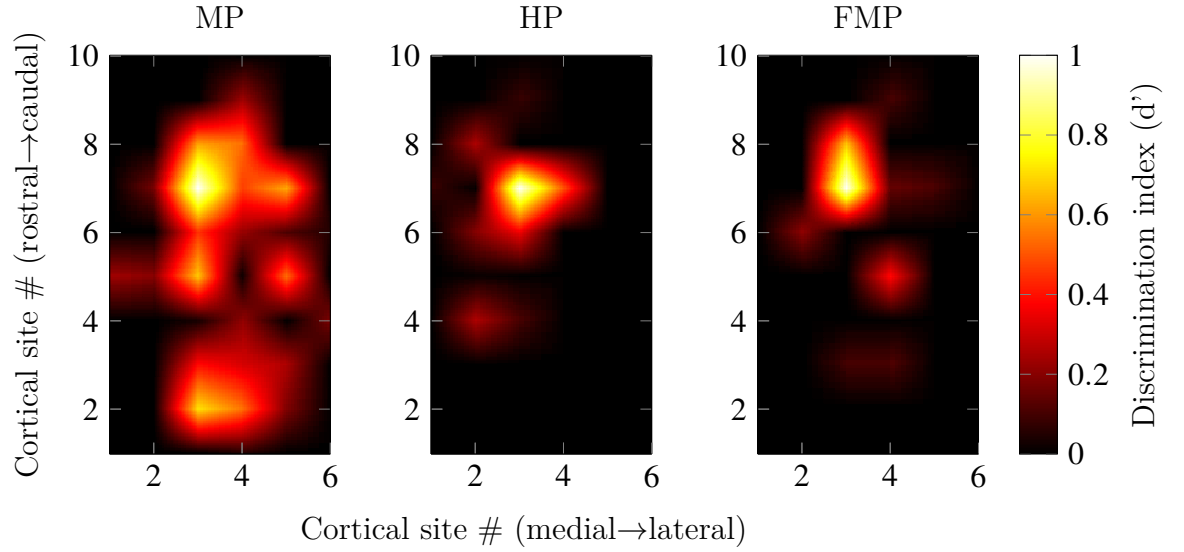
cortical selectivity across all stimulating electrodes indicated a significantly greater reduction in the spread of cortical activation for HP and FMP stimulation than for MP stimulation (Figure 3.9, two-way ANOVA $p < 0.001$ Tukey post-hoc test), and unlike retinal selectivity, there was no significant interaction between distance and stimulation mode ($p > 0.05$).

3.4 Discussion

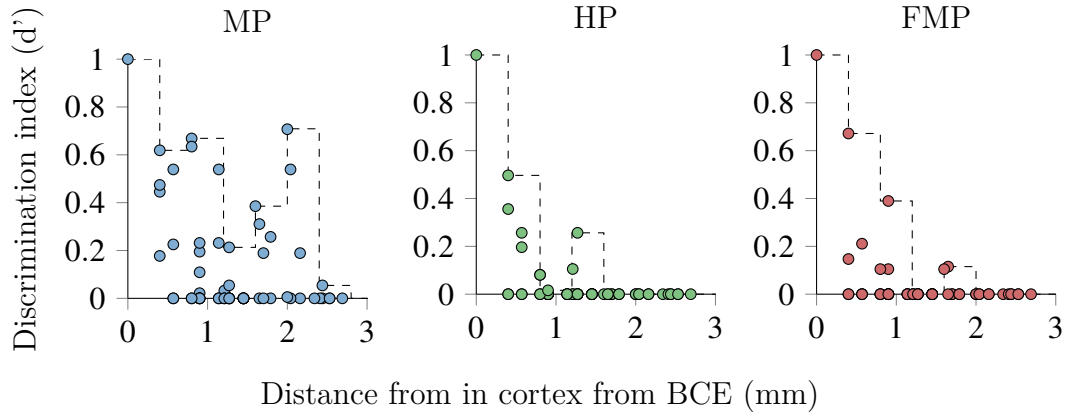
In this study, we investigated the effectiveness of FMP stimulation in reducing the spread of neural activation in the retina and VC compared with MP and HP stimulation. The main finding of this study was that FMP and HP stimulation resulted in significantly restricted spatial spread of neural activation in both the retina and VC when compared to MP stimulation, albeit requiring higher charge levels for FMP and HP stimulation to reach neural activation thresholds. Contrary to our initial hypothesis, no differences in any of the measures were found between FMP and HP stimulation.

3.4.1 Retinal voltage spread

FMP stimulation showed significantly lower voltages on retinal electrodes directly adjacent to the PSE compared to MP stimulation, implying a decrease in current spread in the retina. The restriction of current spread is consistent with computational models for one-dimensional FMP (Frijns et al. 2011, van den Honert et al. 2007) and indicates that FMP is also effective when applied to a two-dimensional electrode array. These data suggest that a reduction in neural recruitment with FMP stimulation can be likely and that more discrete responses would be elicited downstream in the visual pathway. Being able to reduce the spread of the electric field in the retina also suggests that channel interaction may be reduced with a retinal prosthesis when using FMP stimulation (George et al. 2015, Horsager et al. 2011, 2010). FMP has been shown in the cochlear implant to significantly reduce the effects of channel interaction on inferior colliculus activation (George et al. 2015). This has important implications for stimulation strategies that would employ simultaneous stimulation of multiple electrodes (Horsager et al. 2011, 2010, Nirenberg & Pandarinath 2012). Although we did not measure voltage spread for HP stimulation, it is likely that it would also be able to reduce the effects of channel interaction as it had similar effects on retinal and cortical selectivity as FMP did. It has been shown previously that tripolar stimulation in the cochlea (analogous to HP stimulation in



(a) Cortical response maps for MP, HP, and FMP stimulation of the same retinal electrode



(b) Cortical selectivity plots for MP, HP, and FMP stimulation of the same retinal electrode

Figure 3.8: (a) Examples of response patterns on the cortical recording array (6x10 planar) for MP, HP, and FMP stimulation. Colours represent the d' above threshold of responses at the current required to reach d' above threshold on the BCE. MP stimulation elicited a broader region of activation than HP and FMP stimulation. (b) Examples of selectivity plots for responses to MP, HP, and FMP constructed from the data shown in (a). The d' above threshold as a function of distance from BCE. Electrodes that did not reach threshold at any current level were excluded. The dotted line shows the running maximum of each 0.4 mm wide bin.

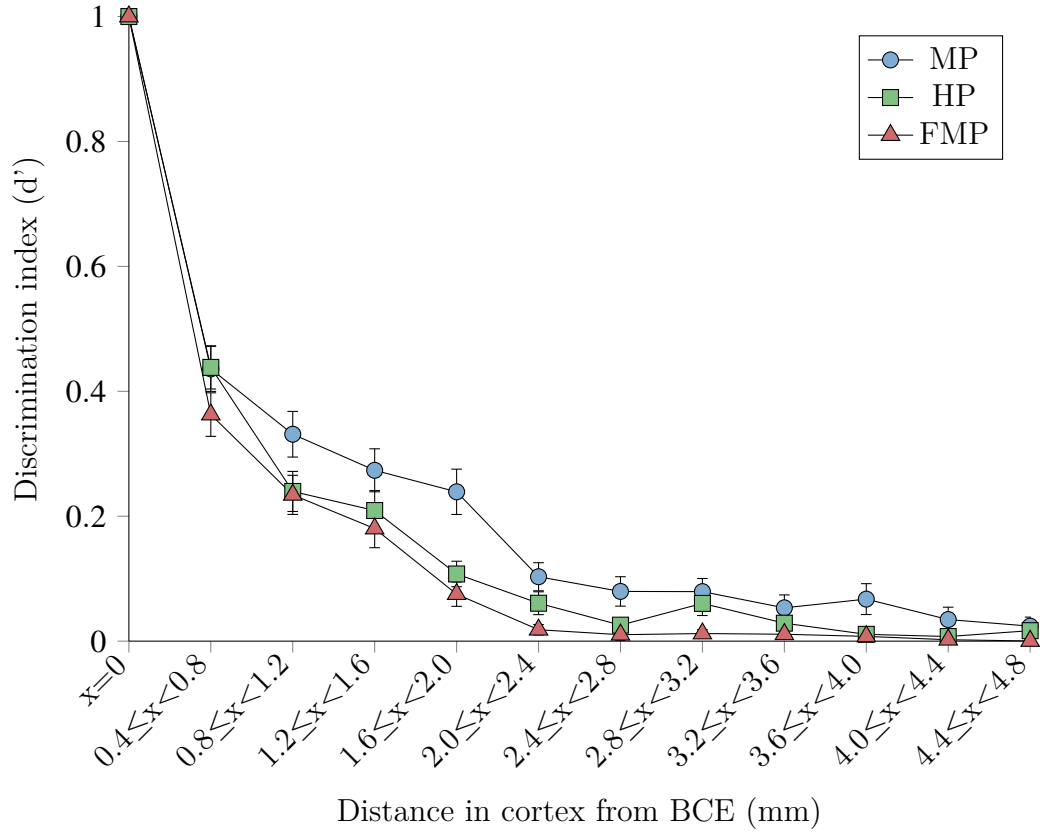


Figure 3.9: Average of the maximum d' values of cortical channels at the current required to reach d' above threshold on the BCE for each stimulation mode. All stimulation modes showed a steady decline in discriminability as the distance from BCE increased. HP and FMP stimulation shows a significantly steeper decline when compared with MP stimulation, with no significant interactions between distance and stimulation mode (two-way ANOVA $p < 0.001$). No significant difference was found between HP and FMP stimulation (two-way ANOVA $p < 0.001$). Error bars show standard error of the mean.

our study) was also able to reduce channel interaction to the same level as that of FMP stimulation (George et al. 2015).

3.4.2 Cortical thresholds

Activation thresholds in the VC for HP and FMP stimulation were shown to be significantly higher than MP stimulation. This is consistent with previous work in the retina and cochlea showing that FMP and discrete return modes, such as HP (Abramian et al. 2011, Cicione et al. 2012, Habib et al. 2013, Matteucci et al. 2013, Shivdasani et al. 2014), tripolar (Bierer 2007, George et al. 2014, 2015), and BP (Gerhardt et al. 2011) stimulation, require higher charge levels to elicit comparable responses. However, compared to previous work with HP stimulation in the retina, threshold differences between MP and HP stimulation were not as pronounced (Cicione et al. 2012, Shivdasani et al. 2014). This study showed a 32% increase in threshold whereas our previous preclinical study reported over 100% increase. The smaller threshold increase is likely due to methodological differences. In particular, the difference in threshold definition, as the previous study defined threshold at 50% of the maximum observed spike rate (Cicione et al. 2012), and the different geometries of the electrode arrays used.

The increased thresholds observed may be due to the more restricted electric field with HP and FMP stimulation being unable to penetrate through to the retina. Current shunting via the highly conductive choroidal blood vessels may also contribute (Gerhardt et al. 2011, Kasi et al. 2011). Higher thresholds may have consequences in a clinical scenario as higher charge levels are more likely to be damaging to the surrounding tissue. The fact that many channels did not reach a saturation of responses at the maximum safe charge injection limits is also concerning as the focusing methods may not be able to reach the same levels of brightness as MP stimulation. A variation of FMP has been trialled in a cochlear implant study termed partial-FMP in order to address the increase in thresholds (George et al. 2015). In this method, weights are calculated the same way, however a multiplier is applied to the diagonal values of the transimpedance matrix, thereby affording a greater ratio of current to the PSE (George et al. 2015). This method is similar to other focusing methods such as partial-tripolar (Wu & Luo 2013) and quasimonopolar stimulation (Khalili Moghaddam et al. 2014, Matteucci et al. 2013). Using this method, lower thresholds could be attained possibly without compromising on selectivity, allowing for titration of the two factors (George et al. 2015). It is likely that a partial-FMP approach could be applied to the retina to compensate for the increased

thresholds however inevitably there may always be a trade-off with threshold and selectivity.

3.4.3 Retinal and cortical selectivity

Retinal and cortical selectivity for HP and FMP stimulation was shown to be significantly higher than for MP stimulation. This shows that the two focusing techniques were effective at restricting the region of neural recruitment in the retina and, by extension, evoked activity in the VC. There was no significant difference between HP and FMP stimulation in terms of retinal or cortical selectivity, suggesting that this application of FMP stimulation did not result in more focused than using a HP return mode. Also with retinal selectivity, there were no significant differences at 2 mm or further from the BRE between the three modes. However, note that d' values determined for all modes at these distances were often very low as even MP stimulation would seldom spread far at these current levels. This is consistent with our previous study where responses on a given cortical channel were much less when an electrode 2 mm or further from the BRE was stimulated (Cicione et al. 2012). Based on the average receptive field of a cat cortical neuron, 1.6° , it is unlikely that a single cortical site would receive projections from an area more than 2 mm away from the BRE (Warren et al. 2001).

Based on our cortical selectivity measures we can estimate that stimulation applied to a single site yielded evoked activity with a radius of 2.4 mm in the cortex for MP stimulation, and 1.6 mm in cortex for HP and FMP stimulation (defined as the bin distance where d' values drop consistently below 0.1. See Figure 3.9). Using a cortical magnification factor of 0.707 mm of cortex/degree of visual field for an eccentricity of 10° , as defined by Tusa et al 1978, these regions of activation equate to approximately 1.69° and 1.13° of visual angle respectively (Tusa 1978), indicating that a higher degree of resolution could be achieved with HP and FMP stimulation.

In regards to HP stimulation, the results of this study are partially consistent with our previous study (Cicione et al. 2012). HP stimulation has been shown in previous work to restrict retinal activation (Cicione et al. 2012, Habib et al. 2013), however it was shown to have no impact on cortical selectivity (Cicione et al. 2012) and anecdotally did not produce smaller phosphenes in patients compared to MP stimulation (Shivdasani et al. 2014). One explanation for this is the method used to match intensity levels. The previous study in animals compared responses at the

same point along the dynamic range of neural responses, determined by % of maximum spike rate (Cicione et al. 2012), as opposed to this study, where we converted responses into d' values to assess the level of discriminability above threshold to simulate a matched ‘brightness’ level in the brain. Also, in patients, while phosphenes were compared at the same level above relative thresholds, these amplitudes may not have been at the same point along the perceptual dynamic range which would have been expected to be different between MP and HP stimulation, based on what we showed in our previous preclinical study (Cicione et al. 2012). This highlights the importance of the method used to determine selectivity, both electrophysiologically and clinically when using different stimulation modes. While we have used two different approaches in our laboratory, clinically, the ideal approach would be to compare phosphene sizes at brightness-matched levels. However, as brightness and phosphene size are known to co-vary with stimulus amplitude, it may be sufficient to compare the degree of growth in both measures when comparing different stimulation modes, where FMP and HP stimulation could be expected to elicit a smaller degree of growth compared to MP stimulation. Another explanation for the divergence in selectivity results between this study and our previous preclinical study is the difference in electrode geometry. Electrodes used in this study had a greater diameter than the previous study however maintained the same distance centre-to-centre (Cicione et al. 2012). This change may account for the different results as reduced edge-to-edge distance between electrodes may have resulted in greater voltage reduction with a HP return arrangement compared to our previous study.

A somewhat surprising finding in our study was that FMP weights applied to channels immediately adjacent to the PSE were significantly higher than estimated HP weights, especially since we found no differences in our cortical measures. This diverges from recordings taken from cochlear implants where the weights of flanking electrodes are typically calculated to be approximately -0.5, dividing current equally between the two channels (Senn 2014, van den Honert et al. 2007). This could possibly be due to the differences between the electrode-tissue interface in the cochlea and the suprachoroidal space. Intracochlear electrodes are often in contact with fluid or relatively consistent impedance whereas the retinal electrodes are in contact with the choroid, which is comprised of a heterogeneous assortment of connective tissue and blood vessels (Gerhardt et al. 2011, Kasi et al. 2011). However, this does indicate that FMP stimulation is not merely replicating HP stimulation. While this study does not detect any significant functional difference between the two modes, it is possible that there would be perceptual differences that we are unable to determine

using our analysis method.

Overall, our results indicate that both HP and FMP stimulation are capable of restricting the spread of neural activation in the retina and VC, which would be expected to result in the perception of more discrete phosphenes when either compared at the same point along their perceptual dynamic range or at an equivalent brightness level compared to MP stimulation.

3.4.4 Future studies

This study demonstrates that HP and, for the first time, FMP stimulation is capable of restricting the spread of activation in two dimensions in the retina. Firstly, this has important implications for stimulation strategies requiring simultaneous stimulation of electrodes. It has been shown previously that channel interaction can be minimised on a linear intracochlear electrode array using tripolar and FMP stimulation (George et al. 2015). It has also been shown that channel interactions can be reduced in the retina using HP stimulation (Matteucci et al. 2016). Further investigation in to the use of these current focusing techniques in reducing electrical crosstalk in the retina could be of great benefit.

Secondly, there are a number of differences between the state of the feline eye in this study and the likely state of a retinal prosthesis patient’s eye that may impact the translation of these findings to patients. Our experiments were conducted following an acute implantation of the suprachoroidal device. In patients there are a number of long term morphological changes that occur in the eye following implantation, such as chronic inflammation and fibrosis, that are not represented in this study (Villalobos et al. 2013). Further studies in long-term implanted animals would provide a more accurate understanding of the efficacy of HP and FMP stimulation in a more representative scenario. Also, the animals used during this study were sighted cats with intact healthy retinas. At least for the near future, the intended recipients for retinal prosthesis are currently patients with severe photoreceptor loss and associated remodelling of the remaining retinal circuitry. Our previous work in a feline model (Aplin et al. 2016b) of photoreceptor degeneration indicates that activation spread is largely unchanged, however it is possible that the increased thresholds of the focusing techniques may have a more pronounced effect in a blind recipient. Further studies in an animal model of retinal degeneration would give a much clearer indication on the effects of HP and FMP stimulation on the blind retina.

3.4.5 Conclusion

This study showed, for the first time, that current focusing techniques reduced voltage spread and neural activation in the retina and, by extension, the spread of evoked activity in the VC, compared to traditional MP stimulation at the expense of higher thresholds. To our knowledge this is also the first instance of FMP being attempted with a two-dimensional electrode array. No significant differences were observed between the two focusing techniques. Clinical application of these techniques would be expected to result in smaller phosphenes, thus reducing phosphene overlap between electrodes and increasing visual resolution for retinal prosthesis recipients. Further study in long-term implanted blind feline models could give a clearer insight in to the translational benefit of these techniques.

3.5 Acknowledgements

The authors wish to thank Stephanie Epp, Ceara McGowan, Dimitra Stathopoulos, Alison Neil, Rodney Millard, Kerry Halupka, Alexia Saunders, and Evgeni Sergeev for technical assistance. Also, thanks to Penny Allen and Chi Luu for implanting the suprachoroidal device. Funding for this research was provided by the National Health and Medical Research Council (NHMRC) Project Grant #1063063. The Bionics Institute acknowledges the support it receives from the Victorian Government through its Operational Infrastructure Support Program.

Chapter 4

Creating virtual electrodes with two-dimensional current steering

This chapter has been published in its entirety as the following peer-reviewed journal article:

Spencer, T. C., Fallon, J. B. & Shivdasani, M. N. (2018). Creating virtual electrodes with 2D current steering. *Journal of Neural Engineering*, 15(3), 35002.

Objective: Current steering techniques have shown promise in retinal prostheses as a way to increase the number of distinct percepts elicitable without increasing the number of implanted electrodes. Previously, it has been shown that virtual electrodes (VEs) can be created between simultaneously stimulated electrode pairs, producing unique cortical response patterns. This study investigated whether VEs could be created using two-dimensional current steering, and whether these VEs can produce cortical responses with predictable spatial characteristics.

Approach: Normally-sighted eyes of seven adult anaesthetised cats were implanted with a 42-channel electrode array in the suprachoroidal space and multi-unit neural activity was recorded from the visual cortex. Stimuli were delivered to individual physical electrodes, or electrodes grouped into triangular, rectangular, and hexagonal arrangements. Varying proportions of charge were applied to each electrode in a group to “steer” current and create virtual electrodes. The centroids of cortical responses to stimulation of virtual electrodes were compared to those evoked by stimulation of single physical electrodes.

Results: Responses to stimulation of groups of up to six electrodes with equal

ratios of charge on each electrode resulted in cortical activation patterns that were similar to those elicited by the central physical electrode (Centroid's: RM ANOVA on Ranks, $p > 0.05$; Neural Spread: ANOVA on Ranks; $p > 0.05$). We were also able to steer the centroid of activation towards the direction of any of the electrodes of the group by applying a greater charge to that electrode, but the movement in the centroid was not found to be significant.

Conclusions: The results suggest that current steering is possible in two dimensions between up to at least six electrodes, indicating it may be possible to increase the number of percepts in patients without increasing the number of physical electrodes. Being able to reproduce the spatial characteristics of responses to individual physical electrodes suggests that this technique could also be used to compensate for faulty electrodes.

4.1 Introduction

Retinal prostheses are currently the only approved treatment for retinitis pigmentosa (RP), a group of hereditary degenerative retinal diseases that affect over one million people worldwide (Hartong et al. 2006, Shepherd et al. 2013, Weiland et al. 2005). These provide artificial vision to patients by electrically stimulating surviving non-photosensitive neurons in the inner retinal layers. Electrical stimulation of the retina elicits the perception of discrete flashes of light (Ahuja et al. 2011, Shivdasani et al. 2014). These percepts, termed phosphenes, are used as building blocks to construct an artificial image, much like pixels on a computer monitor (Shepherd et al. 2013, Weiland et al. 2005). Clinical studies have shown that retinal prostheses can improve patient performance in spatio-motor tasks, pattern recognition, and basic object recognition. However, due to the poor visual horizontal acuity afforded by present-generation devices, more complex tasks such as independent navigation, facial recognition, and reading, barring in a few exceptional patients, are still out of reach. Technical and safety constraints limit the size and density of electrode arrays which, using conventional single electrode stimulation techniques, directly limits the range of percepts that can be elicited.

Current steering refers to a number of stimulation techniques aimed at manipulating the distribution of electrical potential around electrode sites (Chaturvedi et al. 2012, Dumm et al. 2014, Luo et al. 2010). These techniques make use of simultaneous stimulation of electrodes in a controllable fashion to shift the peak of the summated electrical field, in order to target specific neural populations. Interactions between

electrical fields are often considered to be undesirable, as they can result in unpredictable temporal or spatial smearing of the percept (Horsager et al. 2011, 2010, Wilke et al. 2011b), so they are typically circumvented using sequential stimulation presented within the flicker fusion rate of the visual system. However, this limits the number of phosphenes to the number of implanted electrodes, which is in turn subject to engineering and surgical constraints. Incorporating electrical field interactions into the stimulation strategy may allow an increase in the number of unique percepts without significant changes to the electrode array.

The perceptual effects of electrical field interactions between simultaneously stimulated electrodes has been explored in cochlear implants. So called “virtual” electrodes can be created between simultaneously stimulated adjacent electrode pairs, eliciting the perception of an intermediate pitch (Firszt et al. 2009, 2007, Koch et al. 2007). This technique is implemented clinically to increase the auditory spectral resolution afforded to patients (Firszt et al. 2009, Koch et al. 2007). Current steering in deep brain stimulation between up to four linearly arranged electrodes has also been shown to mitigate dyskinesia side effects in Parkinson’s patients, presumably by shifting the peak of the summated electrical field away from undesired neural populations (Barbe et al. 2014, Martens et al. 2011). In the retina there have been a number of pre-clinical studies into the effects of field shaping techniques. Virtual electrodes can be created between two physical electrode pairs (Dumm et al. 2014). Increasing the proportion of charge delivered to one of these electrodes can also shift the virtual electrode towards it (Dumm et al. 2014). Interactions between many simultaneously stimulated retinal electrodes can also be used to reduce current spread, thereby reducing the spread of neural activation (Spencer et al. 2016), and have been shown to be capable of modulating the firing probability of individual retinal ganglion cells (Jepson et al. 2014). Cortical responses to simultaneous stimulation of up to 42 electrodes can also be predicted by constructing a model from cortical responses to white-patterned stimuli (Halupka et al. 2016), demonstrating that neural responses to simultaneous multichannel stimulation are repeatable and consistent. Current steering therefore shows significant promise in increasing the range of percepts that can be presented to patients. However, it is unclear whether these techniques are likely to provide percepts that are useful in presenting complex visual information to patients. The relationship between cortical activity elicited by physical and virtual electrode stimulation has not been extensively studied, and accurate prediction of responses depends on extensive sampling of cortical activity (Halupka et al. 2016).

This study aims to further investigate the creation of virtual electrodes using two-dimensional current steering. In this study we specifically aimed to determine whether we could reproduce the properties of cortical responses to physical electrodes, by steering current between differing numbers of adjacent electrodes. Electrode failure is a significant issue for prosthetic devices and is a major factor in efficacy and lifespan (Ayton et al. 2014, Blomstedt & Hariz 2005). Virtual electrodes have the potential to mitigate this issue by replacing faulty physical electrodes, reducing the functional deficits of reduced phosphene counts and the potential damage associated with repeated repair and reimplantation. We also aimed to confirm that the ability to shift virtual electrodes by altering the proportions of charge is maintained when using different two-dimensional electrode arrangements, and to determine whether the spatial properties of the resultant cortical response could be predicted based only on the knowledge of stimulation weights applied.

4.2 Methods

4.2.1 Anaesthesia and surgery

All procedures were approved by the Bionics Institute Animal Research Ethics Committee (Project #14/304AB) and were in accordance with the Australian Code of Practice for the Care Use of Animals for Scientific Purposes (8th edition) and with the National Institute of Health, USA guidelines regarding the care and use of animals for experimental procedures. Normally sighted adult cats ($n=7$) were used in this study. Due to increased regulation of the supply of pentobarbital in Australia, two different anaesthetic protocols were followed. Five of the seven cats were sedated with ketamine (intramuscular [i.m.] 20 mg kg^{-1}) and xylazil (subcutaneous [s.c.] 2 mg kg^{-1}). Anaesthesia was maintained over the experimental period for up to three days with a continuous intravenous [i.v.] infusion of sodium pentobarbitone ($3\text{--}2 \text{ mg kg}^{-1} \text{ h}^{-1}$) as per our previous studies (Cicione et al. 2014, Shivdasani et al. 2014, Spencer et al. 2016). A continuous i.v. infusion of Hartmann’s solution (sodium lactate, $2.5 \text{ mg kg}^{-1} \text{ h}^{-1}$) was also administered throughout the experiment. The remaining two cats were sedated with ketamine (i.m. 8 mg kg^{-1}), medetomidine (i.m. 0.012 mg kg^{-1}), and methadone (i.m. 0.4 mg kg^{-1}). Anaesthesia was maintained with continuous i.v. infusion of Propofol (24 mg kg^{-1}). A continuous i.v. infusion of methadone in Hartmann’s solution (0.5 ml of 10 mg ml^{-1} Methadone in 250 ml compound sodium lactate, $0.05 \text{ mg kg}^{-1} \text{ h}^{-1}$) was also administered throughout the period of anaesthesia. Due to the depressive effects of Propofol on the respiratory system, tracheostomies were performed and the animals ventilated on

100% oxygen (20-25 breaths/min) (model 6025; Ugo Basile, Monvalle, VA, Italy). Respiration rate, heart rate, end-tidal CO_2 , blood pressure and temperature were monitored consistently throughout the experiment and maintained at normal levels by adjusting the anaesthetic flow rate. Daily injections of dexamethasone (i.m. 0.1 mg kg^{-1}) and clavulox (s.c. 10 mg kg^{-1}) were administered to all seven cats. Pupils were dilated by regular topical application of a mixture of phenylephrine hydrochloride (2.5%) and tropicamide (1%).

The suprachoroidal electrode array was similar to that which has been used in our previous work (Ayton et al. 2014, Villalobos et al. 2013), fabricated on a biocompatible silicon substrate and consisting of 42 platinum electrodes of $600 \mu\text{m}$ diameter spaced 1 mm from centre to centre. The implantation procedure is detailed in our previous studies (Saunders et al. 2014, Villalobos et al. 2013). Briefly, following a lateral canthotomy, scleral incision, and dissection of a pocket between the sclera and choroid, the array was inserted $\sim 15 \text{ mm}$ into the suprachoroidal space until the tip was beneath area centralis. Our aim during implantation is to encompass as much of the area centralis as possible. The array is typically placed over the area centralis, often relatively superior and temporal in order to avoid contact with the optic disc. Electrical connections to the electrodes on the array were tested using an automated impedance monitoring software developed in LabVIEW (National Instruments, Austin, TX, USA) used in previous studies (John et al. 2011). The animal was placed in a stereotaxic frame inside a darkened electrically shielded room. Visual inspection of the eye and fundus photographs were taken to determine the health of the eye and positioning of the array. A craniotomy was then performed spanning 15 mm rostral and 5 mm caudal from the interaural line, and 7 mm lateral from the sagittal suture on the side contralateral to the implanted eye, exposing the visual cortex. A number of studies have shown that, in normally sighted cats, most 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, Payne 1994, Wässle & Illing 1980, Illing & Wässle 1981). The dura mater was carefully excised from the region. Using two parylene-based flexible platinum electrode arrays (as described in Fallon et al. 2016 (Fallon et al. 2016)), electrically evoked potentials (EPs) in response to cathodic-leading biphasic charge-balanced current pulses ($0\text{-}750 \mu\text{A}$, 1 ms per phase) were mapped along the surface of the visual cortex to determine the cortical region with the lowest EP thresholds. Up to two planar “Utah” 36- (6×6) or 60- (6×10) channel penetrating microelectrode arrays (Blackrock Microsystems, Foxborough, MA, USA) were inserted in the regions of Area 17/18 of

the visual cortex with the lowest EP thresholds to a depth of approximately 1 mm. Recording electrodes are 1 mm in length with a pitch of 400 μm . Based on manufacturer estimates, the exposed platinum tip of each electrode is 50 μm long and, at its thickest point, $23 \pm 12 \mu\text{m}$ in diameter. Assuming a uniformly conical tip shape, this translates to an approximate surface area ranging from 2.15 mm^2 to 10.56 mm^2 . The size and number of electrode arrays used for each animal was determined by the prevalence and location of large blood vessels, as care was taken to minimise damage these structures during insertion.

4.2.2 Experimental protocols

Electrical stimuli were generated with a 128-channel IZ2 stimulator (Tucker-Davis Technologies, Alachua, FL, USA). Due to the low maximum current output per channel (300 μA), each group of three channels was combined using a custom-built circuit board bringing the maximum functional number of combined stimulating channels to 42 and the maximum current output to 900 μA per combined channel. Each combined channel was connected directly to an electrode on the suprachoroidal array enabling independent simultaneous stimulation of all 42 electrodes. Pulses were presented at randomly varied currents ranging from zero to 750 μA in 50 μA steps at a repetition rate of 300 Hz. Each current step was repeated 10 times. Stimulus pulses were cathodic-first and symmetrically biphasic with a 1000 μs phase width and 25 μs interphase gap. Either all the charge from each pulse was presented to a single electrode or the total charge was split across groups of electrodes for steered stimulation according to predefined charge ratios. Thus the maximum charge delivered to the retina was capped to 750 nC corresponding to a maximum charge density of 265 nC cm^{-2} (for single electrode stimulation) which is below the safe limit for gassing when using platinum electrodes (Leung et al. 2015). Platinum needle electrodes were placed in the conjunctiva to provide an extraocular return path.

Stimulating electrode groups were chosen in three predefined geometric shapes: triangles, rectangles, and hexagons (shown in Figure 4.1). For each arrangement, it was important that the electrode groups contained a single electrode in its centre that was not part of the group but stimulated on its own as we wanted to assess if a similar cortical response could be obtained between single and steered multi-electrode stimulation. Four stimulus configurations were used for each shape: central physical electrode (CPE) stimulation, whereby the single electrode in the centre of the shape received all the charge. Central virtual electrode (CVE) stimulation, whereby the outer electrodes of the shape received equal proportions of charge, with the intention

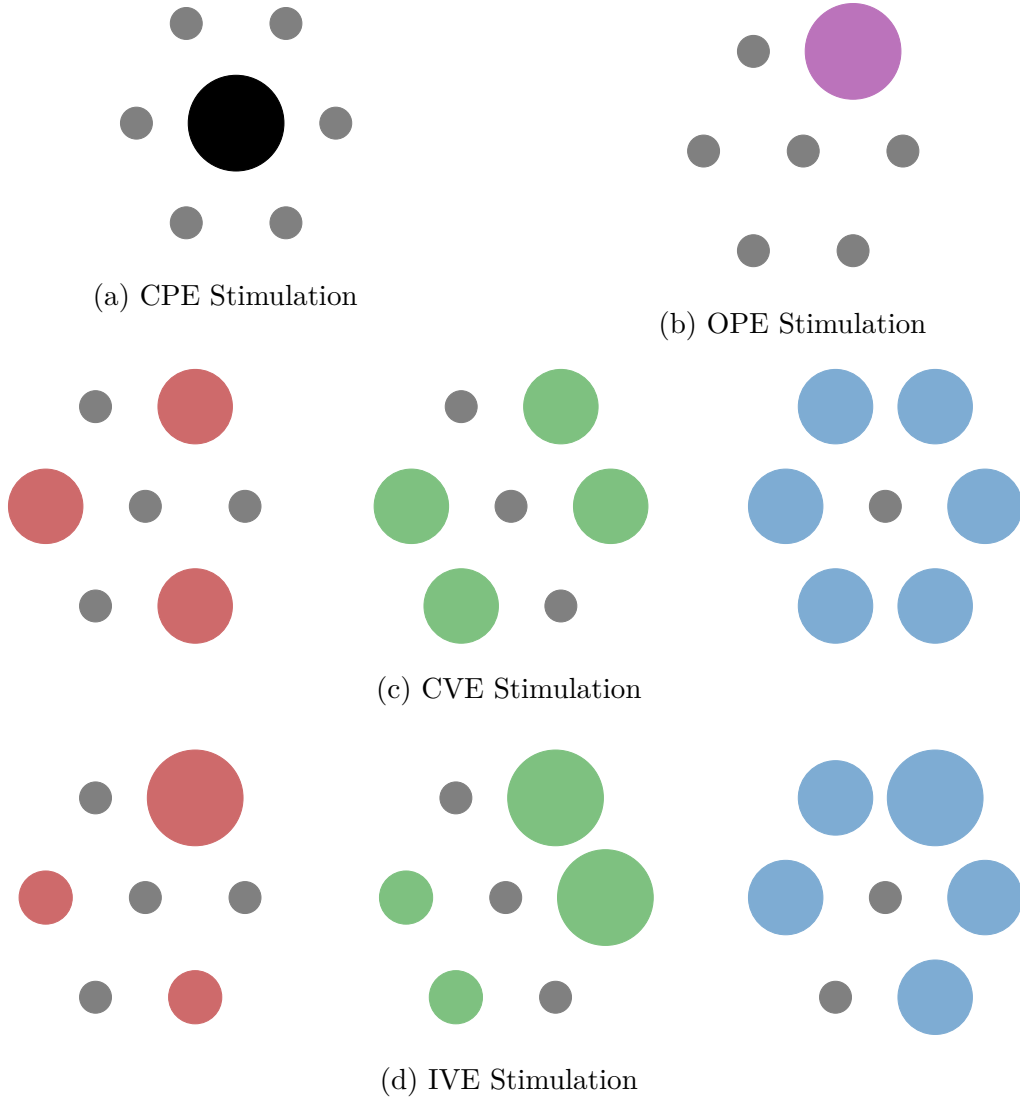


Figure 4.1: Diagram showing the different combinations of electrode shapes and stimulation configurations. Grey circles represent unstimulated electrodes on the stimulating array. Larger circles in other colours represent electrodes being stimulated. The size of the electrodes shows the proportion of charge delivered (not to scale). Central physical electrode (CPE) stimulation is where the central electrode of the shape is stimulated, and outer physical electrode (OPE) stimulation is where an outer electrode of the shape is stimulated. Central virtual electrode (CVE) stimulation is where charge is distributed evenly between the OPEs. Intermediate virtual electrode (IVE) stimulation is where charge is unevenly distributed amongst the OPEs, with the aim of creating a virtual electrode between the CPE and the OPE with the greater proportion of the charge.

of creating a virtual electrode in the same area as the CPE. Intermediate virtual electrode (IVE) stimulation, where certain electrodes received a greater proportion of charge, depending on the geometric shape. For triangular electrode shapes, half the charge was delivered to one electrode, while the other two electrodes each received one quarter of the charge. For rectangular electrode shapes, two electrodes received one third of the charge each, while the others each received one sixth. For hexagonal electrode shapes, only five of the six electrodes received charge with one third of the charge applied to one outer electrode, no charge delivered to the electrode opposite, and the other four electrodes each receiving one sixth. Due to experimental time constraints other charge ratios were not explored. IVE stimulation was repeated for each electrode shape, while rotating the outer electrodes that received proportionally greater charge. Finally, outer physical electrode (OPE) stimulation was also performed where an outer electrode received all the charge. This was done as a control to confirm that responses were different between different single electrodes. OPE stimulation was also repeated for each outer electrode in each shape.

4.2.3 Data analysis

Cortical recordings were analysed using custom scripts written in Igor Pro (WaveMetrics, Lake Oswego, OR, USA) and MATLAB (Mathworks, Natick, MA, USA). Signal artefacts were removed using techniques described by Heffer & Fallon (2008). The signal was bandpass filtered after artefact removal (Butterworth filter 0.3-5 kHz; order 3) and spikes were timestamped when the signal exceeded 4 times the root mean square value. Spikes detected within a 3 ms to 20 ms window were included in the analysis, as it is hypothesised to corresponding to both direct activation of retinal ganglion cells and indirect activation of the network (Boinagrov et al. 2014), and to remain consistent with earlier studies (Cicione et al. 2012, Dumm et al. 2014, Shivdasani et al. 2010, Spencer et al. 2016, Wong et al. 2008). For each recording, the number of spikes were counted 3 ms to 20 ms prior to the stimulus in order to estimate the spontaneous firing rate. The average spontaneous firing rate over the ten stimulus repeats was subtracted from spike rates recorded during the evoked response period. For each recording channel, spike rates averaged across the 10 repetitions of each current step were used to construct an input-output function and a sigmoid curve was fitted. Consistent with our earlier studies (Cicione et al. 2012, Shivdasani et al. 2012), threshold was defined as the level of charge where the sigmoid curve reached 50% of the maximum saturated spike rate on the recording channel. For each stimulus configuration, the recording channel with the lowest threshold was designated the best cortical electrode (BCE). Cortical spatial maps

were constructed by plotting the spike rate across all recording channels at the threshold charge of the BCE.

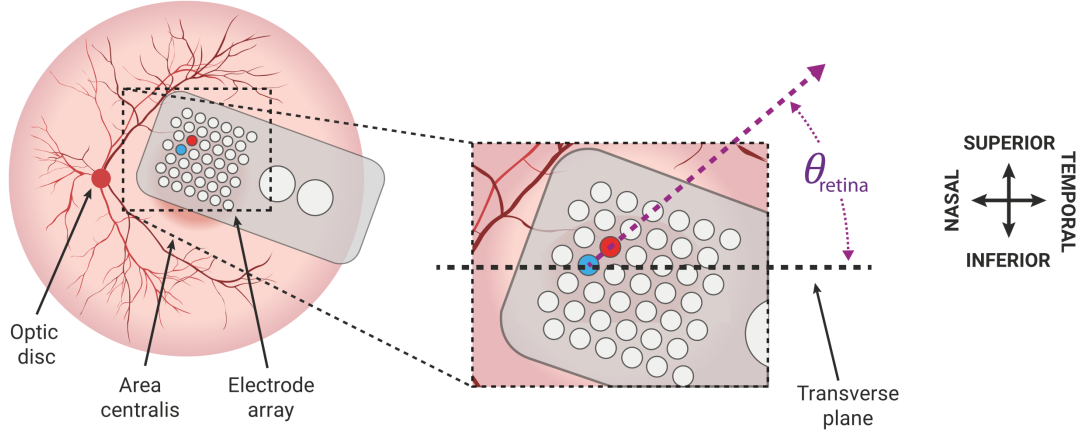
To characterise the spatial characteristics of cortical responses, we calculated the weighted centroid and neural activation spread of the cortical spatial maps. Centroids were calculated as a normalised spike-rate weighted centre of mass across all channels, according to the following formula:

$$\bar{x} = \frac{\sum_{i=1}^n m_i x_i}{\sum_{i=1}^n m_i} \quad \bar{y} = \frac{\sum_{i=1}^n m_i y_i}{\sum_{i=1}^n m_i}$$

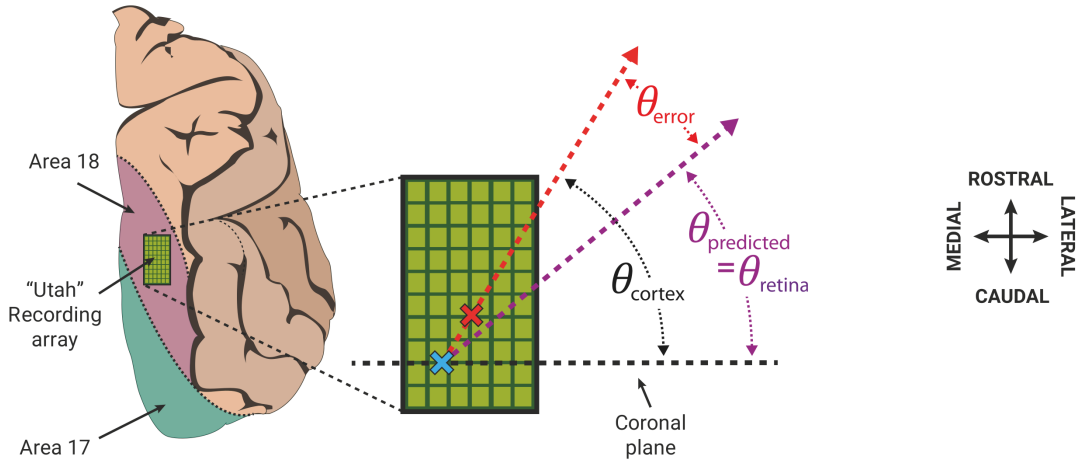
Where $(\bar{x}, \bar{y})$ are the coordinates of the centroid, m_i is the normalised spike rate from each electrode site (i.e. $m_1, m_2, \dots, m_n$), and x_i and y_i are the coordinates of each electrode site (i.e. $(x_1, y_1), (x_2, y_2), \dots, (x_n, y_n)$).

Applying the same stimulation to the retina typically results in similar, but not identical, cortical responses. This can be due to a number of factors, such as changes to impedance in the choroid due to inflammation, sensitisation to previous stimuli, depth of anaesthesia, or central neural influences. As such, cortical responses to two different sets of CPE stimulation were recorded, in order to determine the average degree of inherent variation in centroid position to identical stimulation. Centroids calculated for the repeat of CPE stimulation, as well as for CVE, IVE and OPE stimulation were compared in distance from the centroid calculated for the original CPE stimulation run. As there were multiple iterations of IVE and OPE stimulation for each electrode shape, the distances for each stimulation mode were averaged for each shape. The average shift in cortical centroid for each of the stimulation configurations compared to the original CPE run were compared with a repeated measures analysis of variance (ANOVA). We hypothesised that the average centroid shift between responses elicited by CPE and CVE stimulation should not be greater than the shift calculated between repeats of CPE stimulation. Ideally, IVE stimulation should create a virtual electrode between the central and outer physical electrodes, as such we expected the average centroid shift between responses to CPE and IVE stimulation to be greater than repeats of CPE stimulation, but less than the shift calculated between CPE and OPE stimulation.

The spread of neural activity was calculated for each configuration using the method described by Cicione et al (Cicione et al. 2012). Normalised spike rates on each chan-



(a) Deriving θ_{retina}



(b) Deriving θ_{error}

Figure 4.2: Diagram illustrating our method of predicting the direction of centroid shift. (a) shows the method of deriving θ_{retina} . The blue electrode represents the electrode at the centre of an electrode shape, and the red electrode represents an outer electrode of a shape, which is either being stimulated independently, or has received the greatest proportion of charge as part of IVE stimulation. The angle between these two electrodes relative to the transverse plane of the eye is θ_{retina} . (b) shows the method of deriving $\theta_{prediction}$ from θ_{retina} . The blue and red crosses represent the location of the centroid of the responses elicited by stimulation of the blue and red electrodes from (a). The purple dotted line represents $\theta_{prediction}$, which equals θ_{retina} , relative to the coronal plane of the cortex. The red dotted line represents θ_{cortex} , which is the measured angle between the centroids. The difference between $\theta_{prediction}$ and θ_{cortex} is designated θ_{error} .

nel at the charge required to reach 90% of the maximum saturating spike rate on the BCE were plotted as a function of distance from the BCE. A decaying exponential was fitted and the inverse tau used to quantify cortical selectivity. Activation spread was measured for each stimulus configuration of each electrode shape. Our previous work showed no significant difference in the activation spread of responses elicited by physical electrodes and virtual electrodes created by stimulation of electrode pairs (Dumm et al. 2014). We expected this to be maintained in virtual electrodes created by two-dimensional electrode shapes.

In addition to measuring centroid shift, we also devised a method to predict the expected direction of centroid shift based on work done by Tusa et al on the retinotopic mapping in Brodmann areas 17 (Tusa et al. 1979) and 18 (Tusa et al. 1979). We predicted that superior movement of the peak of the electrical field in the retina would translate to rostral movement in the centroid of contralateral cortical activation, and that temporal movement in the retina would translate to lateral movement in the cortical centroid. The location of each physical electrode in the eye was determined from fundus images taken post-implantation. Using this translational mapping technique, we attempted to predict the direction of the cortical centroid shift based on angles measured in the retina between physical electrodes, and the expected position of virtual electrodes. We expected that the angle between the peaks of the electrical field in the retina (θ_{retina}), relative to the transverse plane of the eye, would be similar to the angle between the centroids of the resultant cortical responses (θ_{cortex}), relative to the coronal plane of the brain (illustrated in Figure 4.2).

To validate our technique, we first attempted to predict the angle of centroid shift between CPE and OPE stimulation, as the locations of physical electrodes are known in the retina. Assuming the peak of the electrical field in the retina is centred at the same location as the stimulated electrodes, we measured the angle between the electrodes from the fundus images. This angle was rotated in accordance with the directional mapping between retina and cortex explained above to give a predicted angle of centroid shift ($\theta_{predicted}$). The actual angle between the two centroids elicited was then measured. The difference (θ_{error}) between $\theta_{predicted}$ and θ_{cortex} were plotted in a polar frequency histogram and placed in bins with a 45° width. Rayleigh's test was used to measure uniformity of the circular distribution and, if shown to be non-uniform, a circular mean was calculated. We expected that θ_{error} values will not be uniformly distributed, and would have a near-zero circular mean if the centroids moved in the same direction as we expected them to. Following successful validation of our technique, we attempted to predict the direction of centroid shift

between CVE stimulation and IVE stimulation, assuming that CVE stimulation would produce an electrical field with a peak over the CPE, and that IVE stimulation would result in a centroid shift in the direction of the electrode or electrodes with the greatest proportion of charge.

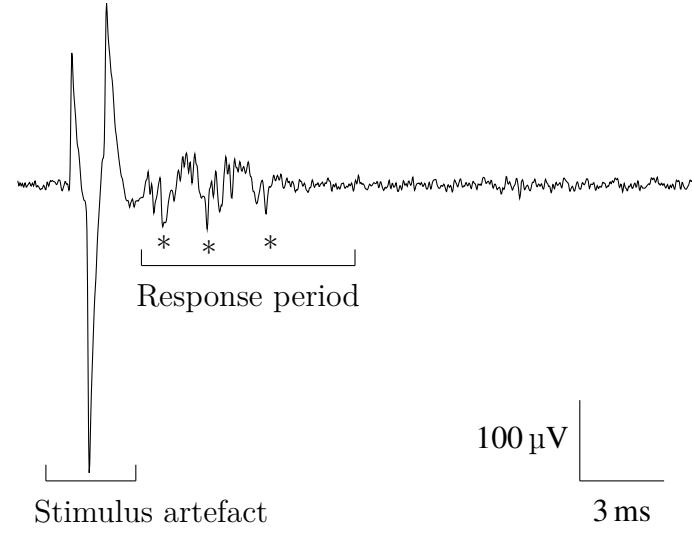
4.3 Results

4.3.1 Differences between anaesthetic protocols

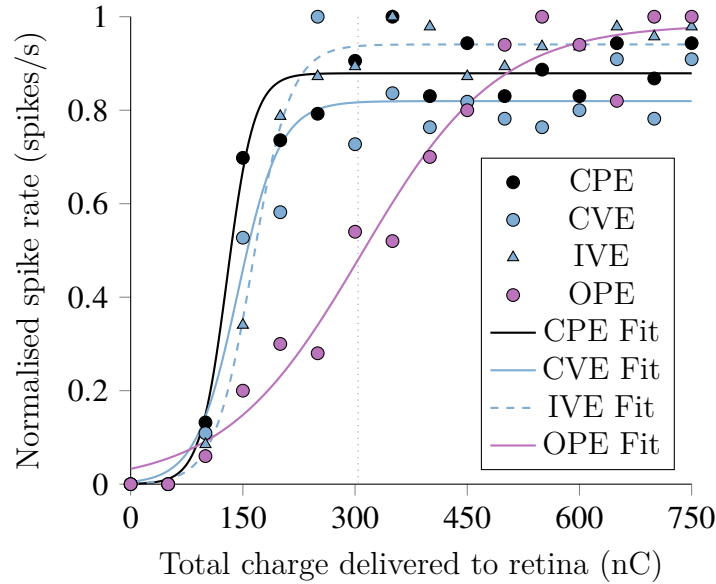
No significant difference was found in the latency or maximum firing rate of electrically-evoked cortical responses between the two different anaesthetic protocols (p 's < 0.05, Mann-Whitney U test). However, there was a statistically significant increase in spontaneous firing rates, rising from an average of 2.18 spikes/sec ($n=39563$ cortical channels) in cats anaesthetised with pentobarbital to 3.07 spikes/sec ($n=8940$ cortical channels) in cats anaesthetised with a combination of propofol and methadone ($p < 0.001$, Mann-Whitney U test). While statistically significant, we do not believe that the rise in spontaneous firing rate between the groups is of a sufficient magnitude to affect our spike analysis methods given we subtracted spontaneous firing from the spike count obtained in the 3 ms to 20 ms window. Given these results, the data collected from all animals were combined and analysed in the same fashion regardless of anaesthetic protocol.

4.3.2 Reproducing responses to physical electrodes

For this analysis, shapes were only included if a threshold could be calculated from at least one recording channel in response to two repeats of CPE stimulation, and at least one run of CVE, IVE and OPE stimulation. Cortical responses were collected from 38 triangular and 19 hexagonal shapes with all modes of stimulation applied, totalling 342 physical electrodes. Unfortunately, two sets of CPE stimulation were not recorded for rectangular shapes due to time constraints and technical issues, and as such are excluded from this analysis. Examples of input-output (IO) plots for each stimulus mode of a hexagonal electrode shape are shown in Figure 4.3. Using these criteria, 18 triangular and 8 hexagonal shapes were included in this analysis. No significant differences in lowest cortical thresholds were found between stimulation of single physical electrodes (i.e. CPE or OPE stimulation, 177 ± 8.4 nC, $n=127$), and virtual electrodes (i.e. CVE and IVE stimulation) created by triangular (174 ± 6.2 nC, $n=122$) or hexagonal (193 ± 9.1 nC, $n=84$) electrode shapes ($p > 0.05$, ANOVA on Ranks). No significant differences in lowest cortical thresholds were found between



(a) Bandpass filtered signal (without artefact removal)



(b) Input-output functions on a single cortical channel

Figure 4.3: Examples of electrically evoked multiunit activity on a single cortical recording channel. (a) Recording of multiunit activity on a single recording channel in response to stimulation of a single retinal electrode. Stimulation artefact and the recorded response are highlighted. The red line denotes the -3RMS threshold, and asterisks denote detected spikes. (b) Input-output functions of a single cortical channel in response to CPE (black), CVE (blue), and OPE (purple) stimulation of the same hexagonal electrode shape. Dots represent the average normalised spike rate at each discrete charge interval. The unbroken lines show the sigmoid fit for each dataset. The dotted lines show the point of the sigmoid that corresponds to the threshold (CPE: 128.8 nC, CVE: 143 nC, IVE: 158 nC, OPE: 308.5 nC). Note the similarity between the threshold and sigmoid fit characteristics between CPE, CVE and IVE stimulation. As the peak of the electrical field shifts to an adjacent electrode, the threshold for this cortical channel increases.

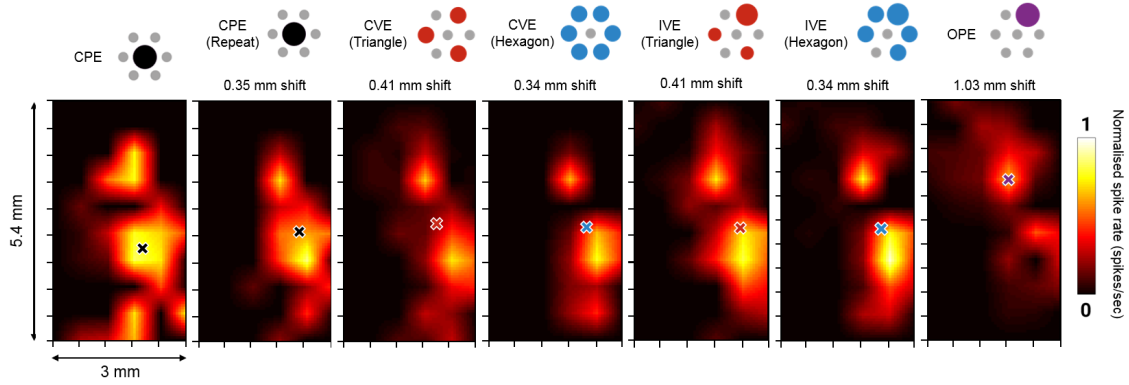


Figure 4.4: Examples of cortical spatial maps constructed in response to the different stimulation configurations. Smoothing has been applied to the maps using linear interpolation of the values at neighbouring grid points in each direction. Recording electrodes lie on the intersections of the axes ticks. For this example, the triangular and hexagonal electrode shapes share the same CPE and OPEs. Weighted centroids are marked on each spatial map by an X. Note the similarities in centroid locations between the virtual electrodes and the CPE repeats but a significant shift in the centroid when one of the OPEs was stimulated. The shift of the centroids from the centroid calculated for the first run of CPE stimulation is labelled above each map.

CPE ($n=42$), CVE ($n=26$), IVE ($n=85$) and OPE ($n=72$) stimulation ($p>0.05$, ANOVA on Ranks).

Spatial maps for an overlapping triangular and hexagonal electrode shape are shown in Figure 4.4. In this example, the cortical response pattern to CPE stimulation was very similar to the repeat of CPE stimulation and the various virtual electrode stimulation configurations, but different to the OPE configuration. It should be noted that not all triangular and hexagonal electrode shapes shared a CPE or OPEs, therefore responses to each shapes were analysed separately. The average shift in cortical centroid location between the two repeats of CPE stimulation was found to be 0.451 ± 0.08 mm and 0.431 ± 0.13 mm for the 18 triangular and 8 hexagonal shapes respectively (shown in Figure 4.5). For both triangular and hexagonal shapes, centroid shift from CPE stimulation significantly depended on the stimulus configuration used (Figure 4.5, $p<0.001$, one-way RM ANOVA). Centroid shift between responses to CPE stimulation and CVE and IVE stimulation were not found to be significantly different to the average shift between CPE repeats (Figure 4.5, $p>0.05$, Tukey post hoc). A significant shift in centroid location was observed between responses to CPE and OPE stimulation (Figure 4.5, $p<0.001$, Tukey post hoc). No significant difference was observed between the spread of cortical activity elicited by each stimulation mode ($p>0.05$, one-way ANOVA, Dunn post hoc). No significant difference was observed in the spread of cortical activity between

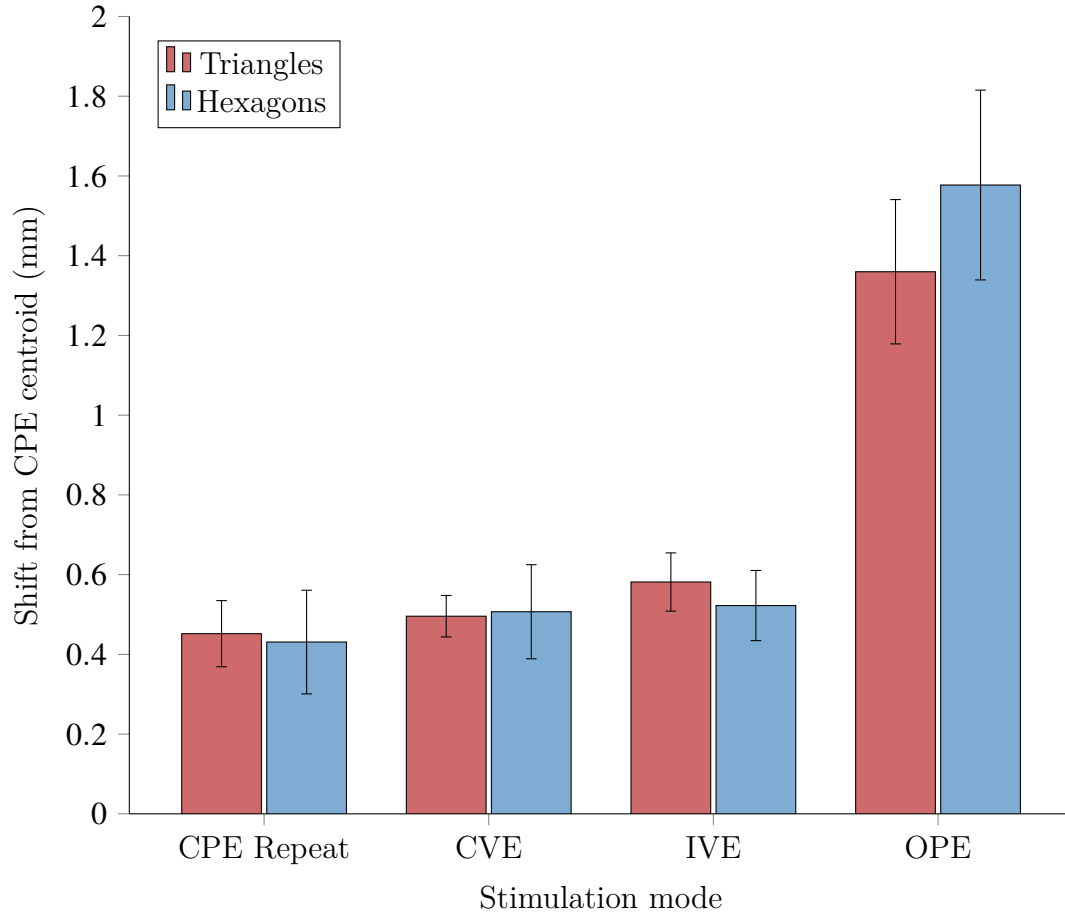


Figure 4.5: Bar chart showing the average distance between the centroid of activation calculated for stimulation of the CPE, and centroids calculated for every stimulation mode, including a repeat of CPE stimulation. Asterisk denotes group showing significant increase (RM ANOVA on Ranks, $p < 0.001$, Tukey post hoc test). No significant difference was observed between the average cortical shift of the repeat of the CPE and the two virtual electrode modes (CVE & IVE) ($p > 0.05$). There was a significant shift between the centroids calculated for CPE stimulation and OPE stimulation ($p < 0.001$). No significant differences were observed between triangular and hexagonal groups (RM ANOVA on Ranks, $p > 0.05$, Tukey post hoc test). Error bars show standard error of the mean.

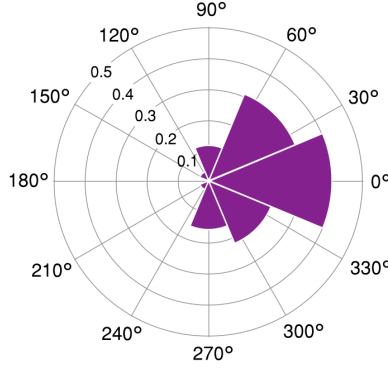


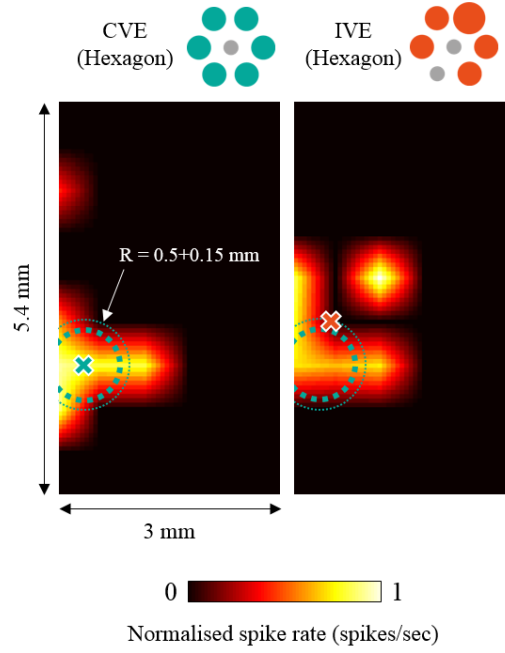
Figure 4.6: Polar probability density histogram showing the distribution of θ_{error} calculated for 41 CPE-OPE pairs, grouped in to 45° bins. Binned values denote the difference between the predicted and measured direction of cortical centroid shift from CPE stimulation to OPE stimulation. The distribution was found to be non-uniform ($p < 0.01$, Rayleigh test). The circular mean for these values was -0.18° . Radial axes show estimated probability density for each bin.

stimulation of single physical electrode stimulation ($1/\tau = 1.84 \pm 0.17$, $n=58$) and virtual electrode stimulation of triangular ($1/\tau = 2.06 \pm 0.17$, $n=57$) or hexagonal ($1/\tau = 2.06 \pm 0.15$, $n=47$) electrode shapes ($p > 0.05$, ANOVA on Ranks).

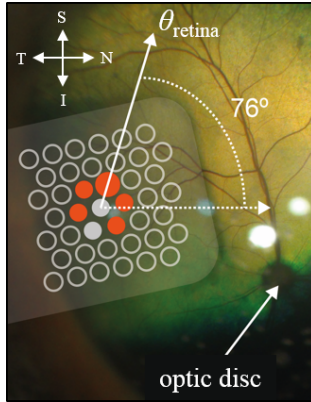
4.3.3 Predicting location of intermediate electrodes

To validate our method of predicting the angle of cortical centroid shift using retinotopy, we first attempted to predict the angle of shift between centroids calculated for responses to CPE and OPE stimulation, as the locations of physical electrodes in the eye can be directly determined from fundus imaging. Responses to CPE and OPE stimulation were recorded from 38 triangular and 19 hexagonal shapes. From these shapes, 41 unique CPE-OPE electrode pairs yielded thresholds. The difference between the expected and measured angles between CPE-OPE centroid pairs θ_{error} is plotted in a binned polar histogram (45° bin width) in Figure 4.6. Statistical analysis showed that the distribution of θ_{error} was not uniform ($p < 0.01$, Rayleigh's test), with a circular mean deviation from the predicted angle of -0.18° .

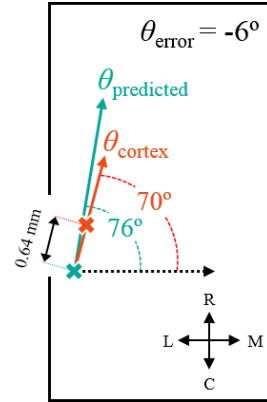
In a similar manner, to determine whether we could predict the angle of centroid shift for responses to intermediate virtual electrodes, shapes were included if a threshold could be calculated from at least one recording channel in response to CVE stimulation and at least one trial of IVE stimulation. CVE-IVE centroid pairs were excluded if the cortical centroid shift between them exceeded the mean average cortical distance between CPE and IVE centroids from the data shown in Figure 4.5



(a) Centroid locations



(b) Retinal direction



(c) Cortical direction

Figure 4.7: Diagrams showing example of centroid shift in the cortex and the methodology of directional prediction. (a) Two spatial maps showing patterns for CVE and IVE stimulation of the same hexagonal shape. The weighted centroids for each pattern are marked by the coloured X. The dotted circles show the mean (thicker line) and upper standard deviation (thinner line) of the average hexagonal IVE centroid shift from Figure 4.5. (b) Fundus image with overlay of the stimulating array in its real position. Orange electrodes show hexagon being stimulated in IVE configuration. The solid white arrow shows the expected direction of shift of the electrical field peak from the peak of field produced by CVE stimulation at an angle of 76° from the horizontal which was used as the direction of the predicted centroid. (c) Diagram comparing the measured direction between the centroids from spatial maps shown in (a), and the predicted direction based on retinal measurements. Note that the measured direction is within only 6° of the predicted direction.

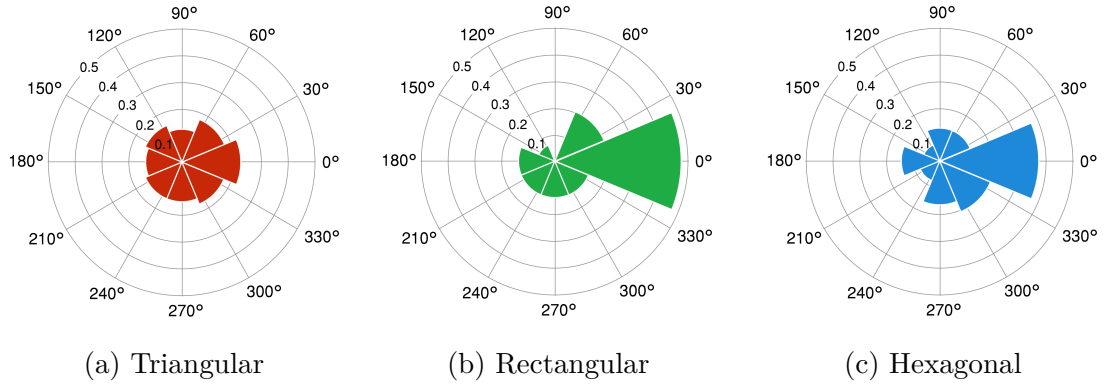


Figure 4.8: Polar probability density histogram showing the distribution of θ_{error} calculated for CVE-IVE pairs, grouped in to 45° bins. (a), (b), and (c) show the differences between the predicted and measured direction of cortical centroid shift from CVE stimulation to IVE stimulation, for triangular ($n=103$), rectangular ($n=19$), and hexagonal ($n=63$) electrode shapes respectively. The distribution for (a) was found to be uniform ($p>0.05$, Rayleigh test), however, (b) and (c) were found to be non-uniform ($p<0.01$, Rayleigh test), with circular means of -13.41° and -20.96° respectively. Radial axes show estimated probability density for each bin.

by one standard error to rule out the effect of responses shifting to neighbouring cortical areas. Using these criteria, 41 triangular, 25 rectangular and 13 hexagonal shapes were included in analysis. Deviation from the predicted direction of centroid shift was calculated for 184 CVE-IVE pairs (103 from triangular, 19 from rectangular, and 62 from hexagonal shapes). Based on the analysis shown in Figure 4.5, where repeated CPE and CVE stimulation resulted in a similar centroid shift, for this analysis we assumed that the peak of the electrical field produced from CVE stimulation would be located at the same point as the CPE for a given shape, and that the peak would shift in the direction of the physical electrode with the greater proportion of charge in the case of IVE stimulation with triangular or hexagonal shapes. For rectangular shapes, we expected the peak to shift toward the midpoint between the two electrodes that received the greater proportion of charge. An example of the method used to predict the angle of cortical centroid shift between a CVE-IVE pair of a hexagonal electrode shape is shown in Figure 4.7.

Contrary to our expectations, for triangular shapes (shown in Figure 4.8a), the θ_{error} calculated for responses between CVE and IVE stimulation were uniformly distributed with no preference in any direction ($p>0.05$, Rayleigh’s test). However, for rectangular and hexagonal shapes (shown in Figure 4.8b & 4.8c), the distribution θ_{error} were found to be non-uniform, with a circular mean deviation from the predicted angle of -13.41° and -20.96° respectively ($p<0.01$, Rayleigh’s test).

4.4 Discussion

In this study, we investigated whether current steering could be extended to a two-dimensional electrode array, allowing us to create virtual electrodes using more than two simultaneously stimulated electrodes. From our data, we have shown that we can reproduce certain spatial characteristics of cortical responses to stimulation of physical electrodes with virtual electrodes created by stimulation of up to six surrounding physical electrodes. While we were not able to create distinct intermediate virtual electrodes when applying unequal stimulation weights, we did show that we could predict the direction of the shift in the centroid of cortical activity based on which electrodes received greater proportions of charge.

4.4.1 Reproducing responses to physical electrodes

We found that equally-weighted simultaneous stimulation of electrodes in either triangular or hexagonal arrangements could elicit cortical responses with similar spatial characteristics to that of stimulation of the electrode in the centre of the shape. Confirming our initial hypothesis, the shift in centroid position between responses to CPE and CVE stimulation was no greater than the shift observed between repeats of CPE stimulation. Shift in cortical centroid position between CPE and OPE retinal stimulation, was significantly higher than between repeats of CPE stimulation. There was also no significant difference in the spread of neural activation elicited by physical electrode or virtual electrode stimulation. This indicates that CVE stimulation of these shapes created virtual electrodes in a similar location to the physical electrode in the centre of the shapes, producing responses with similar spatial characteristics (centroid and cortical spread measures) to CPE stimulation. This is consistent with our previous work with electrode pairs, which showed that cortical responses to physical and virtual retinal electrodes elicited similar levels of cortical activation spread, and that delivering equal proportions of charge to each electrode elicited responses with centroids approximately halfway between centroids calculated for each individual electrode (Dumm et al. 2014). Our results show that the ability to create virtual electrodes with cortical activation spread to physical electrodes is maintained with two-dimensional current steering, and that the spatial linearity of field interactions remains consistent. Somewhat surprisingly, although we predicted IVE stimulation would elicit a centroid shift from CPE stimulation greater than that of repeats of CPE stimulation, we found this was not the case. This data suggests IVE stimulation created a virtual electrode closer to the central electrode compared to the outer electrode receiving the greater charge proportion.

Moreover, the thresholds found between CPE and CVE stimulation in total charge were similar, indicating a much reduced charge requirement on a per electrode basis when performing CVE stimulation. This indicates the CVE stimulation may be an attractive replacement for CPE stimulation when requiring low voltage stimulation, particularly for smaller sized electrodes where the impedances would be high.

4.4.2 Predicting location of intermediate electrodes

Due to the degree of variability in the centroid location calculated for responses to repeated CPE stimulation, it may have been that that virtual electrode produced when performing IVE stimulation did not shift the cortical response enough to be distinguished from this baseline noise, most likely due to an insufficient alteration to charge proportions. As such, we devised a method to determine whether there was a preferred shift toward the electrode/s with the greatest proportion of charge. Our predictive technique is a very crude adaptation of Tusa’s map of visual cortex and does not account for the curvature of the retinotopic map as it approaches the longitudinal fissure and the splenial sulcus (Tusa 1978, Tusa et al. 1979). Our technique also relies on the assumption of the maintenance of similar scaling to the retina in all cortical directions. However, while the theoretical basis for field shaping techniques such as current steering and focusing are predicated on the assumption of linear summation of field potentials, it does not necessarily imply a linear relationship between current weightings and the location of the peak of the summated electrical field. In the absence of a validated model of electrical stimulation from the supra-choroidal space, we cannot know for certain the effects of electrode arrangement on the shape of the resultant electric field.

To validate our technique, we first trialled it by calculating the directionality of the shift in centroids calculated for responses to CPE vs OPE stimulation. We showed that there was a statistical preference for the direction that we predicted, showing that this technique was a valid model. When applied to responses to CVE and IVE stimulation, we found that we also could predict the direction of cortical shift for rectangular and hexagonal shapes, using only the knowledge of which outer electrodes received greater proportions of charge. However this was not the case for triangular shapes. This may have been due to the differing geometry of electrode arrangements and proportions of charge between the shapes. Given our array of electrodes had a 1 mm pitch and assuming absolute linearity in field interactions, the weighted shift in the centre of mass of the electric field in the retina for IVE stimulation for triangular, rectangular, and hexagonal electrode shapes would be

0.25 mm, 0.33 mm, and 0.33 mm respectively from the geometric centre. As there is a reduced shift for stimulation of triangular shapes, it may explain why we were unable to observe a measurable preference in the expected direction. The cortical centroids that did shift in the direction of the electrodes that received the greatest proportion of charge confirmed that IVE stimulation is shifting the electrical field in the retina, consistent with our previous work with electrode pairs (Dumm et al. 2014). However, the absence of significantly observable shifts in cortical centroids with IVE stimulation may also be a result of the generally broad spread of cortical activation elicited by monopolar stimulation. The large spread of the electrical potential in the retina (Matteucci et al. 2013, Spencer et al. 2016), coupled with undesired activation of axons of passage (Schiefer & Grill 2006), may provide insufficient resolution to observe spatially discrete responses to intermediate virtual electrodes. This is consistent with patient reports that phosphenes elicited through monopolar stimulation from the suprachoroidal space often appear large and overlap with phosphenes elicited by neighbouring physical electrodes (Sinclair et al. 2016). Using a stimulating array that has smaller electrodes and is implanted closer to the target neurons, such as devices designed for epi- and sub-retinal placement, may result in more spatially discrete activation patterns. Using longer pulse widths (>25 ms) or sinusoidal stimulation has also shown promise in improving the selectivity of retinal neuron activation and avoiding activation of axons of passage. These techniques may result in more uniform and discrete cortical activation patterns and phosphenes, and allow for more accurate centroid calculations

4.4.3 Implications and future directions

This study demonstrates for the first time that virtual electrodes created by simultaneous stimulation of up to six physical electrodes can reproduce the spatial characteristics of responses to individual physical electrodes. These data also supports other studies that assert that field interactions are both predictable and repeatable, and, rather than a hindrance, have the potential to be a powerful tool in increasing the range of percepts that can be presented to patients.

Electrode failure is a significant issue for many prosthetic devices. Due to the complexity of making miniaturised and biocompatible devices, electrode arrays are fragile and prone to lead wire and electrode breakage. Due to the similarity between thresholds, centroid locations and spread of neural activation, it is possible that CPE stimulation and CVE stimulation of such geometric shapes will elicit similar percepts in patients. Using simultaneous stimulation of adjacent surrounding

electrodes, the percept elicited by the failed electrode in the centre may be able to be replicated, compensating for the loss of sensory input. As it may take a significant amount of time and training for patients to be able to interpret the stimuli they are being presented with, the consistency of percepts elicited by a device will likely play a substantial role in its usefulness (Dagnelie 2008, Geruschat & Deremeik 2011). Using virtual electrodes, device lifespan may be improved and will provide patients with longer-term consistent stimuli. Virtual electrodes may also replace the need for electrode-dense arrays, reducing manufacturing complexity. However, it may be prudent to include redundant physical electrodes available for virtual electrode stimulation in case of electrode failure. In a device with fewer electrodes, breakage of an electrode could have further reaching consequences on phosphene generation than a traditional electrode array, as a single electrode may be responsible for multiple percepts when used as part of different geometric shapes.

Incorporation of current steering in cochlear implant stimulation strategies have been shown to elicit additional pitch percepts (Firszt et al. 2007), however the functional benefit to speech recognition has been minimal (Buechner et al. 2008, Firszt et al. 2009). This may in part be due to broad spread of current, resulting in perceptual overlap (George et al. 2015). As mentioned earlier, psychophysical testing has shown that phosphenes often appear large and overlap with phosphenes elicited by neighbouring physical electrodes (Sinclair et al. 2016). As such, creating additional phosphenes between these phosphenes would likely provide little functional benefit, as patients may have difficulty discriminating between them. In addition, phosphenes are typically elicited sequentially in order to reduce unwanted spatiotemporal electrical interactions (Horsager et al. 2010, 2011). As timing between stimuli is compressed to accommodate greater numbers of phosphenes (those elicited by both physical electrodes and virtual electrodes), these interactions may become more prominent. Future-generation devices with improved electrode geometries and materials may be able to elicit more discrete discriminable phosphenes, however, for present generation devices, current steering may need to be combined with a form of current focusing. Traditional forms of current focusing use local return configurations such as bipolar (Cicione et al. 2012, Gerhardt et al. 2011), hexapolar (Cicione et al. 2012, Habib et al. 2013, Jepson et al. 2014), quasimonopolar (Khalili Moghadam et al. 2014, Matteucci et al. 2013), and common ground stimulation (Cicione et al. 2012). These techniques may prove to be incompatible with this form of current steering as neighbouring return electrodes may need to be recruited to create virtual electrodes and so would be unavailable to act as current sinks. Focused multipolar (FMP) stimulation, which utilises simultaneous stimulation of surrounding

electrodes with different weights and polarities to shape the electrical field, has been shown to yield a similar degree of reduction of spread of neural activation as hexapolar stimulation (Spencer et al. 2016). As the field is actively shaped by altering stimulation weights, it is possible that two-dimensional steering could be incorporated in to the calculation of these weightings. Future studies should investigate the compatibility of these techniques and whether more discrete cortical patterns can be elicited using virtual electrodes.

4.4.4 Limitations

We have used a number of measures such as centroid location, spread of neural activation, and cortical activation threshold in order to quantify the difference between the cortical responses to physical and virtual electrode stimulation. While these metrics show that virtual electrodes can reproduce certain properties of the cortical response, there are many other spatial and temporal components that we did not investigate that may impact on phosphene appearance. As such, we cannot claim with any certainty that phosphenes generated by physical or virtual electrode stimulation using surrounding electrodes would appear the same to patients. However, we believe that our measures still provide a strong argument that virtual electrodes created using two-dimensional current steering can produce phosphenes in a similar location to those elicited by physical electrodes, and share a similar spread and perceptual threshold.

Further investigation should be conducted in to the limitations of this technique, such as the maximum physical distance between electrodes before electrical field interactions diminish. Due to time constraints, this study investigated only a limited number of current proportions. Experimenting with a wider range of unequal current weights may provide a more comprehensive understanding of the generation of intermediate virtual electrodes. Delivery of different current amplitudes to electrodes simultaneously adds the engineering requirement of multiple independent current drives, increasing the technical complexity of the device. Multiple current drives may not be required to deliver equally-weighted stimulation, provided the impedance of the physical electrodes are similar. Stimulation of physical electrodes in irregular geometric arrangements may provide a more comprehensive way to produce virtual electrodes in regions other than the centre of a shape.

The cats used in this study were acutely-implanted and normally sighted and. As

such, there are a number of structural and functional differences between the visual system of these cats and visually-impaired patients. Firstly, there are many effects that occur following implantation in patients, such as chronic inflammation and fibrosis, that were not reflected in this study (Nayagam et al. 2014, Roessler et al. 2009). Secondly, the morphology of a degenerated retina is very different to that of our normally-sighted cats. In addition to the loss of photoreceptors, there is also a significant degree of remodelling and indirect cell death that occurs in the inner layers of the retina (Fariss et al. 2000). There is also substantial attenuation of the retinal vasculature and thinning of the choroid in response to reduced metabolic demand. Due to loss of visual input there is also significant cortical remapping, however, recent studies have shown that prolonged use of retinal prostheses may partially reverse this (Humayun 2017). While we would not expect retinal degeneration to affect the shape of the electrical fields produced by current steering, it is possible that the altered physiological conditions may result in unexpected cortical activity. Our previous work in a cat model of retinal degeneration has shown increased cortical thresholds, decreased cortical spread, and increased size of retinal receptive fields (Aplin et al. 2016b, Halupka et al. 2017). While this may not affect the location of phosphenes elicited by virtual electrodes, shape, spread, and other spatio-temporal properties may be different in degenerated eyes. Future studies in long term implanted blind cats would provide a more representative insight in to the benefits of this technique.

4.4.5 Conclusion

This study shows for the first time that virtual electrodes can be created using two-dimensional current steering with simultaneous stimulation of up to six physical electrodes. Virtual electrodes created using this method produced cortical responses that shared similar thresholds, centroid locations and activation spread to responses elicited by the central physical electrodes surrounded by the electrodes used for steering in the form of a geometric shape. We have also shown that virtual electrodes can be shifted in desired directions by altering the proportions of currents applied to steering electrode groups, however the change in charge proportions to observe this directionality preference needs to be large enough. This technique could provide a greater level of control over the location of phosphenes in patients' visual field, increase the number of percepts that can be presented to patients, as well as compensate for faulty physical electrodes. Further studies in long-term implanted blind feline models could give clearer insights in to the translational benefit of these techniques.

4.5 Acknowledgements

The authors wish to thank Stephanie Epp, Ceara McGowan, Ali Almasi, Felix Aplin, Kerry Halupka, Faith Lamont, Rodney Millard, Alison Neil, Alexia Saunders, Evgeni Sergeev, Dimitra Stathopoulos, Patrick Thien, and Sam Titchener for assistance. Also, thanks to Penny Allen and Chi Luu for implanting the suprachoroidal device. Funding for this research was provided by the National Health and Medical Research Council (NHMRC) Project Grant #1063093 and the Bart Reardon PhD Scholarship. The Bionics Institute acknowledges the support it receives from the Victorian Government through its Operational Infrastructure Support Program.

Chapter 5

Electrical field shaping techniques in a model of retinal degeneration

A subset of the results presented in this chapter have been published as the following full conference paper:

Spencer, T.C., Fallon, J.B., Abbott, C.J., Allen, P.J., Brandli, A, Luu, C.D., Epp, S., Shivdasani, M.N., 2018, ‘Electrical Field Shaping Techniques in a Feline Model of Retinal Degeneration’, in the *40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Honolulu, HI, USA.

Objective: Electrical field shaping techniques such as current focusing and steering have been shown in previous chapters to be promising tools to increase the number of discriminable percepts afforded to retinal implant patients. However, these studies, as well as the majority of studies conducted by other groups assessing the efficacy of such techniques, have been conducted in normally-sighted animals. It is well known that the degenerate visual system exhibits significantly different response properties to electrical stimulation compared to that of a healthy system. This study aimed to reassess the effectiveness of focused multipolar (FMP) stimulation and 2D virtual electrode (VE) current steering using an animal model that is more representative of patients with retinal degeneration, in order to better inform translational endeavours.

Approach: Five cats received intravitreal injections of adenosine triphosphate (ATP) in one eye to induce unilateral photoreceptor degeneration and subsequent retinal remodelling. We then recorded cortical responses to traditional single electrode monopolar stimulation and compared them to responses to FMP stimulation

and VE current steering.

Results: Retinal degeneration was successfully induced for four of the five cats. We found that, while we were able to replicate some of the promising results from previous chapters using the fellow healthy eye of blinded animals, we were not able to focus or steer cortical responses effectively in the degenerated eyes. We also found that responses to stimulation of the degenerate retinae were significantly more discrete compared with those elicited from stimulation of the control retinae. Additionally, cortical mapping of the degenerate retinae was found to be compressed. It is likely that retinotopic distortion, resulting from plastic changes to the visual system in response to loss of afferent input, is responsible for the reduced effectiveness of these stimulation strategies.

Conclusions: Our findings suggest that FMP stimulation and VE current steering are unlikely to provide significant benefit to patients in their current form. Further optimisation of these techniques is required in order to be effectively applied to degenerate retinae. A greater understanding of the remodelling that occurs as a result of photoreceptor degeneration is also essential to provide clearer interpretation of these results and to tailor future stimulation strategies.

5.1 Introduction

Electrical field shaping techniques have shown significant promise in recent years of neural prosthetic research, in terms of increasing the capabilities of devices constrained by surgical and engineering limitations (Habib et al. 2013, Matteucci et al. 2013, Spencer et al. 2016, Halupka et al. 2016, Spencer et al. 2018). Techniques such as current focusing and current steering have been employed clinically in patients who have received cochlear implants and deep brain stimulation implants with varying success (Landsberger & Srinivasan 2009, Buechner et al. 2008, Koch et al. 2007, Chaturvedi et al. 2012, van den Honert et al. 2007). However, in the field of retinal prosthetics, there has been little translation of these techniques from the laboratory to the clinic. While retinal prostheses are targeting neural populations that are not directly affected by diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD), there is significant remodelling and degeneration that occurs at the level of the retina and central visual pathway as a result of photoreceptor death (Marc et al. 2003, Jones & Marc 2005, Jones et al. 2003, Grunwald et al. 1996).

In Chapter 3, it was demonstrated that focused multipolar (FMP) stimulation, a technique pioneered in cochlear implants, could be applied to a two-dimensional retinal prosthesis (Spencer et al. 2016). FMP stimulation works by simultaneously stimulating all electrodes in the array using charge weightings calculated from transimpedance measurements obtained prior to stimulation (van den Honert & Kelsall 2007). These weightings typically present as concentric rings of positive and negative charge relative to the central stimulating electrode at the epicentre of the focused electrical field (Spencer et al. 2016). We showed that FMP stimulation was effective in reducing the spread of electrical charge in the retina, which translated to greater selectivity of cortical responses. Due to the retinotopic nature of the visual cortex, we concluded that FMP stimulation would likely result in more discrete phosphenes compared with monopolar stimulation. In Chapter 4, it was shown that virtual electrodes (VEs) can be created by simultaneously stimulating up to six electrodes, allowing us to elicit cortical patterns that have unique spatial properties, while maintaining similar levels of cortical and retinal selectivity compared to responses elicited by single electrode monopolar (MP) stimulation. The ability to create virtual electrodes alleviates the requirement of additional physical electrodes in an array to elicit a greater number of phosphenes and provides a method for compensating for physical electrodes that break. However, these results were obtained using normally-sighted animal models. Due to the vastly different physiology of a healthy and pathological visual system, it would be prudent to investigate the efficacy these promising stimulation strategies in an animal model that is more representative of target patients.

In collaboration with the Centre for Eye Research Australia (CERA) and University of Melbourne, the Bionics Institute developed a feline model of retinal degeneration using unilateral intravitreal injections of adenosine triphosphate (ATP) to induce photoreceptor death, without directly affecting inner retinal layers (Aplin et al. 2014, 2016a). This technique has been shown to effectively induce partial degeneration of the outer nuclear layer, reduce afferent input to the visual system and, consequently, remodelling of the inner retinal layers (Aplin et al. 2016a). Comparative electrophysiological studies have shown significant differences in cortical responses to electrical stimulation of healthy and degenerate retinæ, such as increased activation thresholds, increased size of electrical receptive fields, and lower saturated spike counts (Aplin et al. 2016b, Halupka et al. 2017). Histological analysis of degenerated retinæ has also shown significant glial scarring, discrete retinal detachments due to photoreceptor outer segment loss, and thinning of the inner retinal layers within 14 weeks post-injection (Aplin et al. 2016a). While the time course of degeneration in-

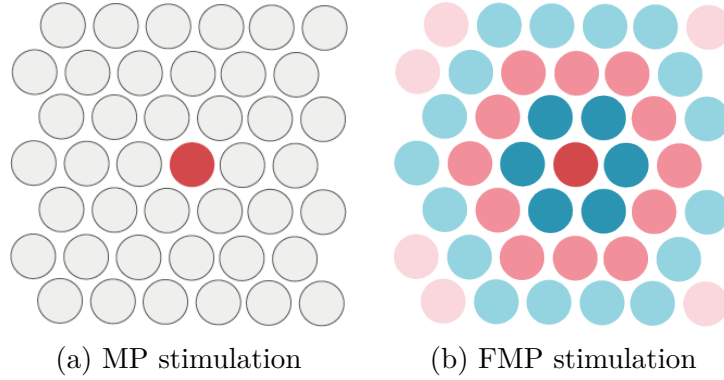


Figure 5.1: Diagrammatic representation of (a) monopolar (MP) and (b) focused multipolar (FMP) stimulation weights on a two-dimensional 42-channel electrode array. Red and blue circles respectively denote similar and opposite polarities on each electrode with respect to the central electrode. The size of the circles shows the relative proportions of charge delivered. As shown in the figure, FMP weights typically present as radiating concentric rings of positive and negative charge.

duced with ATP is significant faster than the degeneration process in RP, they share several features in terms of the morphological changes occurring in retinal neurons. These include significant inner retinal cell loss, breakdown of distinct retinal layers, attenuated vasculature, and new maladaptive cellular connections (Fariss et al. 2000, Jones et al. 2016). As well as retinal remodelling there are also significant effects on cortical and thalamic organisation as a result of reduced afferent input (Eger et al. 2005). In light of the promising results in previous chapters, this study aims to investigate the effectiveness of FMP stimulation and VE current steering using this cat model of retinal degeneration, in order to better inform translational endeavours.

5.2 Methods

All procedures were approved by the Bionics Institute Animal Research Ethics Committee (Project #14/304AB) and were in accordance with the Australian Code of Practice for the Care Use of Animals for Scientific Purposes (8th edition) regarding the care and use of animals for experimental procedures. Five adult domestic cats were used in this study.

The first part of this study presents data collected in response to MP and FMP stimulation of the control and degenerate retinae. The aim of this was to determine whether FMP stimulation is effective in eliciting more discrete regions of retinal and cortical activation than MP stimulation when applied to retinae with significant photoreceptor degeneration.

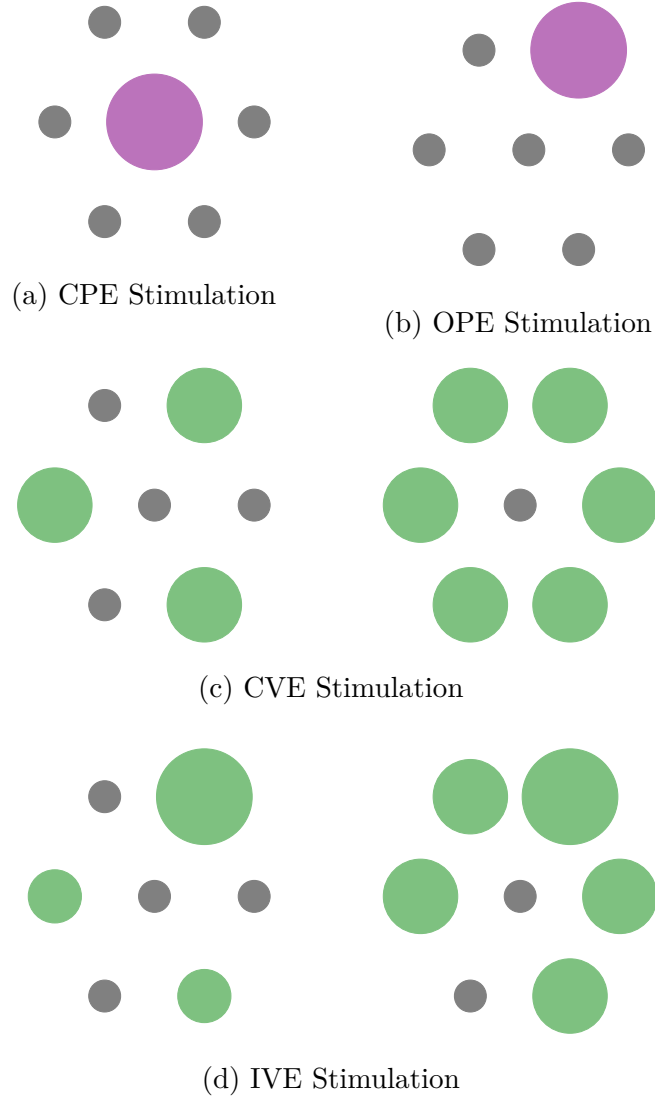


Figure 5.2: Diagrammatic representation of different forms of **(a-b)** physical (PE) and **(c-d)** virtual (VE) electrode stimulation for each electrode shape. The size of the electrodes shows the relative proportions of charge delivered (not to scale). Central physical electrode (CPE) and outer physical electrode (OPE) stimulation refers to stimulation of the central and outer electrodes of the shape. Central virtual electrode (CVE) and intermediate virtual electrode (IVE) stimulation refers to stimulation intended to create virtual electrodes and was carried out using outer electrodes in hexagonal and triangular shapes.

The second part of this study presents data collected in response to physical electrode (PE) VE stimulation of the control and degenerate retinae. The aim of this section is to determine whether VE stimulation continues to be an effective strategy to steer current and reproduce responses to PE stimulation when applied to degenerate retinae.

5.2.1 Blinding procedure

Prior to intravitreal injection, cats were anaesthetised with ketamine (intramuscular [i.m.] 20 mg kg^{-1}) and xylazil (subcutaneous [s.c.] 2 mg kg^{-1}). Baseline retinal health was assessed using spectral-domain optical coherence tomography (OCT), full-field flash electroretinogram (ERG), and colour fundus photography. A paracentesis was performed to prevent dangerous increase in intravitreal pressure after injection of ATP solution. A $200 \mu\text{l}$ solution of three parts sterile saline (0.9% NaCl) and one-part Dexamethasone (4 mg ml^{-1}), containing approximately 200 mM to 217 mM ATP disodium salt (Sigma-Aldrich Corp., St Louis, MO, USA) was injected into the intravitreal space of the eye. Dosages were determined prior to the procedure in collaboration with clinical ophthalmologists. Two weeks following recovery, retinal health was reassessed under anaesthesia in order to determine whether the ATP solution had successfully resulted in photoreceptor death. We considered a minimum 50% reduction in a-wave amplitude of the ERG from baseline to be our threshold for significant global photoreceptor loss. For more localised assessment, outer retinal thickness obtained from OCT and pigment deposits in fundus imaging in the area centralis region of the retina was assessed by a clinical ophthalmologist. OCT image slices were performed at the same retinal locations allowing for direct comparison to baseline measurements prior to ATP injection. Total retinal thickness was measured as the distance between the inner limiting membrane to the border of the retinal pigment epithelium,. Inner retinal thickness was defined as the distance between the inner limiting membrane to the inner nuclear layer (INL), and outer retinal thickness as the distance between the INL and retinal pigment epithelium (RPE). If it was determined, based on these measures, that there was not a sufficient level of degeneration, an additional dose of ATP solution was injected into the eye.

5.2.2 Anaesthesia and surgery

Once significant outer retinal degeneration was observed, we waited at least 14 weeks before conducting the acute electrophysiological experiment in order to allow time for inner retinal remodelling. For the acute experiments lasting 72 hours, cats were

pre-medicated with ketamine (i.m. 8 mg/kg), medetomidine (i.m. 0.012 mg kg⁻¹), and methadone (i.m. 0.4 mg kg⁻¹). Anaesthesia was maintained with continuous intravenous (i.v.) infusion of Propofol (24 mg/kg). A continuous i.v. infusion of methadone in Hartmann's solution (0.25 mL of 10 mg/mL Methadone in 250 mL compound sodium lactate, 0.005 mg kg⁻¹ h) was also administered throughout the period of anaesthesia. Due to the depressive effects of Propofol on the respiratory system, tracheostomies were performed, and the animals ventilated on 100% oxygen (20-25 breaths/min) (model 6025; Ugo Basile, Monvalle, VA, Italy). Respiration rate, end-tidal CO₂, blood pressure and temperature were monitored consistently throughout the experiment and maintained at normal levels by adjusting the anaesthetic flow rate. Daily injections of dexamethasone (i.m. 0.1 mg kg⁻¹) and clavulox (s.c. 10 mg kg⁻¹) were administered to all cats. Pupils were dilated by regular topical application of a mixture of phenylephrine hydrochloride (2.5%) and tropicamide (1%).

The retinal electrode array was of a similar design to that used in our previous work (Ayton et al. 2014, Villalobos et al. 2013), fabricated on a biocompatible silicon substrate and consisting of 42 platinum electrodes of 600 µm diameter spaced 1 mm from centre to centre. The implantation procedure is detailed in our previous studies (Villalobos et al. 2013, Saunders et al. 2014). Briefly, following a lateral canthotomy, scleral incision, and dissection of a pocket between the sclera and choroid, the array was inserted approximately 15 mm into the suprachoroidal space until the tip was beneath area centralis. Electrical connections to the electrodes on the array were tested using an automated impedance monitoring software developed in LabVIEW (National Instruments, Austin, TX, USA), as used in previous studies (John et al. 2011).

The animal was placed in a stereotaxic frame inside a darkened electrically shielded room. Visual inspection of the eye and fundus photographs were taken to determine the health of the eye and confirm positioning of the array. A craniotomy was performed spanning 15 mm rostral and 5 mm caudal from the interaural line, and 7 mm lateral in both directions from the sagittal suture, exposing the dorsal surface of both hemispheres of the visual cortex (VC). The dura mater was carefully excised from the region. Using two custom-fabricated parylene-based flexible platinum electrode arrays (as described in Fallon et al. (2016)), electrically evoked potentials (EPs) in response to cathodic-leading biphasic charge-balanced current pulses (0 µA to 750 µA, 1 ms per phase) applied to the retinal array, were mapped along the surface of each hemisphere of the VC to determine the cortical region with the lowest evoked

potential thresholds. Planar “Utah” 60- (6x10) channel penetrating microelectrode arrays (Blackrock Microsystems, Foxborough, MA, USA) were inserted in to both hemispheres of the visual cortex, to a depth of approximately 1 mm, in the regions with the lowest evoked potential thresholds to stimulation of the contralateral eye. The recording electrodes on each array were separated by a distance of 400 μm and each array sampled 7.2 mm² of cortex.

5.2.3 Electrical stimulation

Electrical stimuli were generated with a 128-channel IZ2 stimulator (Tucker-Davis Technologies, Alachua, FL, USA). Due to the low maximum current output per channel (300 μA), each group of three channels was combined using a custom-built circuit board bringing the maximum functional number of combined stimulating channels to 42, and a maximum current output to 900 μA per combined channel. Each combined channel was connected directly to an electrode on the suprachoroidal array enabling independent simultaneous stimulation of all 42 electrodes. Pulses were presented at randomly varied currents ranging from zero to 750 μA in 50 μA steps at a repetition rate of 1 Hz. Each current step was repeated 10 times. Stimulus pulses were symmetrically biphasic with a 1000 μs phase width and 25 μs interphase gap. Platinum needle electrodes were placed in the conjunctiva to provide an extraocular return path for charge.

Cortical recordings were analysed using custom scripts written in Igor Pro (Wave-metrics, Lake Oswego, OR, USA) and MATLAB (Mathworks, Natick, MA, USA). Signal artefacts were removed using techniques described by Heffer & Fallon (2008). The signal was bandpass filtered after artefact removal (Butterworth filter 0.3 kHz to 5 kHz; order 3) and spikes were timestamped when the signal exceeded 4 times the root mean square value. Spikes detected within a 3 ms to 20 ms window were included in the analysis, as it is hypothesised to corresponding to both direct activation of retinal ganglion cells and indirect activation of retinal circuitry (Boinagrov et al. 2014), and to remain consistent with earlier studies (Shivdasani et al. 2010, Wong et al. 2008, Spencer et al. 2016, Dumm et al. 2014, Cicione et al. 2012). For each stimulus trial, the number of spikes were also counted 3 ms to 20 ms prior to the stimulus in order to estimate the spontaneous firing rate. The average spontaneous firing rate over the ten stimulus repeats was subtracted from spike rates recorded during the evoked response period.

5.2.4 FMP stimulation and analysis

Following implantation, the stimulating electrode array was connected to a 512 cross-point switch matrix (PXI 2523, National Instruments, Austin, TX, USA). Channels of the switch matrix were also connected to an isolated constant current stimulator and a digital multimeter (PXI 4072, National Instruments, Austin, TX, USA), allowing stimulation and voltage recording at any electrode site. Using custom software written in LabVIEW (National Instruments, Austin, TX, USA) voltage waveforms were collected from every electrode in response to MP stimulation of every other electrode, allowing for the construction of a transimpedance matrix. Using these measurements, FMP weights were calculated in the same manner as described in Chapter 3 (see section 3.2.2). As shown in Figure 5.1, stimulation weights typically present as concentric rings of positive and negatively weighted charge radiating from the central electrode. FMP stimulation was then applied to all functional electrodes.

Cortical activity was recorded in response to MP and FMP stimulation of the retinal electrodes. For each stimulating electrode, threshold was defined as the lowest current required to exhibit spike rates greater than the baseline spontaneous activity while showing a monotonic increase in spiking activity at all higher current levels on any cortical channel. As explained in Chapter 3 (see section 3.2.5), due to increased thresholds, it is common for current focusing techniques to fail to produce saturating cortical spike rates at the maximum safe charge limit. This effect was also expected to be exacerbated when stimulating the degenerate retinae, as increased thresholds had been reported (Aplin et al. 2016b). As such, rather than comparing responses between MP and FMP stimulation at the same point along their respective dynamic ranges, we quantified the growth of neural response by calculating the discrimination index (d') of spike rates at each current level compared with spiking activity at threshold, using methodology derived from signal detection theory (Green & Swets 1966).

For each cortical channel, the best retinal electrode (BRE) was defined as the stimulating electrode that required the least current to elicit a spike rate on a cortical channel that yielded $d'=1$ above threshold. In a similar manner, the best cortical electrode (BCE) was defined as the cortical channel that required the smallest current to elicit a spike rate that yielded $d'=1$ above threshold. Retinal and cortical activation spread for MP and FMP stimulation were measured using the same methodology outlined in section 3.2.5). Differences in cortical and retinal selectivity

were determined with two-way ANOVAs, using binned distance and stimulus mode as factors, with post-hoc differences calculated using the Tukey test. Statistical comparison of retinal anatomical changes at each electrode site and cortical response thresholds and spread were assessed using a generalised linear mixed model (GLMM), using eye health, outer nuclear layer (ONL) cell density, and INL cell density as fixed effects, and the subject as a random effect (West et al. 2014).

5.2.5 Virtual electrode stimulation and analysis

Stimulating electrode groups for VE stimulation were chosen in regular hexagonal or triangular shapes (as shown in Figure 5.2). Each shape contained a single central physical electrode (CPE) and six outer physical electrodes (OPEs). Initially, cortical activity was recorded in response to MP stimulation of each CPE and OPE. Applying the same stimulation to the retina typically results in similar, but not identical, cortical responses. As such, cortical responses to stimulation of the CPE were recorded twice, in order to determine the average degree of inherent variation in cortical activity to the same stimulation method.

Two forms of VE stimulation was then applied to each shape. Central virtual electrode (CVE) stimulation, whereby the OPEs of the shape received equal proportions of charge, with the intention of creating a VE in the same area as the CPE, was applied once to each shape. Intermediate virtual electrode (IVE) stimulation, where certain OPEs received a greater proportion of charge, was also applied to each shape. For IVE stimulation of triangular electrode shapes, half the charge was delivered to one OPE, while the other two OPEs each received one quarter of the charge. For IVE stimulation of hexagonal electrode shapes, one third of the total charge was applied to one OPE, no charge delivered to the OPE opposite, and the other four flanking OPEs each received one sixth of the charge. IVE stimulation was repeated for each shape multiple times, rotating which OPE received the greatest proportion of charge. For all PE and VE stimulation, the maximum combined charge delivered to the retina was capped to **750 nC** corresponding to a maximum charge density of **265 μCcm^{-2}** which is below the safe limit for gassing when using platinum electrodes (Leung et al. 2015).

In order to remain consistent with previous studies, and the work outlined in Chapter 4, responses to steered stimuli were characterised using different methodology to that used in current focusing. For each recording channel and each stimulus configuration, normalised spike rates averaged across the 10 repetitions of each cur-

rent step were used to construct an input-output function and a sigmoid curve was fitted. Rather than use our previous definition of threshold, the BCE for each retinal electrode was defined as the cortical channel that required the least charge to elicit 50% of the maximum saturating spike rate (P50). Cortical spatial maps were constructed by plotting the spike rate across all recording channels at the lowest charge required to elicit 90% of the maximum saturating spike rate (P90) on the BCE. To characterise the spatial characteristics of cortical responses, we calculated the weighted centroid and selectivity of the cortical spatial maps. Centroids were calculated as a normalised spike-rate weighted centre of mass across all channels, according to the formula outlined in Chapter 4 (see section 4.2.3). Cortical selectivity was calculated using the method described by Cicione et al. (2012), which is also described in section 4.2.3.

5.2.6 Histological analysis

Animals were euthanised at the completion of the electrophysiological experiment via an overdose of Propofol (24 mg kg^{-1}). Without removing the stimulating arrays, both eyes of each animal were enucleated and placed in Davidson’s fixative for 18 h to 36 h at room temperature. The eyes were then transferred to a 50% ethanol solution for 6 h to 8 h before being stored in 70% ethanol at 4%. Coloured dye was applied to the outer sclera to indicate the location of each implanted electrode before the array was carefully removed from the suprachoroidal space. Full thickness strips of retinal, choroidal, and scleral tissue were cut from the implanted regions and embedded in agar before being transferred to a 10% neutral-buffered formalin solution for 1 h to 3 h. The tissue was then embedded in paraffin in a standard automated cycle, cutting $5 \mu\text{m}$ thick vertical sections with a microtome and staining with hematoxylin and eosin (H&E). Using the scleral dye as reference, $800 \mu\text{m}$ -wide micrographs near each electrode site were taken with an Axio Imager 2 upright microscope (Carl Zeiss AG, Oberkochen, Germany) and Axiovision V4.8.2 software. Dehydration of the tissue due to the use of paraffin embedding resulted in a distortion of retinal layer thicknesses. As such, INL cell counts were obtained through manual counting. ONL cell counts were obtained through the use of custom scripts written in ImageJ (National Institutes of Health, Bethesda, MD, USA) and MATLAB. In many regions of the degenerate retinae there was severe breakdown of outer photoreceptor segments and the nuclear layers leading to cell migration and distortion of the retina’s laminar structure. INL cells were defined in this study as any cell body present between the outer and inner plexiform layers. ONL cells were defined as any cell body present between the outer plexiform layer and the inner limiting membrane. Cell bodies

	S1 (LE)	S2 (RE)	S3 (RE)	S4 (LE)	S5 (LE)
Initial Injection	200 mM	200 mM	217 mM	200 mM	217 mM
Follow up #1	200 mM	217 mM	200 mM	200 mM	Severe retinal detach- ment
Follow up #2	217 mM	74% a-wave reduction	50% a-wave reduction	85% a-wave reduction	
Follow up #3	63% a-wave reduction				

Table 5.1: Molar concentrations of ATP in each intravitreal injection. Each injection had a uniform volume of 200 μ l containing three-parts sterile saline and one-part dexamethasone to reduce inflammation. Dosages and retinal health was determined for each subject in collaboration with clinical ophthalmologists. Injections were repeated until a reduction in ERG a-wave amplitude of at least 50% was observed or if complications arose. Severe retinal detachment was only observed in S5, resulting in exclusion from the remainder of the study.

that had migrated to the plexiform layers or in to the space left vacant by absent outer segments were not counted. In the few cases of extreme degeneration and remodelling, counting was undertaken with advice from a histologist.

5.3 Results

5.3.1 Retinal degeneration and remodelling

Retinal degeneration was induced successfully in four of the five cats used in this study without any serious adverse effects. For one cat, significant detachment of the retina was observed two weeks following a single injection of ATP solution. This animal was excluded from the rest of this study. The remaining four cats required at least two intravitreal injections of ATP to induce a level of retinal degeneration that met our criteria (dosages shown in Table 5.1). Subject #1 (S1) was the only animal to require an additional third injection. Due to scheduling constraints, we were unable to standardise the time between the observation of degeneration and the electrophysiological experiment. Counting from the final injection for each subject, experiments were conducted 97, 166, 103, and 110 days following for S1, S2, S3, and

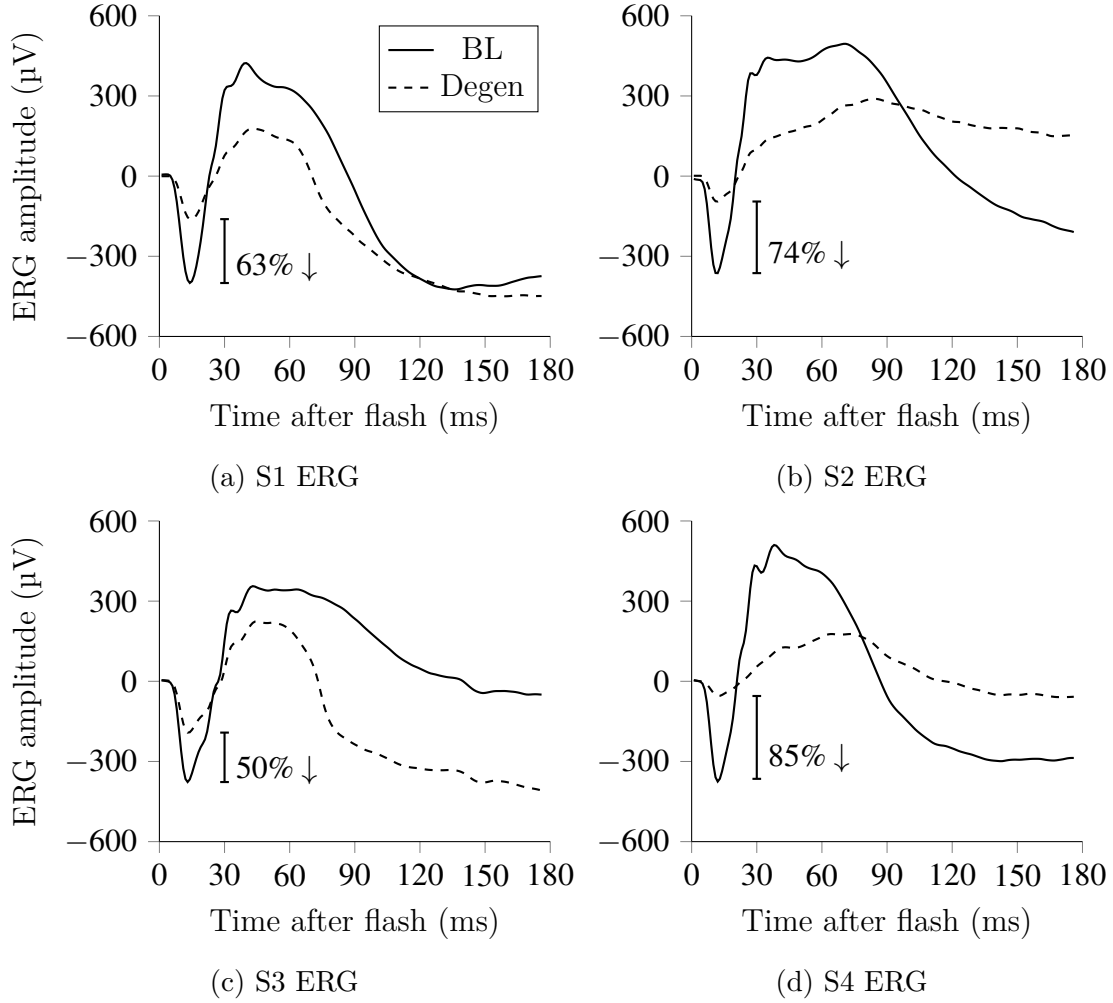
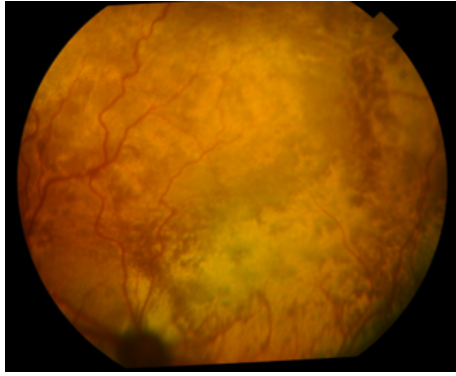


Figure 5.3: ERGs of each subject before and after retinal degeneration. The blue line represents the baseline ERG trace obtained prior to the initial intravitreal injection of ATP. The red line represents the ERG trace obtained at the conclusion of each subjects' final retinal health follow up. Reduction in a-wave amplitude is shown in each graph. All subjects shown above exhibited at least 50% reduction in a-wave amplitude from baseline.



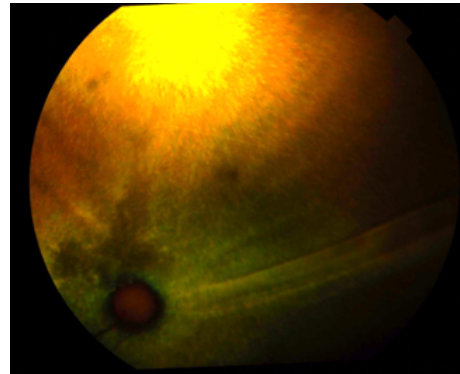
(a) S1 Fundus



(b) S2 Fundus

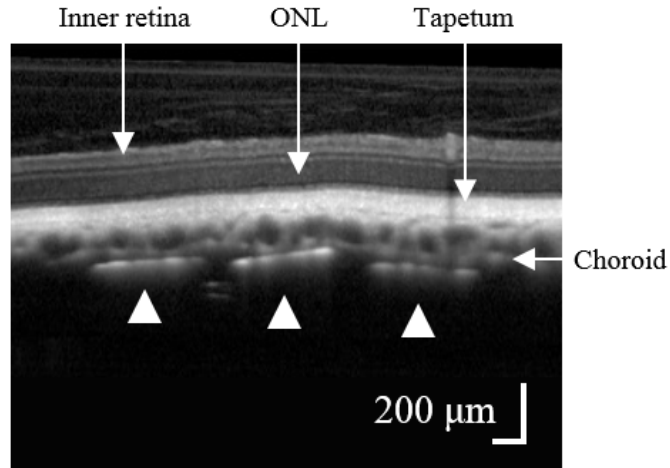


(c) S3 Fundus

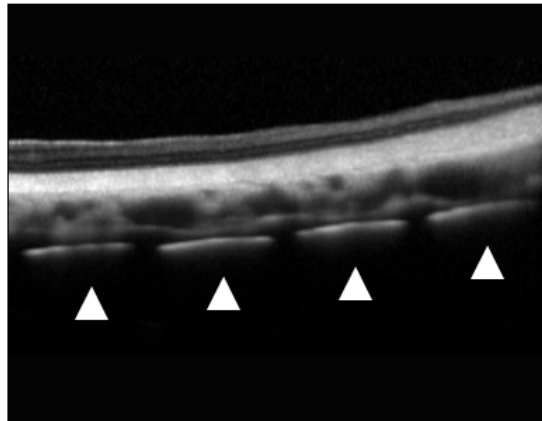


(d) S4 Fundus

Figure 5.4: Fundus images of the degenerated retinae from all four subjects post-implantation. Dark patches of pigment deposits can be observed as a result of photoreceptor apoptosis. The white asterisk shows a small haemorrhage due to surgery in S3. This did not appear to significantly impact the experiment



(a) OCT image of an implanted control eye



(b) OCT image of an implanted degenerate eye

Figure 5.5: Example OCT images of the (a) control and (b) degenerate retinae of S4 following implantation of the stimulating electrode arrays in both eyes. The inner retinal layers can be seen as a light band, whereas the ONL is darker in colour. It is evident in this example that the retina of the ATP-injected eye is far thinner than the retina of the fellow control eye. The implanted platinum electrodes can be seen beneath the choroid and are highlighted by white triangles.

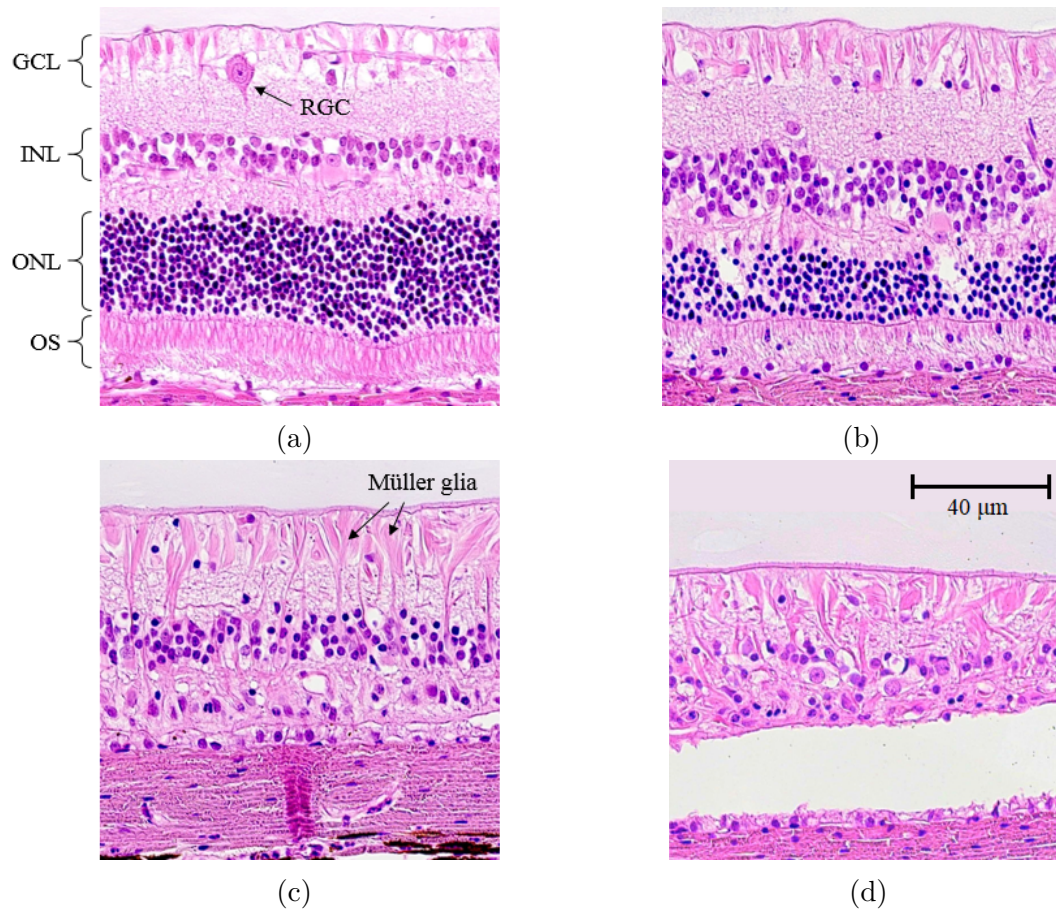


Figure 5.6: H&E sections of (a) control and (b-d) degenerate retinæ of S4, showing the range of retinal structural changes in response to ATP-induced photoreceptor degeneration. Note the reduction in ONL cell numbers and breakdown of the distinct retinal layers. In more degenerated regions such as in (c) and (d) there is also loss of inner retinal cells and increased Müller glial activation.

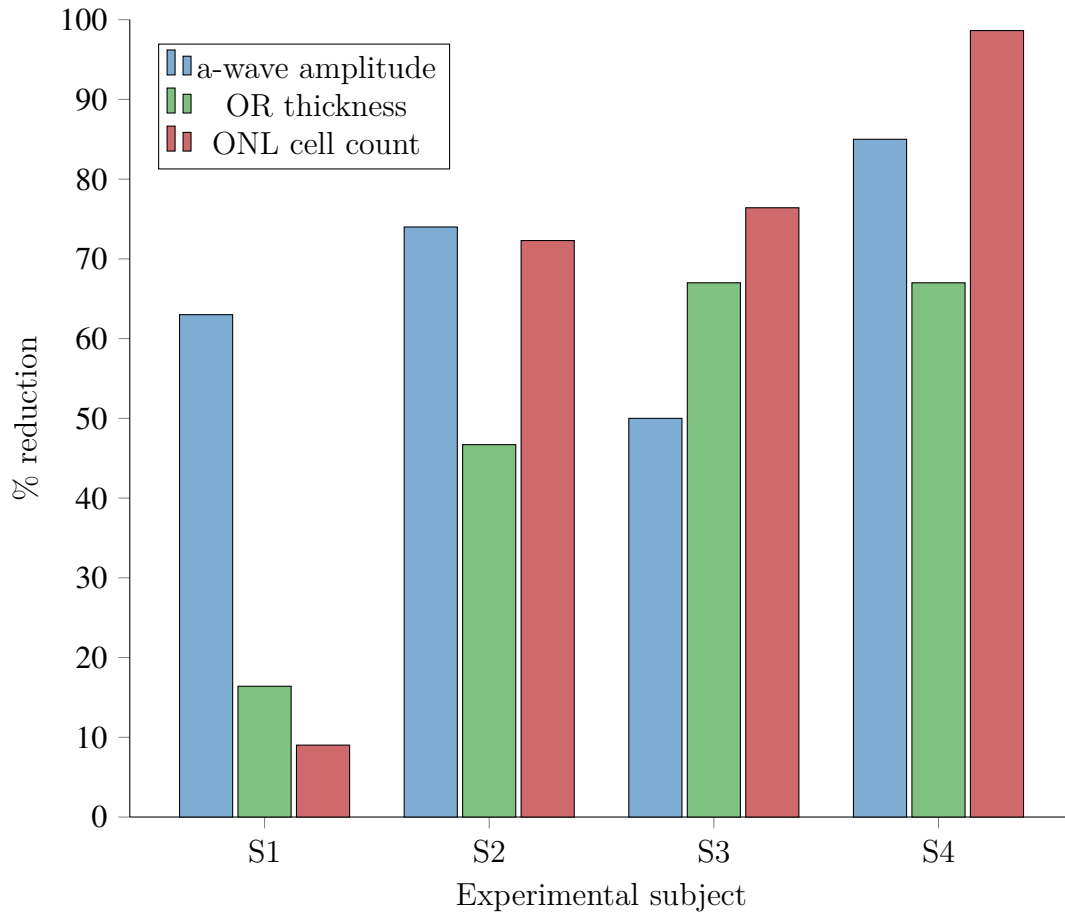


Figure 5.7: Bar chart showing % reduction of health indicators of the degenerate retinæ. A-wave amplitudes and OR thicknesses were measured using ERG and OCT prior to the electrophysiological experiment and compared to baseline measurements from before the initial ATP injection. ONL cell counts were obtained following the experiment using histological preparations of the retinæ and compared with the fellow control eye.

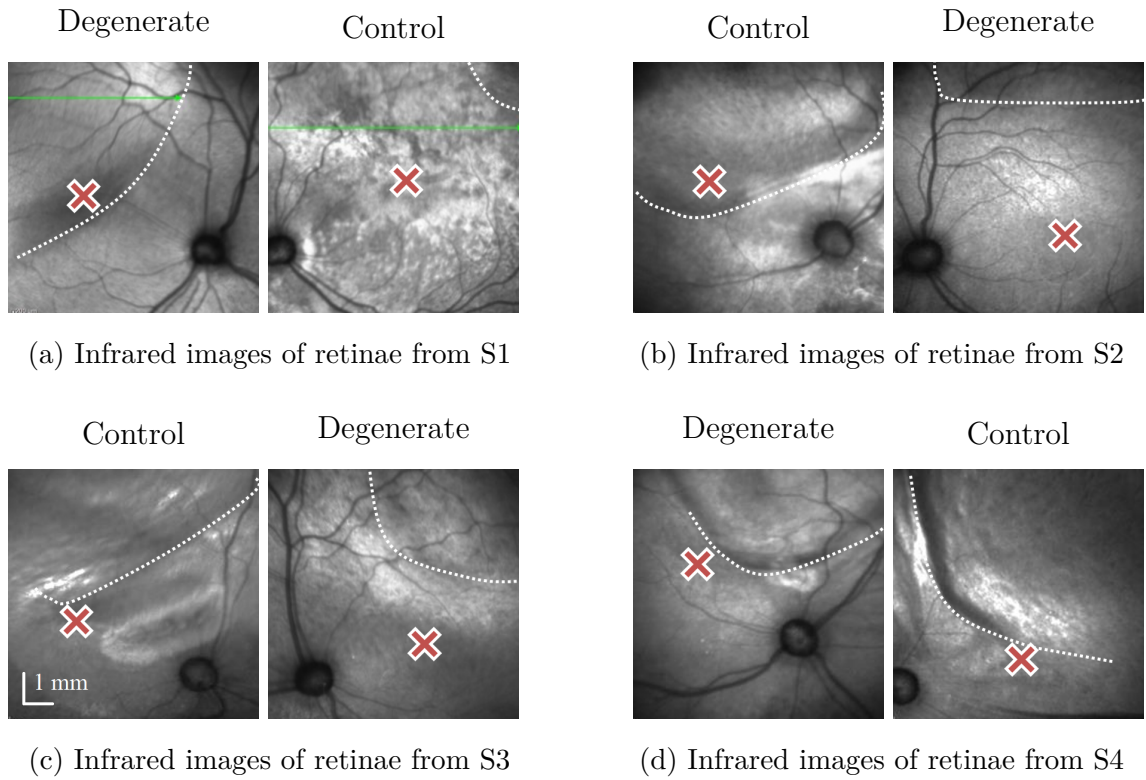
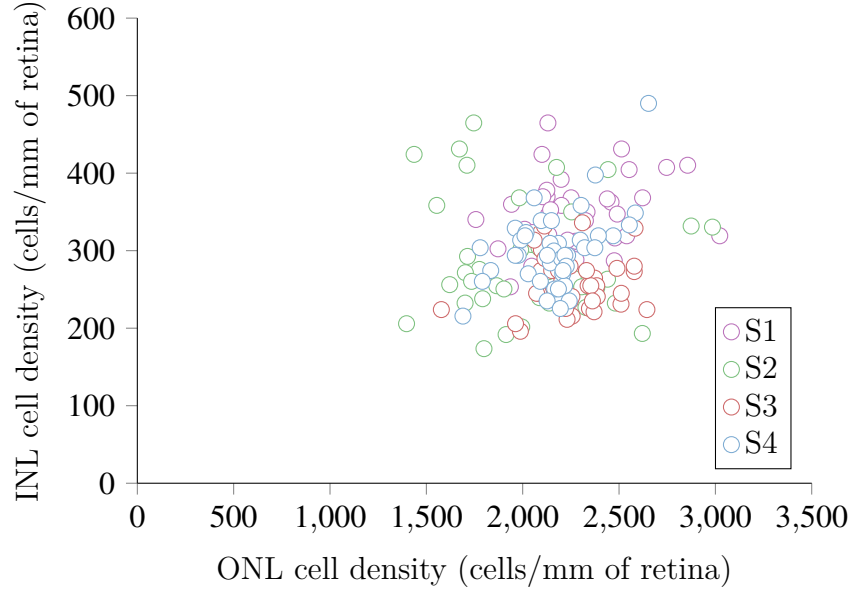


Figure 5.8: Infrared images of both the normal and degenerated retinæ for all four animals post implantation. The white dotted lines denote an approximation of the location of the stimulating arrays underneath the choroid. The red crosses denote the approximate location of area centralis.

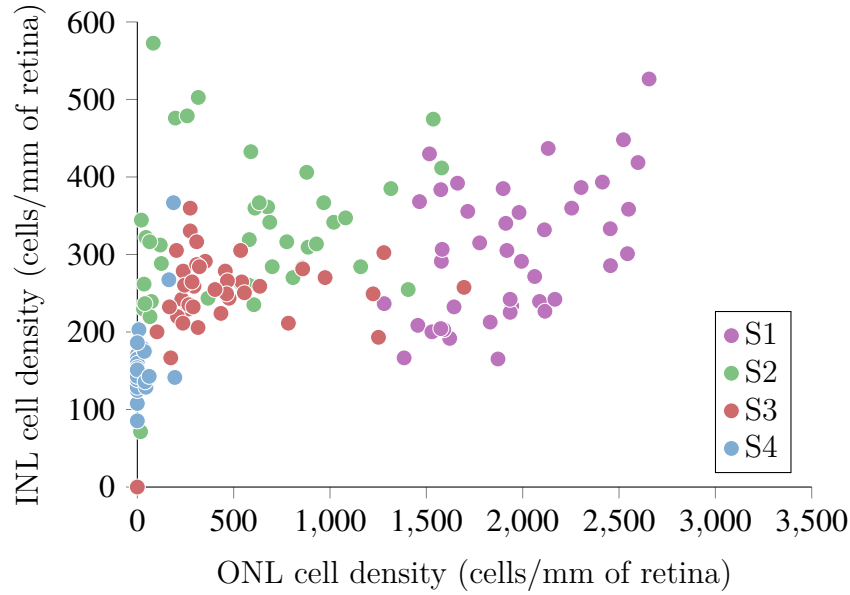
S4 respectively. All these durations were greater than the longest time point of 12 weeks for animals used in our previous studies with this model.

Each of the four animals exhibited at least 50% loss in a-wave ERG amplitude and darkened regions of pigment deposit in fundus images. (shown in Figures 5.3 & 5.4). Reduced outer retinal (OR) thicknesses compared with baseline measurements were also observed (example shown in Figure 5.5). Histological evidence following the acute experiment showed significant ONL cell density loss and varying degrees of INL cell loss. There was also substantial variation in the level of degeneration between different electrode sites in the same eye. Figure 5.6 shows example H&E micrographs of different locations within the same degenerate eye, highlighting the different degrees of cell death and gliosis. Figure 5.7 provides a comparison between the reduction of ERG a-wave amplitude and OR thickness from baseline measurements, along with the difference in ONL cell densities between the control and degenerate retinae. Due to the inconsistency of degeneration of the retina, there was a clear discrepancy between a-wave amplitude reduction, a global measure of retinal function, and the reduction in OR thickness and cell counts derived from OCT and histological analysis of the region directly beneath the electrode array. This is especially evident for S1, where a 63% reduction in a-wave amplitude was observed yet there was very little reduction in retinal thickness or cell counts near the array. Figure 5.8 shows the locations of the stimulating arrays within each eye in relation to area centralis. Generally, the arrays were positioned successfully over centralis or close by in a superior-temporal location.

Figure 5.9 shows the relationship between INL and ONL cell densities beneath each electrode site for both the control and degenerate retinae. While there was a significant correlation found between ONL and INL cell density across all animals ($p < 0.001$, $\rho = 0.55$, Spearman's Rho), there was no significant correlation found on a per-electrode basis within each animal with the exception of S1 ($p < 0.005$, $\rho = 0.43$, Spearman's Rho). The use of 1 ms pulse widths has been previously found to result in the activation of bipolar (BP) cells, as such we expected a positive correlation between INL cell loss beneath a given electrode and its lowest cortical threshold. However, as shown in Figure 5.10, no correlation was found between ONL or INL cell densities for each electrode site and the cortical activation thresholds for MP stimulation ($p > 0.05$, GLMM).

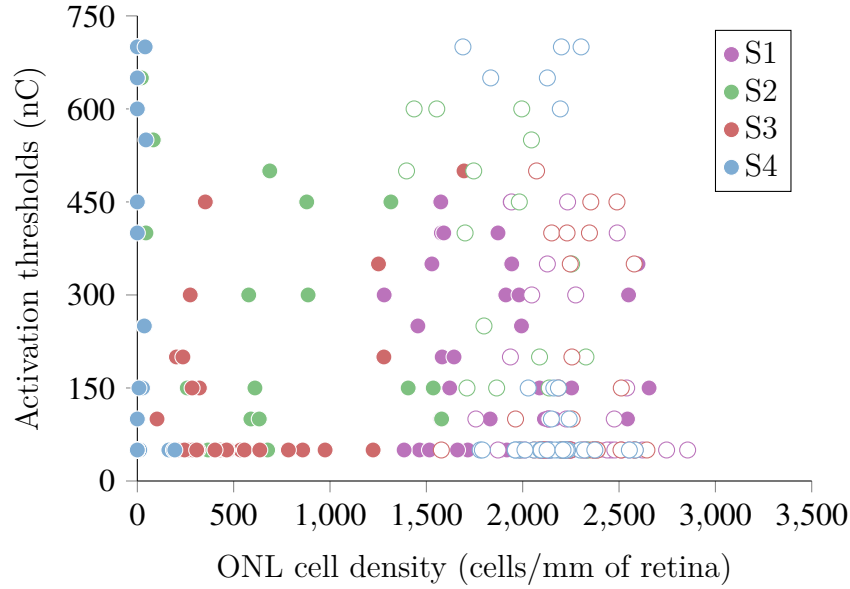


(a) Cell densities beneath electrodes in the control retinæ

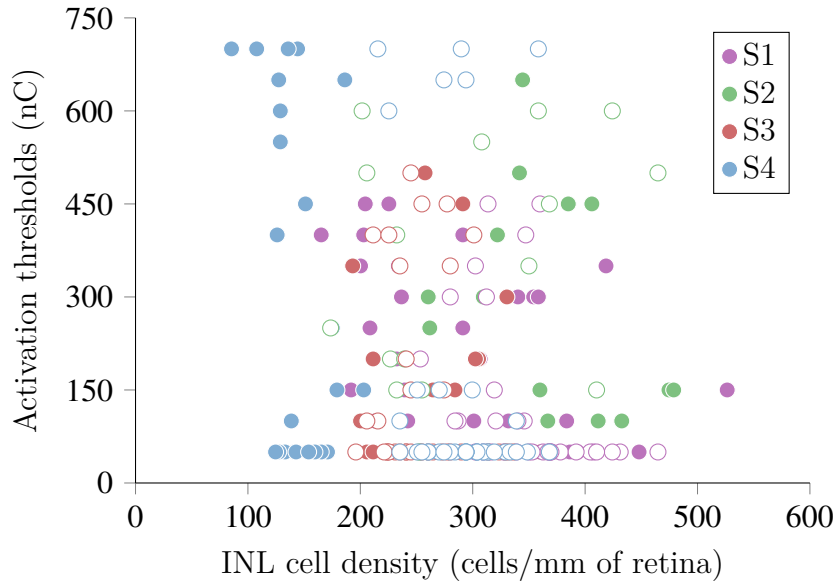


(b) Cell densities beneath electrodes in the degenerate retinæ

Figure 5.9: Scatter plots showing the relationship between ONL and INL cell densities at each electrode site in the (a) control and (b) degenerate retinæ of the four subjects. Each stimulating array contained 42 electrodes.



(a) Activation thresholds as a function of ONL cell density



(b) Activation thresholds as a function of INL cell density

Figure 5.10: Scatter plots showing, for each electrode, the relationship between (a) ONL and (b) INL cell densities at each electrode site and the lowest charge required to reach activation threshold on at least one cortical recording channel. Empty and filled circles respectively represent stimulating electrodes implanted in the control and degenerate retinae. No correlations were observed between threshold and ONL or INL cell densities in either the control ($n=123$) or degenerate ($n=135$) retinae ($p>0.05$, GLMM).

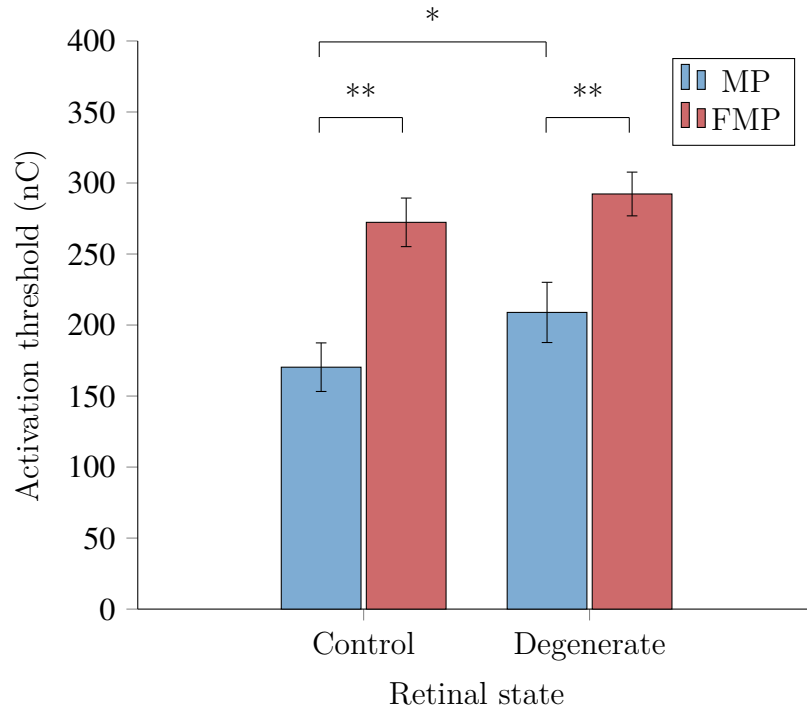
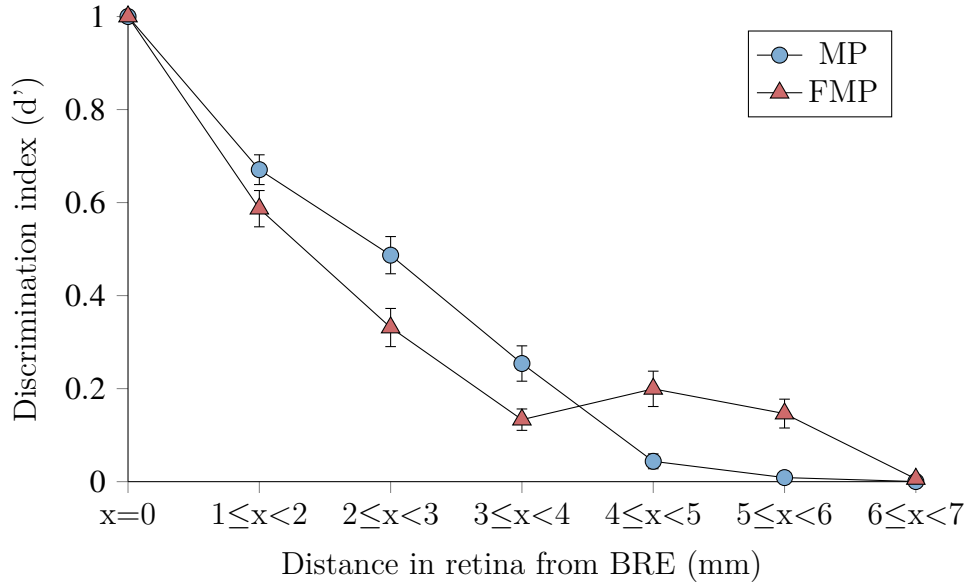
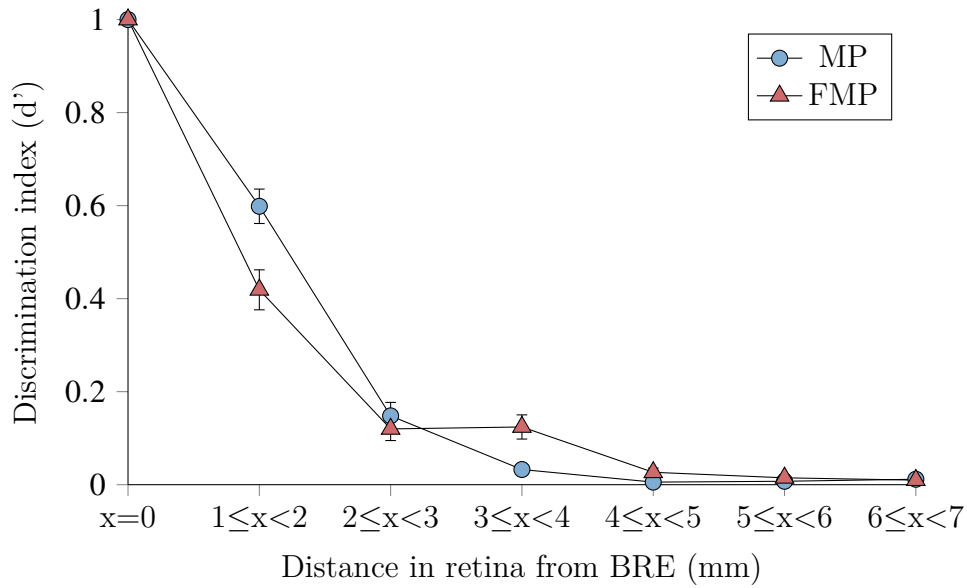


Figure 5.11: Average contralateral cortical thresholds for MP and FMP stimulation of the control (MP: $n=123$, FMP: $n=65$) and degenerate (MP: $n=135$, FMP: $n=123$) retinae. For both control and degenerate retinae, higher thresholds were observed for FMP stimulation than MP stimulation ($p<0.001$). Higher thresholds were also seen for MP stimulation of the degenerate retinae than MP stimulation of the fellow control retinae ($p<0.05$). No significant difference was observed between FMP stimulation of the normal and degenerated retinae ($p<0.05$). Error bars show standard error of the mean (* $p<0.01$ ** $p<0.001$, Welch's one-way ANOVA, Games-Howell post hoc).



(a) Retinal selectivity plot for MP and FMP stimulation of the control retinæ



(b) Retinal selectivity plot for MP and FMP stimulation of the degenerate retinæ

Figure 5.12: Scatter plots showing, for each electrode, the relationship between (a) ONL and (b) INL cell densities at each electrode site and the lowest charge required to reach activation threshold on at least one cortical recording channel. Empty and filled circles respectively represent stimulating electrodes implanted in the control and degenerate retinæ. No correlations were observed between threshold and ONL or INL cell densities in either the control ($n=123$) or degenerate ($n=135$) retinæ ($p>0.05$, GLMM).

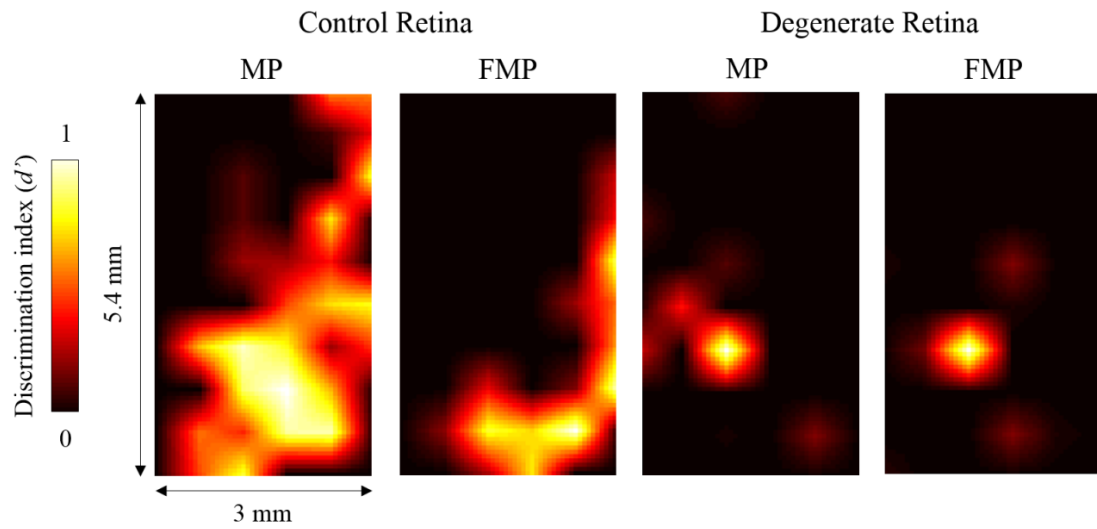
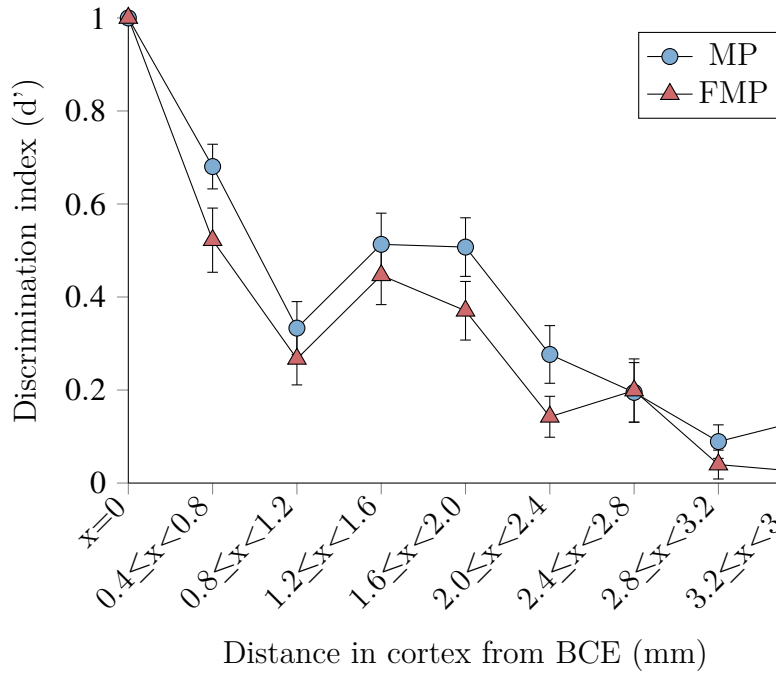
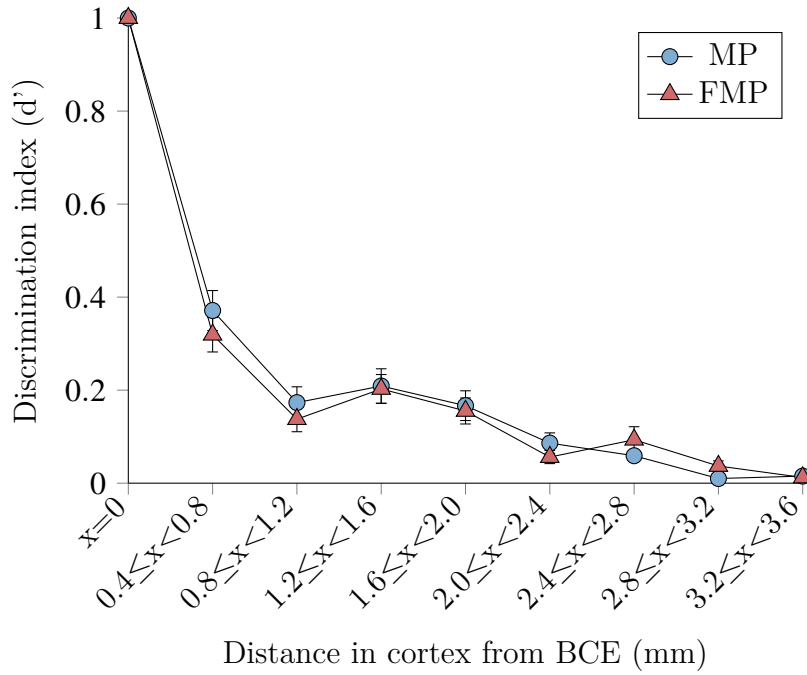


Figure 5.13: Examples of d' -based spatial maps of cortical responses to MP and FMP stimulation of a normal and degenerated retina. Colours represent the d' above threshold of responses at the current required to reach $d'=1$ above threshold on the BCE. These maps have been smoothed using linear interpolation. In these examples, MP stimulation is shown to elicit a broader spread of activation than FMP stimulation when applied to the normal retina. However, the spread of activation between these two modes is far more similar when applied to the degenerated retina. Overall, activation spread for both modes of stimulation of the degenerated retina is less than that elicited by stimulation of the healthy retina.



(a) Cortical selectivity plot for MP and FMP stimulation of the control retinae



(b) Cortical selectivity plot for MP and FMP stimulation of the degenerate retinae

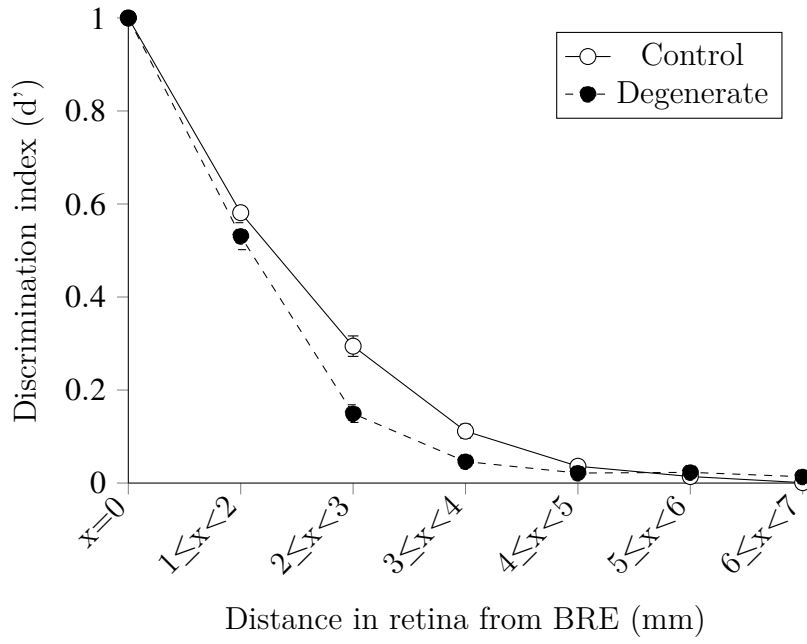
Figure 5.14: Average of the maximum d' values elicited on cortical channels at the current required to reach $d'=1$ above threshold on the BCE. (a) d' -based cortical selectivity plots for MP and FMP stimulation of healthy retinae ($n=18$ retinal electrodes). There was a significant decrease in spread of cortical activation for FMP stimulation compared to MP stimulation, with no significant interactions between distance and stimulation mode ($p<0.05$, two-way ANOVA, Tukey test). (b) Cortical spatial spread plots for MP and FMP stimulation of healthy retinae ($n=53$ retinal electrodes). There was no significant difference between the values for each bin ($p>0.05$, two-way ANOVA). Error bars show standard error of the mean.

5.3.2 Responses to focused multipolar stimulation

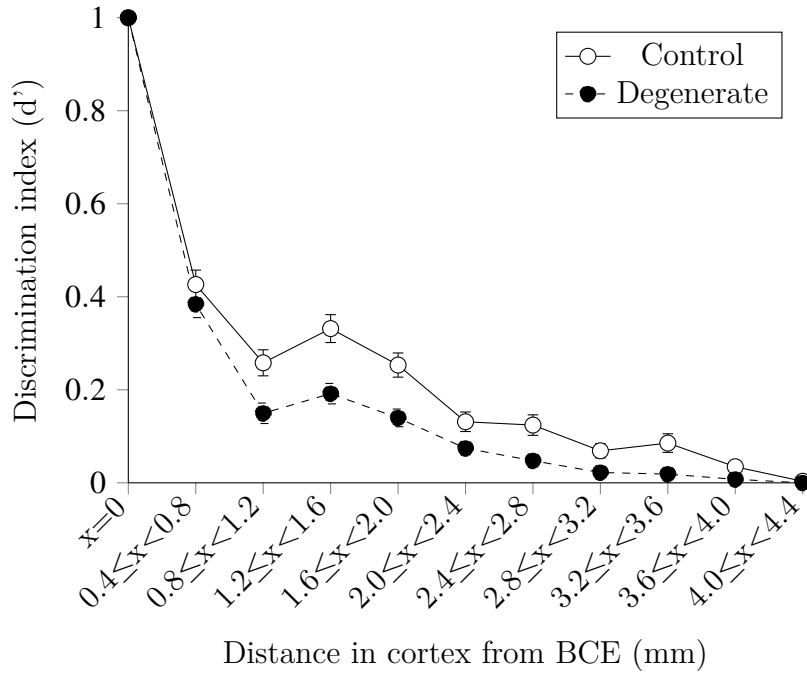
Cortical responses were collected to MP stimulation of 128 retinal electrodes in control eyes of all four animals. Of these electrodes, 94 (73.4%) elicited a response of $d'=1$ above threshold on at least one contralateral cortical channel. Due to difficulty calculating FMP weights for the control eyes of two animals, FMP stimulation was only applied to 84 retinal electrodes. Of these electrodes, 28 (33%) elicited a cortical response of $d'=1$ above threshold. Only the 18 electrodes that satisfied this criterion for both MP and FMP stimulation were included in the analysis. Neural activity was also recorded in response to MP and FMP stimulation of a combined 143 retinal electrodes implanted across all four degenerate eyes. The number of these electrodes in the degenerate eyes that elicited a response of $d'=1$ above threshold was 106 (74.1%) for MP stimulation and 65 (45.4%) for FMP stimulation. Responses to 53 retinal electrodes in the degenerate eyes that satisfied this criterion for both MP and FMP stimulation were included in analysis.

As shown in Figure 5.11, there was a significant increase in cortical activation thresholds for FMP stimulation when compared to MP stimulation of the control retinae ($p<0.001$, Welch's one-way ANOVA, Games-Howell post hoc, MP: 170 ± 17 nC, FMP: 272 ± 21 nC). The same result was observed for stimulation of the degenerate retinae ($p<0.001$, MP: 209 ± 17 nC, FMP: 292 ± 15 nC). Thresholds for MP stimulation were also higher when applied to the degenerate retinae compared with the control retinae ($p<0.01$). In both the control and degenerate retinae, there was a significant reduction in retinal activation spread when FMP stimulation was used compared to MP stimulation. Retinal selectivity plots in Figure 5.12 show significant reduction in spiking activity (two-way RM analysis of variance (ANOVA), $p<0.001$ Tukey post-hoc test) for FMP stimulation compared with MP stimulation, when electrodes up to 4 mm and 2 mm from the BRE for each cortical channel were stimulated in the control and degenerate retinae respectively.

Figure 5.13 shows cortical spatial maps in response to MP and FMP stimulation of both control and degenerate retinae. In these examples, while FMP stimulation elicited activation patterns with less spread than MP stimulation for control retinae, there was very little difference between the spatial spread of responses to MP and FMP stimulation of the degenerate retinae. In addition, both MP and FMP stimulation of the degenerate retinae resulted in responses with less spread than those evoked when stimulating the control retinae. The cortical selectivity plots in Figure 5.14 show that, overall, there was significantly reduced cortical activation spread



(a) Retinal selectivity plot for MP stimulation of the control and degenerate retinæ



(b) Cortical selectivity plot for MP stimulation of the control and degenerate retinæ

Figure 5.15: (a) Retinal selectivity plot of responses to MP stimulation of the control (n=102 cortical channels) and degenerate (n=170 cortical channels) retinæ. There was significantly reduced retinal activation spread from 2 mm onwards from the BRE for stimulation of the degenerate retina compared with the control retinæ (p<0.001, two-way ANOVA, Tukey test). (b) Cortical selectivity plot of responses to MP stimulation of the control (n=94 retinal electrodes) and degenerate (n=106 retinal electrodes) retinæ. There was significantly reduced retinal activation spread from 2 mm onwards from the BCE for stimulation of the degenerate retina compared with the control retinæ. (p<0.001, two-way ANOVA, Tukey test).

for FMP stimulation of the control retinae when compared with MP stimulation ($p < 0.05$), but that there was no significant difference in spread for MP and FMP stimulation of the degenerated retinae (two-way ANOVA, $p > 0.05$). MP stimulation of the degenerate retinae also resulted in significantly less retinal and cortical activation spread than MP stimulation of the control retinae. This was maintained when including responses to all electrodes that elicited a d' of 1 on at least one contralateral cortical channel using MP stimulation (two-way ANOVA, $p < 0.001$ Tukey post-hoc test, shown in Figure 5.15).

Scatter plots showing cortical spread of responses to MP and FMP stimulation of each retinal electrode (represented as the area under the selectivity curve [$d' \cdot \text{mm}$]) as a function of ONL and INL cell densities are presented in Figure ???. No significant correlation was found between MP or FMP cortical selectivity and ONL or INL cell densities ($p > 0.05$, GLMM).

5.3.3 Responses to virtual electrode current steering

Cortical activity was recorded in response to stimulation of 7 hexagonal and 14 triangular electrodes shapes in control retinae. Each hexagonal electrode shape overlapped with two triangular shapes, thereby sharing the same CPE and OPEs. Of the 7 unique CPEs that were stimulated, none elicited a saturating cortical response at the highest safe charge level. In order to use the same methodology presented in Section 4.2.3, calculation of the centroid of responses to two applications of CPE stimulation is required to measure the variability in centroid location for responses to identical stimulation. Without this measure, current steering analysis of responses to these shapes was not possible. Overall, however, saturating responses were obtained for 69 applications of PE stimulation and 98 applications of VE stimulation of the control retinae, allowing us to at least compare P50 threshold values between PE and VE stimulation in the control retinae.

Responses were also collected to CPE, CVE, IVE, and OPE stimulation of 17 hexagonal and 33 triangular electrode shapes in the degenerate retinae. Each hexagonal shape overlapped with up to two triangular electrode shapes. Of the 17 unique CPEs stimulated, only 6 (35.2%) elicited a saturating cortical response on at least one contralateral cortical channel for two applications of identical stimulation and were used for centroid analyses. Overall, saturating responses were obtained for 66 applications of PE stimulation, and 126 applications of VE stimulation which were used to compare P50 thresholds.

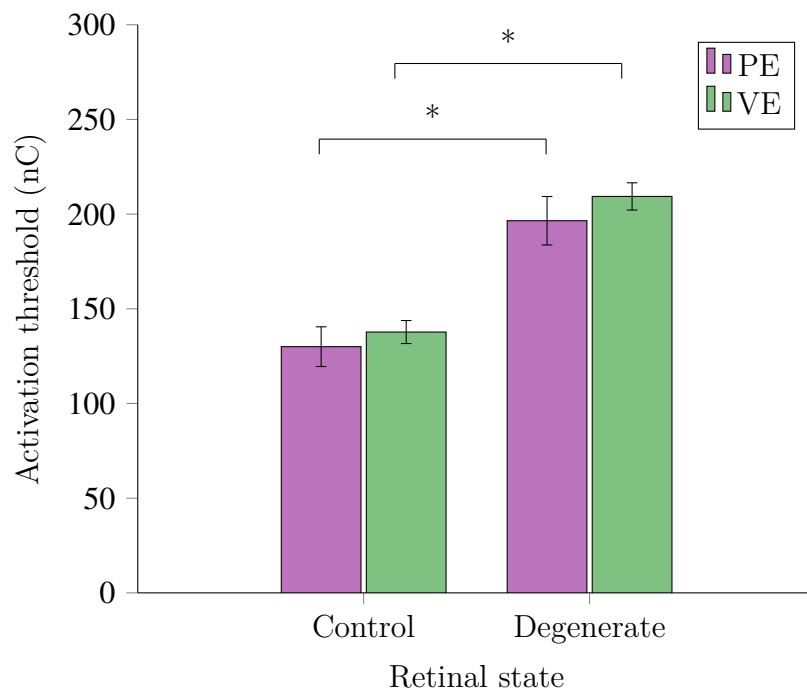


Figure 5.16: Bar chart of the average charge required to elicit a P50 response on at least one contralateral channel for PE and VE stimulation of the control (PE: n=69, VE: n=98) retinae and degenerate (PE: n=66, VE: n=126) retinae. PE and VE stimulation of the degenerate retinae required a significantly higher charge level to elicit a cortical P50 response than stimulation of the control retinae (* $p < 0.001$). No significant difference was observed between PE and VE stimulation of either the control or degenerate retinae ($p < 0.05$, one-way ANOVA, Tukey post hoc). Error bars show standard error of the mean.

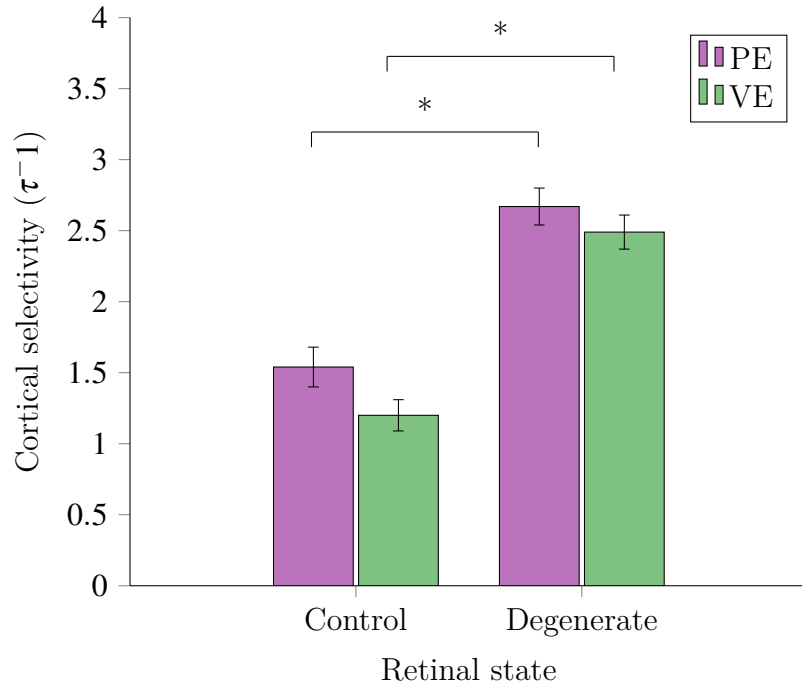


Figure 5.17: Bar graph showing average cortical selectivity values ($\bar{\tau} - 1$) of P90 responses to PE and VE stimulation of control (PE: $n=38$, VE: $n=71$) and degenerate (PE: $n=44$, VE: $n=74$) retinal states. There was a significant increase in the selectivity of responses to both PE and VE stimulation of the degenerate retinal states in comparison to the control retinal states (* $p < 0.005$). No significant difference was found between PE and VE stimulation of the same retinal states ($p > 0.05$, one-way ANOVA, Tukey post hoc). Error bars show standard error of the mean.

In agreement with our analysis of MP and FMP thresholds, there was a significant increase in P50 charge level for both PE and VE stimulation of the degenerate retinæ, compared to stimulation of control retinæ ($p < 0.01$, one-way ANOVA, Tukey post hoc, shown in Figure 5.16). No difference was observed between P50 charge levels required for PE and VE stimulation within the control or degenerate retinæ ($p > 0.05$). P90 cortical selectivity was also found to be significantly higher for both PE and VE stimulation of the degenerate retinæ, also in agreement with the MP and FMP analyses (Figure 5.17, $p < 0.005$, one-way ANOVA, Tukey post hoc). No difference in cortical selectivity was observed between PE and VE stimulation for either the control or degenerate retinæ ($p > 0.05$) suggesting that VEs did not produce more spread than PEs as observed in 4.

In the study presented in Chapter 4, electrode shapes were only included in further analysis if a saturating response could be obtained for two applications of CPE stimulation, and at least one application of CVE, IVE, and OPE stimulation. Unfortunately, in this study, only 4 (23.5%) hexagonal shapes and 7 (21.2%) triangular shapes in the degenerate retinæ met these criteria. In order to improve the number of samples and increase statistical power, the inclusion criteria was altered for this study. Shapes that elicited saturating responses for at least one application of CPE stimulation, and at least one application of CVE, IVE, or OPE stimulation, were included in order to increase the number of comparable samples. As many electrode shapes partially overlapped, responses had been recorded to two applications of stimulation of 17 OPEs. As saturating responses could only be obtained from stimulation of 6 CPEs, the variation between the two applications of stimulation to these OPEs were also included in this dataset, bringing the total number of included responses to multiple applications of the same stimulation to 23.

Spatial maps showing responses to CPE, CVE and OPE stimulation of a hexagonal electrode shape are shown in Figure 5.18. In this example, contrary to what was expected based on the results in Chapter 4, the cortical response patterns for CPE and CVE stimulation yielded different centroid locations. The centroid calculated for CVE stimulation seemed far closer to that calculated for the pattern elicited in response to stimulation of one of the OPEs of this shape that elicited responses with the lowest P50 charge level out of all the OPEs forming this shape.

Responses to CVE stimulation of 7 (41.2% of total) hexagonal and 15 (45.4%) triangular electrode shapes were included in the analysis. Centroids calculated for IVE stimulation of 9 (52.9%) hexagonal and 17 (51.6%) triangular shapes were also in-

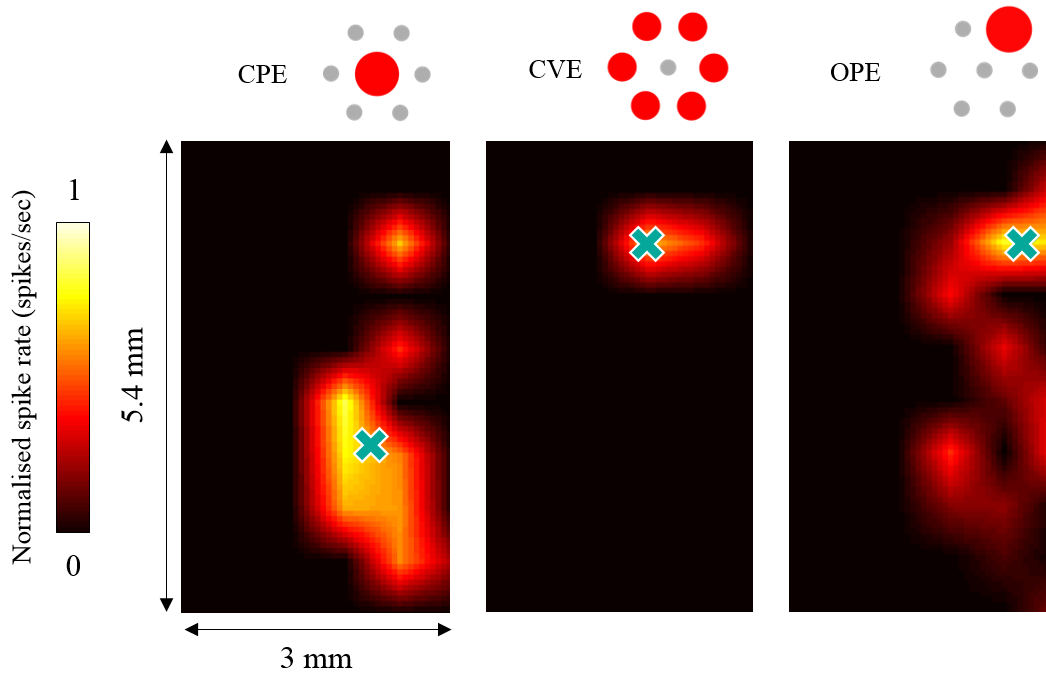


Figure 5.18: Example cortical spatial maps of P90 responses to CPE, hexagonal CVE, and OPE stimulation of the same electrode shape. Blue crosses show the weighted centroid location calculated for each response. The centroid for the response to CVE stimulation in this example is far closer to the centroid calculated for OPE stimulation than that for CPE stimulation, contrary to what was expected. In this OPE example, the outer electrode that was stimulated had the lowest P50 charge level of all the outer electrodes for that shape.

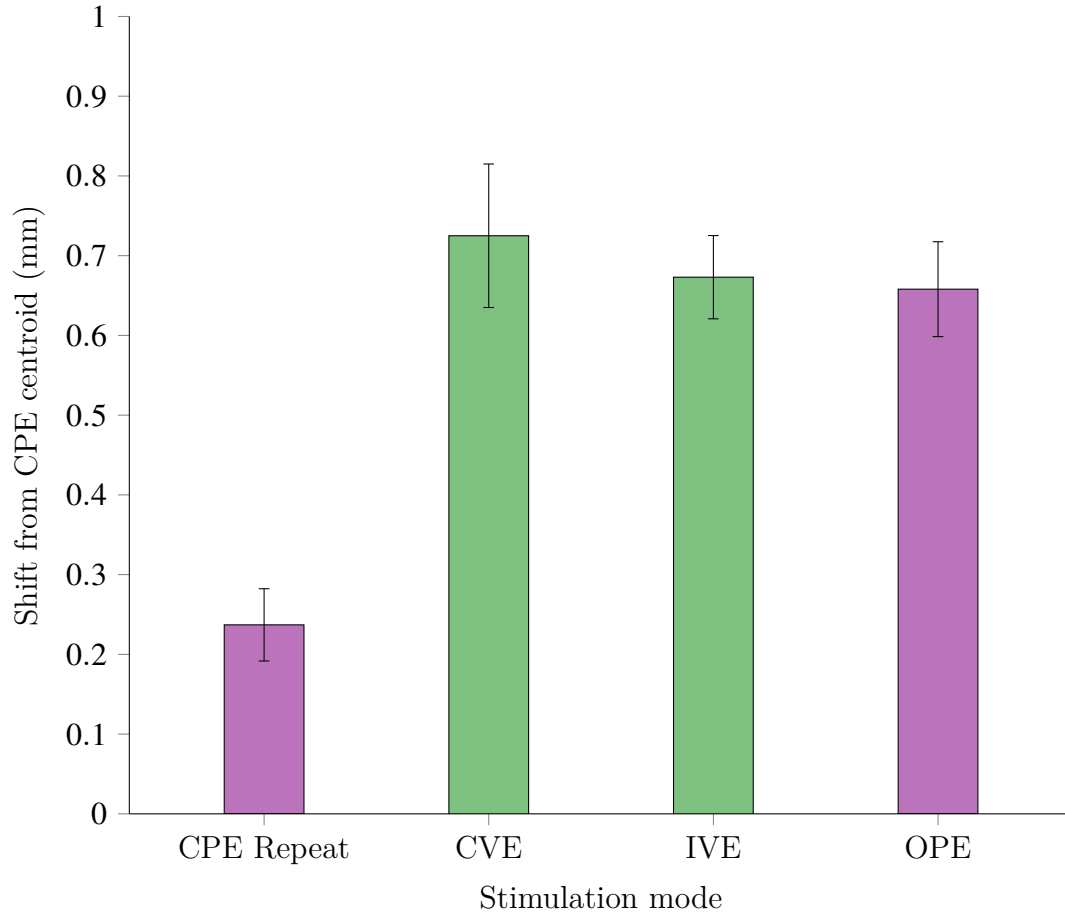


Figure 5.19: Bar graphs showing the average distance between the centroid of activation calculated for stimulation of the CPE, and centroids calculated for CPE, CVE, IVE and OPE stimulation. There was a significantly higher degree of centroid shift when CVE, IVE, or OPE stimulation was applied, compared with the second application of CPE stimulation (* $p < 0.001$, ANOVA on Ranks, Tukey post hoc test). No significant difference was observed between CVE, IVE or OPE stimulation ($p > 0.05$). No significant differences were observed between VE stimulation of hexagonal and triangular electrodes shapes (ANOVA on Ranks, $p > 0.05$), as such, the values in these datasets were combined. Error bars show standard error of the mean.

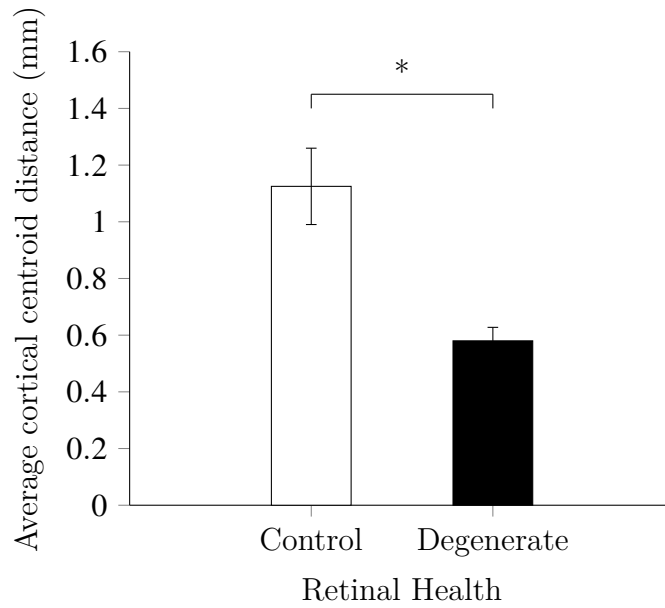


Figure 5.20: Bar graphs showing the average distance between centroids calculated for responses to independently stimulated adjacent electrodes in the control (n=105 pairs) and degenerate (n=59 pairs) retinae. There was as significant reduction in adjacent centroid distance for responses to stimulation of the degenerated retinae (*p<0.001, two-tailed t-test). Error bars show standard error of the mean.

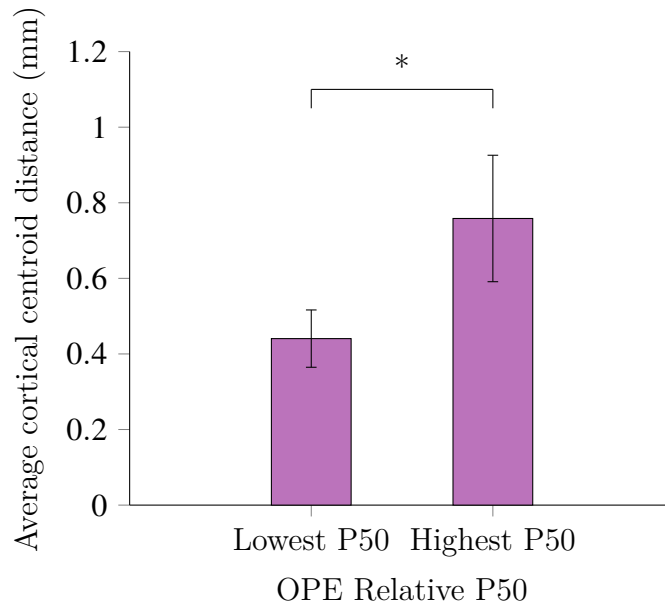


Figure 5.21: Bar graphs showing the average distance between centroids calculated for responses to CVE stimulation, and centroids calculated for stimulation of the OPE with the highest and lowest P50 thresholds (n=10). Centroids calculated for CVE stimulation were significantly closer to the centroids of the response to stimulation of the OPE with the lowest threshold over that of the response to stimulation of the OPE with the higher threshold (*p<0.005, paired t-test). Error bars show standard error of the mean.

cluded. Of the 102 unique stimulated OPEs, 32 (31.3%) responses met the inclusion criteria. As there were no significant differences in the results for triangular and hexagonal stimulation of the electrode shapes, the data for CVE and IVE stimulation were combined in Figure 5.19. The average shift in cortical centroid location between the two repeats of CPE stimulation was found to be 0.23 ± 0.05 mm, significantly less than observed in the study presented Chapter 4 (see Figure 4.5) using healthy eyes ($p < 0.001$, two-way t-test). To test the hypothesis that this is an effect of the retinal degeneration, we compared the average centroid shifts between PE stimulation of pairs of adjacent electrodes in the control and degenerate retinae. As shown in Figure 5.20, the centroid shift was significantly reduced for stimulation of the degenerate retinae, compared with control.

As the centroids calculated for CVE and VE stimulation were similar in distance from the centroid for the response to stimulation of the CPE, we theorised that there may be a relationship between OPE thresholds and the location of the CVE centroids. Figure 5.19 shows that centroids calculated for responses to CVE stimulation were significantly closer to centroids calculated for responses to stimulation of the OPE with the lowest P50 charge level in the same shape, than the OPE with the highest P50 charge level ($p < 0.005$, paired t-test).

5.4 Discussion

This study investigated whether FMP stimulation and VE current steering maintained the effectiveness demonstrated in previous chapters within a cat model of retinal degeneration. Retinal degeneration was successfully induced in four cats with varying degrees of ONL and INL cell loss. While we were able to replicate some of the encouraging results presented in earlier chapters when stimulating the control eyes of these cats, the findings of this study indicate these techniques were significantly less effective when applied the degenerate retinae.

5.4.1 Anatomical retinal changes following degeneration

Retinal degeneration was successfully induced in four of the five cats that were injected with ATP. All subjects required multiple injections to induce a level of degeneration that met our predefined clinical criteria. Significant retinal detachment occurred in one subject, resulting in exclusion from the remainder of this study. During the development of this model, multiple injections were often required in order to induce significant degeneration when applying the dosage we chose to use (Aplin

et al. 2014, 2016a). While higher concentrations of ATP improve the likelihood of significant degeneration, it also increases the prevalence of localised retinal detachments (Aplin et al. 2014). It is theorised that the high rate of photoreceptor death, as well as the direct action of ATP, impairs fluid transfer at the retinal pigment epithelium leading to retinal elevation and detachment (Mitchell & Reigada 2008, Aplin et al. 2016a). In each cat in this study, and previous studies, the distribution and severity of photoreceptor death was not consistent across the retina (Aplin et al. 2014, 2016a). This is highlighted in Figure 5.7, where there is a clear discrepancy between a significant 63% loss in ERG a-wave amplitude and very little ONL cell loss for S1. The ERG measures photoreceptor function across the entire retina, however when investigating locally it was found that very little photoreceptor death occurred in the region where the electrode array was implanted. Figure 5.6 also highlights the degree of variation that can be observed in the same retina in only the small region occupied by the array. The viscosity of the intravitreal fluid may impair widespread and equal diffusion of the ATP solution to the photoreceptors. Hydraulic flow within the eye differs depending on location and has significant effects on the rate of clearance of introduced compounds (Missel 2002). It is possible that the location of the hypodermic needle tip during injection may have influenced distribution. Hydraulic flow is also influenced by intraocular pressure changes (Missel 2002). As such, the volume of injected solution, the size of hypodermic needle, and the timing of the paracentesis procedure may have had substantial effects on intravitreal diffusion. Diffusivity and retinal permeability properties of the solution are also significant factors (Kathawate & Acharya 2008, Xu et al. 2000).

5.4.2 Responses to electrical stimulation of the degenerate retinae

In agreement with our previous work using this model, the lowest charge required to elicit cortical thresholds and P50 responses were significantly higher in response to stimulation of the degenerate retinae, compared with the control retinae (Aplin et al. 2016b). In our previous work, RGC density and the degree of inner retinal gliosis were shown to be significant influences on cortical P50 charge levels (Aplin et al. 2016a). As immunohistochemical analysis was outside of the scope of this thesis, we were not able to accurately quantify RGC density and the degree of gliosis in this study. Also, due to the use of paraffin fixation, we were unable to derive reliable ONL and INL thickness measurements from histological sections. As such, retinal layer thicknesses at each electrode site were measured using OCT imaging immediately post implantation and compared to previously obtained baseline measures. ONL

and INL cell densities were obtained histologically. In agreement with our previous work (Aplin et al. 2016a), no significant correlation between these measures with P50 charge levels was found.

We also observed a significant increase in cortical selectivity for MP stimulation of the degenerate retinae compared to the control retinae, using both selectivity measures from Chapters 3 and 4. Our previous work with this model did not report a significant increase in P90 cortical selectivity (Aplin et al. 2016b). Without accurate quantification of other retinal health indicators such as retinal ganglion cell (RGC) densities and gliosis it is difficult to compare the level of inner retinal remodelling to the past studies. Considering that the time period between the final ATP injections and the electrophysiological experiments were substantially longer than previous cohorts of animals, it may be that a greater degree of remodelling has occurred. We also found that there was a significant reduction in the distance between the centroids of responses to MP stimulation of adjacent electrodes in the degenerate retinae. This suggests that the retinotopic map of the degenerate retinae is compressed at the cortical level compared to the healthy control at least when mapped using electrical stimulation of 1 mm spaced electrodes within the retina. Cortical remapping has been shown to be rapid, even in adult subjects, following retinal lesions. In mice, deafferented regions of the cortex following retinal lesions are quickly remapped to respond to stimuli from adjacent regions (Mitchell & Reigada 2008). Complete replacement of dendritic spines to the previously silenced cortical region can occur within two months (Mitchell & Reigada 2008). However, this did not occur when the all afferent input from the eye was ablated (Mitchell & Reigada 2008). There is also evidence that cortical retinotopic reorganisation and perceptual distortion occurs in humans following vision loss (Dilks et al. 2007). As the ATP-induced retinal degeneration was non-uniform and likely resulted in continuing visual input from less degenerated regions of the retina, it is possible significant distortion of cortical retinotopy had occurred by the time of the acute experiment. Further immunohistochemical analysis of the retinal tissue sections would allow for more in-depth quantification of inner retinal changes in response to degeneration. It would also serve as a better comparison to animals from previous studies.

5.4.3 Responses to FMP stimulation

Average cortical thresholds were found to be higher for FMP stimulation than MP stimulation in both the control and degenerate retinae. This is in agreement with not only the study presented in Chapter Chapter 3, but also with previous work with

FMP stimulation and other focusing techniques in other prosthetic devices (Spencer et al. 2016, George et al. 2014, Matteucci et al. 2013, Berenstein et al. 2008). MP stimulation of the degenerate retinae also showed significantly increased thresholds compared with the control retinae. This was also to be expected and was demonstrated in our previous work with this model (Aplin et al. 2016b). FMP stimulation did not result in higher thresholds in the degenerate retinae than when applied to the control retinae. However, a smaller percentage of stimulating electrodes elicited a cortical response with a discernible threshold in the degenerate retinae compared with control when FMP was applied. It may be that, due to the already elevated thresholds with FMP stimulation, that we encountered a ceiling effect and that FMP thresholds for many retinal electrodes in the degenerate retinae would have exceeded the upper charge limit. This could potentially exclude a greater number of high threshold responses to FMP stimulation of the degenerate retinae than of the control retinae, making the average appear more similar.

In agreement with the results presented in Chapter 3, FMP stimulation of control retinae resulted in significantly higher retinal and contralateral cortical selectivity than MP stimulation. FMP stimulation of the degenerate retinae also resulted in increased retinal selectivity compared with MP stimulation. However, significant reduction in retinal selectivity was only observed up to 2 mm from the BRE, compared with up to 4 mm for stimulation of the control retinae. This may be partially related to the finding shown in Figure 5.15, where MP stimulation of the degenerate retinae resulted in higher retinal selectivity compared with MP stimulation of the control retinae. When applied to the degenerate retinae, FMP did not elicit more restricted spread of activation compared to MP stimulation. As with retinal spread, the spread of cortical responses to MP stimulation were also significantly reduced in the degenerate eye. It should be noted, that statistical comparisons made between MP and FMP stimulation were paired in order to account for differences in the cortical magnification factor for regions of the retina at different degrees of eccentricity. This is not possible to account for when making comparisons between responses to stimulation of different retinae where stimulating and recording arrays are not identically placed.

The insignificant contributions of FMP stimulation in the increasing selectivity over MP stimulation of the degenerate retina would suggest there would be no further reduction in the size of phosphenes and therefore no greater ability to discriminate between percepts. Due to the already highly selective nature of the degenerate visual system, there may be a limit to how discrete a phosphene can be when using a

stimulating array such with these dimensions and when placed in the suprachoroidal space. We have shown the increase in retinal selectivity for FMP stimulation compared with MP stimulation is maintained in the degenerate retinae, indicating that the electrical field is still being constrained using the FMP methodology. As the increased cortical selectivity that FMP affords even in the control retinae is admittedly small, a remodelled visual system may not have the resolution to detect such a small change in retinal activation at the cortical level. FMP stimulation may be more effective with electrodes of different geometry, arrangement, or spacing, and when placed in a different anatomical location. As FMP stimulation is intended to nullify voltages at each surrounding electrode site, reducing the spacing of the electrodes on the array may improve the ability of the technique to focus current. Testing this technique using arrays designed for different implantation locations, such as epiretinal devices, may provide a better understanding of the differing effectiveness of FMP when smaller and denser electrode arrays closer to the target neurons are used. It may also be possible to modify the methodology behind the calculation of FMP weights in order to further focus the electrical field. However, it is likely that this could only be achieved at the expense of further increase in neural activation thresholds.

5.4.4 Responses to VE current steering

In agreement with the results of Chapter 4, we found that thresholds and spatial selectivity of responses did not change between PE and VE stimulation. However, as described earlier, thresholds and spatial selectivity were higher for both forms of stimulation in the degenerate retinae. Using the previously successful 2D VE current steering methodology shown in Chapter 4, we were unable to reproduce cortical activation patterns to stimulation of physical electrodes using virtual electrodes. Using the centroid of the CVE as a reference point, when performing VE stimulation, centroids shifted by about the same amount as when performing OPE stimulation. In addition, these new CVE centroids were located closer to the centroids of the lowest threshold OPEs compared to the highest threshold OPEs. These results indicate that this method of steering current is less effective when applied to a degenerate retinae. Due to the finding of compressed cortical retinotopy, this methodology may also be impaired by reduced resolution.

In the results presented in Chapter 4, all the electrode shapes that were included in the analysis elicited cortical response patterns for two applications of CPE stimulation. This was not the case in this chapter, as only 6 of the 17 unique CPEs

elicited responses. This does make statistical comparison of these data less reliable, however the low number of CPEs to elicit responses may be significant. Centroids for CVE stimulation were found to be closest to centroids calculated for stimulation of the OPEs with the lowest P50 charge level in their respective shapes. While this is likely that retinal activation also preferences lower threshold retinal regions in normally-sighted animals to some degree, this may have a more significant effect in degenerate retinae due to overall increased thresholds and non-uniform nature of degeneration, especially as past work with this model of retinal degeneration has shown that inner retinal remodelling contributes to increased thresholds.

Optimisation of this technique is likely required in order to be effectively used in degenerate retinae. Altering stimulus weights applied during VE stimulation based on P50 charge levels of OPE stimulation may compensate for variation in retinal sensitivity. Neural responses or perceptual thresholds would need to be obtained and analysed prior to VE stimulation in order to calibrate steering weights.

5.4.5 Limitations

The resolution of our cortical recording apparatus may not be able to detect significant changes in cortical responses to stimulation of the degenerate retina with sufficient accuracy. The increased spatial selectivity seen in the visual cortex when stimulating the degenerate retinae and compression of cortical retinotopy may impair our ability to detect small changes in selectivity and centroid location using a planar array with 400 μm pitch electrodes. This would be supported by the very low d' values in the second bin in Figure 5.14b compared with Figure 5.14a. As our recording technique may not be adequate to measure differences in responses with such high precision, alternate techniques may provide greater insight to these findings. Penetrating recording arrays with reduced electrode pitch may provide greater resolution, however, due to their fragility may be difficult to successfully implant in to brain tissue without damage. Two-photon excitation microscopy has been shown great promise as a way to image neuronal networks in vivo (Stosiek et al. 2003, Flusberg et al. 2005). More modern imaging techniques such as this may be able to supplement electrophysiological investigation of neural tissue and allow for the measurement of activity with far greater spatial resolution. Imaging techniques would also be able to achieve this without causing tissue damage, as is the case with our penetrating electrode arrays.

In-depth immunohistochemical analysis, as was performed for retinae during the development of this model, was outside of the scope of this PhD. These analyses would provide a far greater level of understanding of retinal changes resulting from ATP exposure, such as changes in RGC densities, level of glial activation, and would allow for greater accuracy in the identification of inner retinal cell types. It is also possible that changes to non-retinal structures such as the choroid, tapetum or the RPE that were not detectable using our histological methods could alter current paths and field interactions. More data on the composition of cell types and degree of degeneration and remodelling at each electrode site could provide greater insight in to the failure of these electrical field shaping techniques to reproduce the promising results from previous chapters. RGC density and glial activation were considered to be significant factors that influenced cortical response properties to electrical stimulation in our previous work, however we were unable to quantify these differences using basic histological techniques (Aplin et al. 2016a). We theorise that our observation of increased spatial selectivity of the degenerate retinae, contrary to findings from our previous work, is potentially related to a greater degree of inner retinal remodelling than had occurred before. Only through further tissue analysis can this be explored. It is hoped that, in the future, these more advanced analyses will be performed on the preserved tissue sections and will aid in the future optimisation of the FMP and VE stimulation strategies. Further development of the ATP-induced model of retinal degeneration may benefit from investigation of the time course of retinal and cortical remodelling.

While the intention of this study was to re-evaluate the effectiveness of stimulation strategies in a model more representative of blind patients, our model of retinal degeneration is still different to the natural human presentation of retinal degenerative diseases. Remodelling of inner retinal layers is believed to occur regardless of the underlying cause of photoreceptor death (Jones et al. 2003, Marc et al. 2003, 2008), however the rate of photoreceptor death is significantly higher in the ATP-injected cats compared with human RP patients. Patients with retinal diseases such as RP or AMD progressively lose their vision over the course of years to decades, whereas degeneration occurs within our model over days (Hartong et al. 2006, Jager et al. 2008). Rapid loss of photoreceptors is believed to cause a higher degree of immune response and discrete retinal detachments (Mitchell & Reigada 2008, Aplin et al. 2014). Remodelling of the visual pathway may also occur in a very different way than it would for patients who do not experience the same sudden loss of afferent input. Remodelling in response to extreme light damage to photoreceptors has been shown to be far more aggressive than most genetic or immunological forms

of retinal degeneration, and bears closer resemblance to severe cases of late stage atrophic AMD (Marc et al. 2008). The model is also currently unable to induce the same degree of widespread degeneration as seen in the majority of implanted patients. While there are few cases of implanted patients with residual vision, the majority of patients receive implants during the late stages of their disease and typically experience only bare light perception (Shepherd et al. 2013). Further study and development of this model will aid future research into stimulation strategies and help place it in a clearer context of the human pathology.

5.4.6 Implications for retinal prostheses

The failure to reproduce the results from preceding chapters in cats with retinal degeneration is further evidence of substantial difference in the response of the degenerate visual system to electrical stimulation. It also sheds doubt on whether results of preclinical experimentation using normally-sighted animals are applicable to a human pathological visual system. It has long been known that significant remodelling occurs in the neural retina following photoreceptor death in many animal models (Marc et al. 2003, Jones & Marc 2005). More recently this has also been confirmed to be the case for humans at even very early stages of the disease (Fariss et al. 2000, Jones et al. 2016). While normally-sighted animals have been very useful models during the development of visual prostheses, these results suggest that it would be prudent to carry out experiments in animal models that closer resemble the human physiology these devices must interact with.

It is established that, just as remodelling occurs due to lack of sensory input, remodelling also occurs in response to the reintroduction of sensory input. Sensorineural hearing loss also results in significant deafferentation and remapping of central auditory structures, impairing a patient's ability to interpret sounds once implanted with a cochlear implant (Shepherd & Hardie 2001). It has been shown that following implantation, plasticity is observed in the auditory cortex (Mut et al. 1999, Giraud & Truy 2002), and regions associated with language (Giraud et al. 2001). However, prolonged sensory deprivation impairs the development of these structures and inhibits the potential for learning (Shepherd & Hardie 2001). Implanting early in life or earlier in the time course of the disease has been shown to be more effective in influencing remodelling in the auditory system (Sharma et al. 2002, Gantz et al. 2005, Fallon et al. 2009). A solution to retinal remodelling may be to implant patients earlier with prosthetic devices so as to influence plasticity before significant maladaptive remapping occurs. Suprachoroidal and subretinal devices would likely

be better suited to this task as they would not occlude light and allow patients to continue to use their natural vision in conjunction with prosthetic vision as the disease continues to progress.

Assuming these methods can be optimised for use with the degenerate retinae, the implementation of these techniques to a fully implantable device may require more adaptable stimulator hardware than some devices are capable of. Devices designed to employ FMP and VE stimulation will need to be capable of simultaneously stimulating multiple electrodes with unequal charge weights. One way to achieve this would be to have numerous current sources so that each electrode can be independently driven. This would likely become technically difficult for highly dense electrode arrays, as FMP stimulation presently employs stimulation of all electrodes to focus current. Recently it has also been shown that single-source multipolar stimulation is possible using a variable impedance bank (Senn et al. 2018). Single-source multipolar stimulation was able to focus voltage spread in a similar manner to FMP stimulation on a linear electrode array using the same weight calculation method as described by van den Honert & Kelsall (Senn et al. 2018, van den Honert & Kelsall 2007). Our findings have been that charge weights are typically very low for electrodes far from the stimulating electrode. As such, increasing the number of focusing electrodes beyond a certain distance from the central electrode may not provide significant focusing benefits. Optimisation of the FMP and VE current steering methodology in future studies will likely inform the minimum requirements for use in devices.

5.4.7 Conclusion

Our findings suggest that FMP stimulation and VE current steering would not be effective strategies to focus or steer current in a predictable fashion in their present implementation. It is likely that, without further development and optimisation, they would not be able to provide a significant improvement to visual horizontal cellity afforded to patients. This study also provides further evidence that the responses of degenerate retinae to electrical stimulation are significantly different to that of healthy retinae. Future preclinical studies into development of new visual prosthetic devices or stimulation strategies would likely benefit by using models that closer represent the physiology of patients with retinal degeneration in order to better inform translational endeavours. A greater understanding of the remodelling that occurs as a result of photoreceptor degeneration is essential to provide clearer

interpretation these results and to tailor future stimulation strategies.

Chapter 6

General discussion

The studies in this thesis have investigated two electrical field shaping techniques involving simultaneous stimulation of multiple electrodes designed to improve the resolution of artificial images presented to recipients of retinal prostheses. These studies attempted to provide solutions to two major issues in retinal prosthetics; that patients experience difficulty in distinguishing between different percepts, and that devices are unable to elicit a sufficient number of discriminably unique percepts to convey complex visual information.

Electrical field interactions due to simultaneous multichannel stimulation have commonly been seen as detrimental in neural prosthetic devices due to their seemingly unpredictable nature. As such, the use of sequential stimulation strategies aim to reduce these interactions (Horsager et al. 2011, Matteucci et al. 2016, Wilke et al. 2011b). Our work, and other recent work, with electrical field interactions suggests that rather than aiming to avoid channel interactions, new stimulation strategies should aim to utilise these interactions to enhance the repertoire of discriminable percepts that can be elicited by devices (Dumm et al. 2014, Halupka et al. 2016, Jepson et al. 2014, Maturana et al. 2016). In the preceding chapters, focused multipolar (FMP) stimulation and virtual electrode (VE) current steering have been shown to effective techniques in restricting and steering electrical fields in two dimensions and eliciting predictable neural responses when applied to healthy retinae. While Chapter 5 indicates that further optimisation may be required in order for these specific techniques to be effectively applied to degenerate retinae, our results show nonetheless that electrical field shaping strategies have the potential to confer far greater control over the spatial properties of electrical fields and the resultant neural activation patterns. Our findings also provide a greater understanding of the differences between the responses of the healthy degenerate visual system to electri-

cal stimulation, which we hope will inform further development and optimisation of these techniques.

6.1 Future directions

Throughout these studies, we trialled FMP stimulation and VE current steering using cathodic-first biphasic single pulses. These pulse parameters were chosen to remain consistent with the methodology from our previous preclinical retinal stimulation studies and allow for better comparison. However, clinically, neural stimulation often employs pulse trains in order to maintain a persistent percept or effect (Ayton et al. 2014, Benabid 2003, Berenstein et al. 2008, da Cruz et al. 2016). Some devices also employ differently shaped current waveforms, such as monophasic or triphasic waveforms, biphasic waveforms with asymmetrical phases, or employ differently ordered polarities. Stimulation rate has significant effects on the brightness of percepts and also contributes to the adaptation of neural tissue to electrical stimuli, resulting in perceptual fading (Shivdasani et al. 2014, Stronks & Dagnelie 2014, Wilke et al. 2011c). Further investigation of electrical field shaping strategies should include reevaluation at clinically relevant stimulation rates and using alternative waveform shapes. It is also likely that, due to the use of relatively long 1 ms pulses that inner retinal neurons were activated along with retinal ganglion cells (RGCs) (Jensen et al. 2003, 2005, Fried et al. 2006). It may be beneficial to reevaluate FMP stimulation and VE current steering using shorter pulses (50 μ s to 100 μ s) to examine whether their effectiveness is maintained when RGCs are preferentially activated (Jensen et al. 2003). While we used symmetrically biphasic current pulses, it is unclear what effect electrical field interactions from multipolar stimulation using high numbers of electrodes may have on charge recovery. Chronic intracochlear FMP stimulation has been shown to not adversely affect neural thresholds or spiral ganglion neuron survival (Shepherd et al. 2017). Long-term chronic safety studies may be required to assess the potential for tissue damage resulting from charge leakage.

Due to the processing of visual information that occurs between phototransduction and the visual cortex, our method of inferring retinal activation spread from cortical responses results in an estimation at best. Recording directly from the retina would provide a more precise picture of how our techniques affect retinal neural responses. Because intense artefacts caused from retinal stimulation obscure neural signals, we can only record signals from higher structures that occur after the signal-to-noise

ratio returns to a workable level. For future studies, imaging techniques that are not affected by stimulation artefacts such as calcium imaging or two-photon microscopy, may prove to be more effective in capturing the retinal neural response.

Investigating the effects of electrode size, arrangement, and geometries on these strategies is important to fully understand their limitations and best forms of implementation. The distance between electrodes would likely be a significant factor in the degree individual electrical fields interact. Decreasing the distance between electrodes may allow for electrical field shaping with greater resolution. Conversely, increasing the distance between electrodes will likely decrease the interactions between electrical field and reduce the effectiveness of both techniques. Implantation location may also significantly alter the effectiveness of both techniques due to the differences in tissue impedance and proximity to target cells. Field interactions may be more predictable closer to the electrode site as there may be a greater propensity for current to leak to neighbouring regions of tissue further from the electrode site, especially considering the high-impedance heterogeneous nature of retinal structures like the choroid, or if long pulse widths are used (Savage et al. 2013). Epiretinal or subretinal devices may be better placed for electrical field shaping due to their close proximity to bipolar (BP) cells and RGCs. Investigation of the application of these strategies to other implantable devices such as deep brain stimulation (DBS) and peripheral nerve stimulation devices may also be beneficial.

6.1.1 Alterations to FMP stimulation

Our data suggests that, while capable of restricting the spread of neural activation compared with monopolar (MP) stimulation, FMP stimulation in its present form shows no significant benefit over more technically simple forms of current focusing such as hexapolar (HP) or quasimonopolar stimulation. Our data from our model of retinal degeneration also suggests that current focusing is significantly less effective when applied to degenerate retinæ. Unlike HP and quasimonopolar strategies, the versatile nature of the FMP methodology leaves room for the possibility of substantial modification and optimisation. The results from Chapter 3 shows that FMP significantly reduces voltage spread in the retina, which demonstrates the effectiveness of FMP stimulation in two-dimensions. Further investigation of the effects of alterations to the calculation of charge weightings could improve our understanding of the full potential and limitations of FMP stimulation.

One alteration that could be made would be modifying the method behind estimation of the diagonal values of the transimpedance matrix. Due to polarisation during stimulation, it is not possible to accurately measure the voltage change of the tissue local to a stimulating electrode. As such, we estimate these values using a version of van den Honert, Carlyon, Long, Smith, Shelton & Kelsall (2007) linear extrapolation technique. To adapt this technique to a 2D array, we expanded the extrapolation methodology to use transimpedance values from up to twelve neighbouring electrodes in different directions, as opposed to four electrodes on each opposing side on a linear array. As all charge weightings are calculated by normalising rows of transadmittance values to the diagonal values, the methodology behind the estimation of these values would have a significant impact on the effectiveness of the technique. Alternative forms of estimation may include the use of different forms of extrapolation, curve fitting, or possibly the incorporation of electrode impedance measurements. The effect of intentionally altering the diagonal values of the matrix has been explored using cochlear implants (George et al. 2015). Applying a positive multiplier to these values resulted in reduced effectiveness in focusing neural activation and also a reduction in activation thresholds, proportional to the magnitude of “defocusing” multiplier applied (George et al. 2015). This indicates that the degree of focusing can be modulated in order to reduce neural thresholds to safer levels. Exploration of whether this can be applied to our 2D implementation of FMP would be useful to inform clinical translation. It may also be possible to apply a negative multiplier to the diagonal values in order to increase the degree of focusing. This would likely only be achieved to the further expense of increased thresholds. However, we did find that charge weights applied to electrodes immediately adjacent to the PSE were higher than what we estimate for HP stimulation, which showed the same degree of focusing as FMP stimulation did when applied to healthy retinæ. This may indicate that simply increasing the weightings is not enough to significantly impact the effectiveness of the technique. However, if possible, this would provide a significant advantage over other current focusing techniques where little of the methodology can be modified to increase the degree of focusing.

In order to present meaningful information to patients, it is necessary to be able to elicit the perceptions of multiple phosphenes simultaneously. The use of sequential stimulation is becoming an increasingly unsustainable practice as visual prostheses groups continue to aim to increase the number of elicitable percepts. As the number of sequentially delivered percepts increase, the timing between them becomes increasingly compressed. While our work suggests that simultaneous stimulation can be utilised in a predictable way, under the present implementation of our strate-

gies each phosphene would still need to be elicited sequentially in order to avoid undesired interactions. As timing is compressed further with the addition of new physical and VEs, undesired channel interaction will become an even greater issue as the phase shift between stimuli is reduced (De Balthasar et al. 2003). FMP stimulation has been shown in cochlear implants to be capable of producing multi-peaked electrical fields, allowing for the selective activation of multiple frequency domains in the inferior colliculus simultaneously without the spectral smearing observed using simultaneous MP stimulation (George et al. 2015). It may be possible to achieve the same results using our 2D implementation of FMP stimulation. Further investigation in to the ability of FMP stimulation to create multiple distinct regions of activation could allow multiple phosphenes to be elicited simultaneously and alleviate the timing constraints of sequential stimulation.

Calculation of FMP weights

Over the course of the studies presented in Chapters 3 and 5, we have found that significant variation in electrode transimpedance can impair calculation of FMP weights. Appendix A shows some examples of abnormal transimpedance and transadmittance matrices that resulted in the calculation of charge weightings far beyond safe injection limits. Through a combination of in vivo and in vitro testing, we have found that outlying transimpedance values are often due to abnormally high variance in electrode impedances (see Appendix A for further details). This could be either due to electrode corrosion, acute inflammatory responses, or poor contact with the underlying tissue. We also theorise that, due to the heterogenous nature of the choroid, tissue impedance is likely to be varied depending on electrode location. This issue has not been reported by in vivo studies using cochlear implants (George et al. 2014, 2015), however, high levels of variation in electrode impedance is not likely to be an issue following acute cochlear implantation due to the highly conductive nature of the perilymph. Although, intracochlear electrode impedance are known to increase following long-term implantation and stimulation due to fibrous tissue growth (Shepherd & Clark 1990, Newbold et al. 2004).

Electrode impedances are often unstable following acute suprachoroidal implantation (Shivdasani et al. 2010). Allowing time for the array’s position and the electrode-tissue interface to stabilise often resulted in more uniform impedance values, and more typical transimpedance values. When this was not possible, electrodes related with consistently high transadmittance values were removed from the matrix and excluded from the phased array. Removal of electrodes typically resulted in the

calculation of FMP charge weights within safe injection limits and present in a more archetypal manner, at the expense of fewer electrodes in the phased array. In this study, we were unable to calculate FMP weights for two control eyes. As our model of retinal degeneration is not easily obtained, to minimise the risk of device failure following implantation we reserved the use of arrays that were fabricated closer to the date of the experiment for implantation in to the degenerated eye. The use of older electrode arrays in the follow control eye may have increased the chance of high transadmittance values due to stimulation-induced electrode corrosion.

As shown in Senn (2014) and Appendix A, transimpedance matrices collected just hours apart can show significant variation, raising the question of how often a transimpedance matrix must be recollected and analysed in order to maintain optimal and consistent current focusing. It should be noted that these matrices were constructed from recordings of acutely implanted electrode arrays subject to a more variable biological environment than chronically implanted devices. The magnitude of transimpedance variability over time may be inflated as a result of acute inflammatory processes and small movements of the electrode array that are less likely to be occurring in long-term implanted patients. Regardless, periodic recalibration will likely be a requirement for the use of FMP stimulation clinically. Depending on the sophistication of the impedance monitoring apparatus and the number of electrodes in the array, this may be a lengthy process and prevent the patient from using their device for extended periods of time. Fibrous tissue growth may also result in greater disparity in transimpedance values, further impairing reliable calculation of FMP weights (Villalobos et al. 2012). Further study of FMP stimulation in chronically implanted animals could provide a greater understanding of the effects of chronic implantation on transimpedance measurements and calculation of FMP weights.

6.1.2 VE current steering

VE current steering may provide a way to circumvent electrode breakage by replacing these non-functional physical electrodes with VEs. Phosphenes elicited by damaged electrodes could be reproduced using VE stimulation from neighbouring electrodes. Following implantation, patients must undergo a long period of training to learn to interpret the percepts they are being presented with. The consistency of these percepts is likely to be of great importance to the patients' continued ability to utilise their artificial vision (Spencer et al. 2018). Not only may repeated explantation and reimplantation of devices pose a risk to the health of the eye, but, as it is unlikely that arrays will be placed in exactly the same location as before, it may

result in very different percepts to those that the patient had been training to interpret. Using VEs, device lifespan may be improved and could provide patients with longer-term consistent stimuli. According to our data from normally-sighted cats, assuming that electrodes are of equal distances from each other, equally-weighted stimulation of adjacent electrodes would produce neural activation patterns similar to those elicited by the faulty electrode. This could be easily implemented as equally-weighted stimulation could be achieved with a single current source. However, our work in our model of retinal degeneration did not indicate this was possible when applied to degenerate retinae.

While VE current steering could be used to replace faulty electrodes and extend the lifespan of devices, the overall aim of this thesis was to investigate the potential for these techniques to improve the resolution of retinal prostheses. Reliable and predictable creation of VEs allows devices to elicit a greater number of unique percepts. Our data from both normally-sighted cats and our model of retinal degeneration indicates that VEs produce cortical patterns with similar spatial selectivity as those elicited by physical electrode (PE)s, suggesting that the summated electrical field created by VE current steering is of a similar size and shape to those created by PE stimulation. While our data from our model of retinal degeneration showed that we were unable to accurately predict their location, the spatial spread of responses remained similar to those elicited by PE stimulation. Optimisation of the steering strategy would likely mean adjusting the weights of stimulation to account for variation in neural thresholds. However, our model exhibits a pattern of photoreceptor degeneration that is atypical of patients with RP or AMD. It is possible that due to the more consistent nature of advanced-stage retinal degenerative diseases that VE current steering will be more effective in patients than observed using our model. This is supported by the preliminary data shown in Figure 1.X, where current steering did appear to produce phosphenes in expected locations.

While current steering is currently clinically utilised in cochlear implants and is known to elicit a greater range of pitch percepts (Firszt et al. 2007), there has been no significant improvement in outcomes for speech recognition (Buechner et al. 2008, Firszt et al. 2009). Preclinical evidence suggests that perceptual overlap, resulting from the broad spread of current in the cochlea may, in part, be responsible (George et al. 2015). In order to present complex and meaningful information to patients, a large repertoire of percepts must be elicited. However, in order to be unique, the patient must be able to discriminate between them. Improvements in electrode materials, allowing smaller electrodes with a greater safe charge density limits may

allow for the development of arrays that elicit more discrete phosphenes (Spencer et al. 2018). However, for contemporary devices, it is likely that current steering and current focusing will need to be utilised together in order to produce spatially distinct percepts that patients can discriminate between. FMP stimulation may prove advantageous in this regard as current focusing techniques that utilise local returns may not be feasible if neighbouring electrodes have been recruited into a VE stimulation strategy. Instead, incorporating the VE strategy in to the calculation of FMP weights could produce focused retinal activation between electrodes (Spencer et al. 2018).

While our data in normally-sighted cats showed that centroids moved in a predictable direction in the cortex when IVE stimulation was applied, it should be noted that this may not translate to the location of phosphenes. Patient reports of phosphene locations in visual space do not always correlate well with the predicted position of phosphenes (Dagnelie 2008, Sinclair et al. 2016). This may be in part due to remodelled cortical retinotopy, as also evidenced by our own findings, and the work of others, using our cat model of retinal degeneration (Halupka et al. 2017). Optimisation of this technique will likely require psychophysical experimentation to better understand how VE current steering alters the location of phosphenes.

6.1.3 Implications of retinal degeneration

In agreement with past work using this model (Aplin et al. 2016b, Halupka et al. 2017), we have found that photoreceptor degeneration and subsequent inner retinal remodelling significantly impacts the response of the visual system to electrical stimulation. These studies provide further evidence that future preclinical studies in to development of new visual prosthetic devices or stimulation strategies could benefit from being either repeated, or exclusively carried out, in models that closer represent the physiology of patients with retinal degeneration in order to better inform translational endeavours. We have demonstrated significant distortion of cortical retinotopy, increased spatial selectivity, increased activation thresholds, and thinning of inner retinal layers post-partial photoreceptor death. Combined with other findings such as progressive death of RGCs, severe gliosis, increased ipsilateral activation, and increased retinal receptive fields, there may be significant limitations on what retinal prostheses will be capable of in patients who have been blind for long periods of time (Aplin et al. 2016a,b, Halupka et al. 2017). Further investigation in to how these differing responses to electrical stimulation relate to the anatomical and physiological changes present following degeneration and remodelling would aid

the optimisation and development of stimulation strategies and devices. Correlating electrophysiological properties to immunohistological characterisation of tissue is one method to determine the mechanisms responsible for these changes in neural responses (Aplin et al. 2016a). Calcium imaging of retinae in vitro has also been shown to be a useful tool in mapping neuronal connections and could prove an effective way of investigating the extent and manner of inner retinal remodelling (Behrend et al. 2009, Briggman & Euler 2011).

Continued development of the feline model of retinal degeneration is also important as a way to further investigate the extent and nature of remodelling in the visual system and also for use in the development of stimulation strategies and devices. One major issue with the model is a high failure rate. One out of our five subjects experienced severe retinal detachment, which had also been observed during the development of the model (Aplin et al. 2014). Adverse effects such as these are costly and ethically concerning. We also observed very different levels and patterns of degeneration between subjects and required differing numbers of injections in order to induce significant degeneration. Further development of the model should seek to establish factors that influence successful degeneration and undesirable effects, such as localised or total retinal detachments, and determine how to optimise the procedure.

6.1.4 Device design

For multichannel electrical field shaping techniques to be utilised in patients, devices will need to be driven by stimulators with multiple current sources. This would be a significant technical consideration considering that this requirement will likely increase the engineering complexity, cost, and power consumption of a device. MP and HP stimulation require only a single current source. quasimonopolar stimulation or variations would require two current sources. Our 2D implementation of FMP stimulation utilised all 42 electrodes on the array, requiring 42 independent current sources, however it is likely that only one or two rings of electrodes surrounding the epicentre would be required to elicit the desired effect. As demonstrated by the example transimpedance matrices in Appendix A, the third ring of electrodes often had very low levels of charge delivered and were unlikely to have a significant effect on the distribution of electrical potential. To implement unequally-weighted current steering to create VEs, the number of current sources would need to equal the number of electrodes required to create the desired VEs. This may depend on electrode arrangement and the number of VEs that are required to be elicited

simultaneously. In order to make a device as versatile as possible for clinical and research purposes it would be favourable to have an independent current source for each electrode, however this will likely become even more technically difficult for high density electrode arrays. Alternatively, it has been shown that single-source multipolar stimulation is possible using a variable impedance bank (Senn et al. 2018). This technique makes it possible to stimulate multiple electrodes simultaneously with different weights without the need for multiple current sources. Single-source multipolar stimulation was shown to focus voltage spread and neural activation in a similar manner to FMP stimulation on a linear electrode array using the same weight calculation method as described by van den Honert & Kelsall (2007) (Senn et al. 2018).

In order to calculate charge weights for FMP stimulation, it is necessary to collect transimpedance data from the electrode array. This may place additional hardware and software requirements on devices. Transimpedance data must be collected from each electrode on an array in response to independent MP stimulation of each electrode on the array. This either requires the inclusion of a sophisticated multichannel data acquisition system to record voltages from many channels simultaneously, or lengthily construction of a matrix one electrode pair at a time. Without the inclusion of specialised hardware and software, there may be long periods of time during transimpedance collection where the patient will not be able to make use of their device. These long recording periods will only become more problematic when applied to devices with greater numbers of electrodes, as the time required to collect transimpedance data from one electrode at a time increases exponentially with each additional electrode.

The use of an optimised VE current steering strategy may alleviate the need for large numbers of physical electrodes in future devices. Additional electrodes can increase engineering complexity, device fragility, and exacerbate surgical issues. Using VE current steering, a device could elicit the same number of phosphenes with significantly fewer electrodes. However, if a single electrode is involved in the elicitation of multiple phosphenes, it may be necessary to include redundant electrodes to prevent significant impairment to the VE strategy in the event of electrode breakage (Spencer et al. 2018).

6.2 Final conclusion

In this thesis, data was presented from three studies with the overall aim of investigating the potential of novel electrical field shaping techniques in providing recipients of retinal implants a greater range of discriminable percepts, in order to present patients more information about the visual scene around them. The first study determined that FMP stimulation was capable of eliciting more focused activation of retinal and cortical neural populations, than the more widely used MP stimulation technique, indicating that this technique could be used to elicit discrete phosphenes. The second study established that current steering in two dimensions was possible and that VEs can be created that mimic the cortical spatial characteristics to those elicited by nearby physical electrodes. This indicated that this technique could be used to reproduce phosphenes elicited by broken electrodes, or to produce additional intermediate phosphenes without additional electrodes. The third study re-evaluated these techniques when applied to a feline model of retinal degeneration, demonstrating that their effectiveness is severely diminished when applied to degenerate retinæ. Ultimately, FMP stimulation and VE current steering show significant promise as potential strategies to improve the number and discriminability of phosphenes. However, significant optimisation will likely be required in order to translate these techniques to a clinical setting.

Chapter 7

Bibliography

- Abbott, C. J., Nayagam, D. A., Luu, C. D., Epp, S. B., Williams, R. A., Salinas-LaRosa, C. M., Villalobos, J., McGowan, C., Shivdasani, M. N., Burns, O., Leavens, J., Yeoh, J., Brandli, A. A., Thien, P. C., Zhou, J., Feng, H., Williams, C. E., Shepherd, R. K. & Allen, P. J. (2018), ‘Safety studies for a 44-channel suprachoroidal retinal prosthesis: A chronic passive study’, *Investig. Ophthalmol. Vis. Sci.* **59**(3), 1410–1424.
- Abramian, M., Lovell, N. H., Morley, J. W., Suaning, G. J. & Dokos, S. (2011), ‘Activation of retinal ganglion cells following epiretinal electrical stimulation with hexagonally arranged bipolar electrodes.’, *J. Neural Eng.* **8**(3), 035004.
- Ahuja, A. K., Dorn, J. D., Caspi, A., McMahon, M. J., Dagnelie, G., Dacruz, L., Stanga, P., Humayun, M. S. & Greenberg, R. J. (2011), ‘Blind subjects implanted with the Argus II retinal prosthesis are able to improve performance in a spatial-motor task.’, *Br. J. Ophthalmol.* **95**(4), 539–43.
- Aplin, F. P., Fletcher, E. L., Luu, C. D., Vessey, K. A., Allen, P. J., Guymer, R. H., Shepherd, R. K. & Shivdasani, M. N. (2016b), ‘Stimulation of a suprachoroidal retinal prosthesis drives cortical responses in a feline model of retinal degeneration’, *Investig. Ophthalmol. Vis. Sci.* **57**(13), 5216–5229.
- Aplin, F. P., Luu, C. D., Vessey, K. a., Guymer, R. H., Shepherd, R. K. & Fletcher, E. L. (2014), ‘ATP-Induced Photoreceptor Death in a Feline Model of Retinal Degeneration’, *Invest. Ophthalmol. Vis. Sci.* **55**(12), 8319–8329.
- Aplin, F. P., Vessey, K. A., Luu, C. D., Guymer, R. H., Shepherd, R. K. & Fletcher, E. L. (2016a), ‘Retinal Changes in an ATP-Induced Model of Retinal Degeneration’, *Front. Neuroanat.* **10**(April), 1–13.

- Ayton, L. N., Blamey, P. J., Guymer, R. H., Luu, C. D., Nayagam, D. A. X., Sinclair, N. C., Shivdasani, M. N., Yeoh, J., McCombe, M. F., Briggs, R. J., Opie, N. L., Villalobos, J., Dimitrov, P. N., Varsamidis, M., Petoe, M. A., McCarthy, C. D., Walker, J. G., Barnes, N., Burkitt, A. N., Williams, C. E., Shepherd, R. K. & Allen, P. J. (2014), 'First-in-human trial of a novel suprachoroidal retinal prosthesis.', *PLoS One* **9**(12), e115239.
- Barbe, M. T., Maarouf, M., Alesch, F. & Timmermann, L. (2014), 'Multiple source current steering - a novel deep brain stimulation concept for customized programming in a Parkinson's disease patient', *Park. Relat. Disord.* **20**(4), 471–473.
- Behrend, M. R., Ahuja, A. K., Humayun, M. S., Weiland, J. D. & Chow, R. H. (2009), 'Selective labeling of retinal ganglion cells with calcium indicators by retrograde loading in vitro', *J. Neurosci. Methods* **179**(2), 166–172.
- Benabid, A. L. (2003), 'Deep brain stimulation for Parkinson's disease', *Curr. Opin. Neurobiol.* **13**(6), 696–706.
- Berenstein, C. K., Mens, L. H. M., Mulder, J. J. S. & Vanpoucke, F. J. (2008), 'Current steering and current focusing in cochlear implants: comparison of monopolar, tripolar, and virtual channel electrode configurations.', *Ear Hear.* **29**(2), 250–60.
- Bertschinger, D. R., Beknazar, E., Simonutti, M., Safran, A. B., Sahel, J. A., Rosolen, S. G., Picaud, S. & Salzmann, J. (2008), 'A review of in vivo animal studies in retinal prosthesis research', *Graefe's Arch. Clin. Exp. Ophthalmol.* **246**(11), 1505–1517.
- Bierer, J. A. (2007), 'Threshold and channel interaction in cochlear implant users: evaluation of the tripolar electrode configuration.', *J. Acoust. Soc. Am.* **121**(3), 1642–1653.
- Bierer, J. A. & Middlebrooks, J. C. (2004), 'Cortical Responses to Cochlear Implant Stimulation: Channel Interactions', *JARO - J. Assoc. Res. Otolaryngol.* **5**(1), 32–48.
- Blamey, P. J., Sinclair, N., Slater, K., McDermott, H., Perera, T., Dimitrov, P. N., Varsamidis, M., Ayton, L., Guymer, R. & Luu, C. D. (2013), 'Psychophysics of a suprachoroidal retinal prosthesis', *ARVO Meet. Abstr.* **54**(6), 1044.
- Blomstedt, P. & Hariz, M. I. (2005), 'Hardware-related complications of deep brain stimulation: A ten year experience', *Acta Neurochir. (Wien).* **147**(10), 1061–1064.

- Boex, C., De Balthasar, C., Kos, M.-I. & Pelizzone, M. (2003), ‘Electrical field interactions in different cochlear implant systems’, *J. Acoust. Soc. Am.* **114**(4), 2049.
- Boinagrov, D., Pangratz-Fuehrer, S., Goetz, G. & Palanker, D. (2014), ‘Selectivity of direct and network-mediated stimulation of the retinal ganglion cells with epi-, sub- and intraretinal electrodes.’, *J. Neural Eng.* **11**, 026008.
- Bonham, B. H. & Litvak, L. M. (2008), ‘Current focusing and steering: modeling, physiology, and psychophysics.’, *Hear. Res.* **242**(1-2), 141–53.
- Boycott, B. B., Hopkins, J. M. & Sperling, H. G. (1987), ‘Cone Connections of the Horizontal Cells of the Rhesus Monkey’s Retina’, *Proceedings of the Royal Society of London. Series B, Biological Sciences* **229**(1257), 345–379.
URL: <http://www.jstor.org.ezp.lib.unimelb.edu.au/stable/36187>
- Brendel, M., Frohne-Buechner, C., Stoever, T., Lenarz, T. & Buechner, A. (2009), ‘Investigation of pitch discrimination and the effect of learning for virtual channels realized by current steering.’, *Acta Otolaryngol.* **129**(12), 1425–33.
- Briggman, K. L. & Euler, T. (2011), ‘Bulk electroporation and population calcium imaging in the adult mammalian retina’, *J. Neurophysiol.* **105**(5), 2601–2609.
- Brindley, G. S. & Lewin, W. S. (1968), ‘The sensations produced by electrical stimulation of the visual cortex’, *J. Physiol.* **196**(2), 479–493.
- Buechner, A., Brendel, M., Krüeger, B., Frohne-Büchner, C., Nogueira, W., Edler, B. & Lenarz, T. (2008), ‘Current steering and results from novel speech coding strategies’, *Otol. Neurotol. Off. Publ. Am. Otol. Soc. Am. Neurotol. Soc. [and] Eur. Acad. Otol. Neurotol.* **29**, 203–207.
- Butson, C. R. & McIntyre, C. C. (2008), ‘Current steering to control the volume of tissue activated during deep brain stimulation’, *Brain Stimul.* **1**(1), 7–15.
- Chaturvedi, A., Foutz, T. J. & McIntyre, C. C. (2012), ‘Current steering to activate targeted neural pathways during deep brain stimulation of the subthalamic region’, *Brain Stimul.* **5**(3), 369–377.
- Chen, S. C., Suaning, G. J., Morley, J. W. & Lovell, N. H. (2009), ‘Simulating prosthetic vision: II. Measuring functional capacity.’, *Vision Res.* **49**(19), 2329–43.

- Chen, W., Zhu, X.-H., Thulborn, K. R. & Ugurbil, K. (1999), ‘Retinotopic mapping of lateral geniculate nucleus in humans using functional magnetic resonance imaging’, *Proc. Natl. Acad. Sci.* **96**(5), 2430–2434.
- Cicione, R., Fallon, J. B., Rathbone, G. D., Williams, C. E. & Shivdasani, M. N. (2014), ‘Spatiotemporal interactions in the visual cortex following paired electrical stimulation of the retina’, *Investig. Ophthalmol. Vis. Sci.* **55**(12), 7726–7738.
- Cicione, R., Shivdasani, M. N., Fallon, J. B., Luu, C. D., Allen, P. J., Rathbone, G. D., Shepherd, R. K. & Williams, C. E. (2012), ‘Visual cortex responses to suprachoroidal electrical stimulation of the retina: effects of electrode return configuration.’, *J. Neural Eng.* **9**(3), 036009.
- da Cruz, L., Dorn, J. D., Humayun, M. S., Dagnelie, G., Handa, J., Barale, P. O., Sahel, J. A., Stanga, P. E., Hafezi, F., Safran, A. B., Salzmann, J., Santos, A., Birch, D., Spencer, R., Cideciyan, A. V., de Juan, E., Duncan, J. L., Elliott, D., Fawzi, A., Olmos de Koo, L. C., Ho, A. C., Brown, G., Haller, J., Regillo, C., Del Priore, L. V., Arditi, A. & Greenberg, R. J. (2016), ‘Five-Year Safety and Performance Results from the Argus II Retinal Prosthesis System Clinical Trial’, *Ophthalmology* **123**(10), 2248–2254.
- Dagnelie, G. (2008), ‘Psychophysical evaluation for visual prosthesis’, *Annu. Rev. Biomed. Eng.* **10**, 339–68.
- Dagnelie, G., Christopher, P., Arditi, A., da Cruz, L., Duncan, J. L., Ho, A. C., Olmos de Koo, L. C., Sahel, J. A., Stanga, P. E., Thumann, G., Wang, Y., Arsiero, M., Dorn, J. D. & Greenberg, R. J. (2017), ‘Performance of real-world functional vision tasks by blind subjects improves after implantation with the Argus® II retinal prosthesis system’, *Clin. Exp. Ophthalmol.* **45**(2), 152–159.
- Daschner, R., Rothermel, A., Rudolf, R., Rudolf, S. & Stett, A. (2018), ‘Functionality and Performance of the Subretinal Implant Chip Alpha AMS’, *Sensors Mater.* **30**(2), 179.
- De Balthasar, C., Boex, C., Cosendai, G., Valentini, G., Sigrist, A. & Pelizzone, M. (2003), ‘Channel interactions with high-rate biphasic electrical stimulation in cochlear implant subjects’, *Hear. Res.* **182**(1-2), 77–87.
- De Balthasar, C., Patel, S., Roy, A., Freda, R., Greenwald, S., Horsager, A., Mahadevappa, M., Yanai, D., McMahon, M. J., Humayun, M. S., Greenberg, R. J., Weiland, J. D. & Fine, I. (2008), ‘Factors affecting perceptual thresholds in epiretinal prostheses’, *Investig. Ophthalmol. Vis. Sci.* **49**(6), 2303–2314.

- Dilks, D. D., Serences, J. T., Rosenau, B. J., Yantis, S. & McCloskey, M. (2007), ‘Human Adult Cortical Reorganization and Consequent Visual Distortion’, *J. Neurosci.* **27**(36), 9585–9594.
- Dobelle, B. Y. W. H., Mladejovsky, M. G., Avenue, E. O. & City, S. L. (1974), ‘Phosphenes produced by electrical stimulation of human occipital cortex and their application to the development of a prostheses for the blind’, *October* pp. 553–576.
- Dobelle, W. H. (2000), ‘Artificial Vision for the Blind by Connecting a Television Camera to the Visual Cortex’, *Am. Soc. Artif. Intern. Organs J.* **46**, 3–9.
- Dobelle, W. H., Mladejovsky, M. G., Evans, J. R., Roberts, T. S. & Girvin, J. P. (1976), ‘“Braille” reading by a blind volunteer by visual cortex stimulation’, *Nature* **259**(5539), 111.
- Dryja, T. P., McGee, T. L., Reichel, E., Hahn, L. B., Cowley, G. S., Yandell, D. W., Sandberg, M. a. & Berson, E. L. (1990), ‘A point mutation of the rhodopsin gene in one form of retinitis pigmentosa.’, *Nature* **343**(6256), 364–366.
- Dumm, G., Fallon, J. B., Williams, C. E. & Shivdasani, M. N. (2014), ‘Virtual electrodes by current steering in retinal prostheses’, *Investig. Ophthalmol. Vis. Sci.* **55**(12), 8077–8085.
- Duncan, J. L., Richards, T. P., Arditi, A., da Cruz, L., Dagnelie, G., Dorn, J. D., Ho, A. C., Olmos de Koo, L. C., Barale, P. O., Stanga, P. E., Thumann, G., Wang, Y. & Greenberg, R. J. (2017), ‘Improvements in vision-related quality of life in blind patients implanted with the Argus II Epiretinal Prosthesis’, *Clin. Exp. Optom.* **100**(2), 144–150.
- Edwards, T. L., Cottrill, C. L., Xue, K., Simunovic, M. P., Ramsden, J. D., Zrenner, E. & MacLaren, R. E. (2018a), ‘Assessment of the Electronic Retinal Implant Alpha AMS in Restoring Vision to Blind Patients with End-Stage Retinitis Pigmentosa’, *Ophthalmology* **125**(3), 432–443.
- Edwards, T. L., Cottrill, C. L., Xue, K., Simunovic, M. P., Ramsden, J. D., Zrenner, E. & MacLaren, R. E. (2018b), ‘Assessment of the Electronic Retinal Implant Alpha AMS in Restoring Vision to Blind Patients with End-Stage Retinitis Pigmentosa’, *Ophthalmology* **125**(3), 432–443.
- Eger, M., Wilms, M., Eckhorn, R., Schanze, T. & Hesse, L. (2005), ‘Retino-cortical information transmission achievable with a retina implant’, *BioSystems* **79**(1-3 SPEC. ISS.), 133–142.

- Fallon, J. B., Irvine, D. R. F. & Shepherd, R. K. (2009), ‘Cochlear implant use following neonatal deafness influences the cochleotopic organization of the primary auditory cortex in cats’, *J. Comp. Neurol.* **512**(1), 101–114.
- Fallon, J. B., Irving, S., Pannu, S. S., Tooker, A. C., Wise, A. K., Shepherd, R. K. & Irvine, D. R. (2016), ‘Second spatial derivative analysis of cortical surface potentials recorded in cat primary auditory cortex using thin film surface arrays: Comparisons with multi-unit data’, *J. Neurosci. Methods* **267**, 14–20.
- Fan, V., Grosberg, L. E., Madugula, S., Hottowy, P., Dabrowski, W., Sher, A., Litke, A. M. & Chichilnisky, E. J. (2017), ‘Optimizing Selectivity of Epiretinal Stimulation using Local Returns’, *Invest. Ophthalmol. Vis. Sci.* **58**(8), 4194.
- Fariss, R. N., Li, Z. Y. & Milam, A. H. (2000), ‘Abnormalities in rod photoreceptors, amacrine cells, and horizontal cells in human retinas with retinitis pigmentosa’, *Am. J. Ophthalmol.* **129**(2), 215–223.
- Firszt, J. B., Holden, L. K., Reeder, R. M. & Skinner, M. W. (2009), ‘Speech Recognition in Cochlear Implant Recipients: Comparison of Standard HiRes and HiRes 120 Sound Processing’, *Otol Neurotol* **30**(2), 146–152.
- Firszt, J. B., Koch, D. B., Downing, M. & Litvak, L. (2007), ‘Current steering creates additional pitch percepts in adult cochlear implant recipients.’, *Otol. Neurotol.* **28**(5), 629–36.
- Flusberg, B. A., Jung, J. C., Cocker, E. D., Anderson, E. P. & Schnitzer, M. J. (2005), ‘In vivo brain imaging using a portable 3.9 gram two-photon fluorescence microendoscope’, *Opt. Lett.* **30**(17), 2272–2274.
- Foerster, O. (1929), ‘Beitrage zur Pathophysiologie der Sehbahn und der Sehsphare’, *J. Psychol. Neurol., Lpz.* **39**, 463.
- Fried, S. I., Hsueh, H. a. & Werblin, F. S. (2006), ‘A Method for Generating Precise Temporal Patterns of Retinal Spiking Using Prosthetic Stimulation’, *J. Neurophysiol. by guest* **95**(October 2005), 970–978.
- Frijns, J. H. M., Dekker, D. M. T. & Briare, J. J. (2011), ‘Neural excitation patterns induced by phased-array stimulation in the implanted human cochlea’, *Acta Otolaryngol.* **131**(4), 362–370.
- Frumkes, T. E., Miller, R. F., Slaughter, M. & Dacheux, R. F. (1981), ‘Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy

- retina. III. Amacrine-mediated inhibitory influences on ganglion cell receptive-field organization: a model.’, *Journal of Neurophysiology* **45**(4), 783–804.
- Fujikado, T., Kamei, M., Sakaguchi, H., Kanda, H., Morimoto, T., Ikuno, Y., Nishida, K. K., Kishima, H., Maruo, T., Konoma, K., Ozawa, M. & Nishida, K. K. (2011), ‘Testing of semichronically implanted retinal prosthesis by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa’, *Investig. Ophthalmol. Vis. Sci.* **52**(7), 4726–4733.
- Fujikado, T., Morimoto, T., Kanda, H., Kusaka, S., Nakauchi, K., Ozawa, M., Matsushita, K., Sakaguchi, H., Ikuno, Y., Kamei, M. & Tano, Y. (2007), ‘Evaluation of phosphenes elicited by extraocular stimulation in normals and by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa’, *Graefes Arch. Clin. Exp. Ophthalmol.* **245**(10), 1411–1419.
- Gantz, B. J., Turner, C., Gfeller, K. E. & Lowder, M. W. (2005), ‘Preservation of Hearing in Cochlear Implant Surgery : Advantages of Combined Electrical and Acoustical Speech Processing’, (May), 796–802.
- George, S. S., Wise, A. K., Fallon, J. B. & Shepherd, R. K. (2015), ‘Evaluation of focused multipolar stimulation for cochlear implants in long-term deafened cats’, *J. Neural Eng.* **12**(3), 036003.
- George, S. S., Wise, A. K., Shivdasani, M. N., Shepherd, R. K. & Fallon, J. B. (2014), ‘Evaluation of focused multipolar stimulation for cochlear implants in acutely deafened cats’, *J. Neural Eng.* **11**(6), 065003.
- Gerhardt, M., Alderman, J. & Stett, A. (2010), ‘Electric field stimulation of bipolar cells in a degenerated retina—a theoretical study.’, *IEEE Trans. Neural Syst. Rehabil. Eng.* **18**(1), 1–10.
- Gerhardt, M., Groeger, G. & Maccarthy, N. (2011), ‘Monopolar vs. bipolar subretinal stimulation-an in vitro study.’, *J. Neurosci. Methods* **199**(1), 26–34.
- Geruschat, D. R. & Deremeik, J. (2011), Activities of daily living and rehabilitation with prosthetic vision, in ‘Vis. Prosthetics’, Springer, pp. 413–424.
- Giraud, A. L., Price, C. J., Graham, J. M., Frackowiak, R. S. J. & Lise, A. (2001), ‘Functional plasticity of language-related brain areas after cochlear implantation’, pp. 1307–1316.
- Giraud, A. L. & Truy, E. (2002), ‘Imaging Plasticity in Cochlear Implant Patients’, pp. 381–393.

- Godement, P., Salaün, J. & Mason, C. A. (1990), ‘Retinal axon pathfinding in the optic chiasm: Divergence of crossed and uncrossed fibers’, *Neuron* **5**(2), 173–186.
- Grant, C. & Berson, E. L. (2001), ‘Treatable forms of retinitis pigmentosa associated with systemic neurological disorders’, *Int. Ophthalmol. Clin.* .
- Green, D. M. & Swets, J. A. (1966), *Signal Detection Theory & Psychophysics*, Robert E. Krieger Publishing Co, New York.
- Grunwald, J. E., Maguire, A. M. & Dupont, J. (1996), ‘Retinal hemodynamics in retinitis pigmentosa’, *Am. J. Ophthalmol.* **122**(4), 502–508.
- Guillery, R. W. (1982), The Optic Chiasm of the Vertebrate Brain, in W. D. B. T. C. t. S. P. Neff, ed., ‘Contributions to Sensory Physiology’, Vol. 7, Elsevier, pp. 39–73.
URL: <http://www.sciencedirect.com/science/article/pii/B9780121518073500082>
- Habib, A. G., Cameron, M. a., Suaning, G. J., Lovell, N. H. & Morley, J. W. (2013), ‘Spatially restricted electrical activation of retinal ganglion cells in the rabbit retina by hexapolar electrode return configuration.’, *J. Neural Eng.* **10**(3), 036013.
- 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. & Shivdasani, M. N. (2017), ‘Neural Responses to Multielectrode Stimulation of Healthy and Degenerate Retina’, *Investig. Ophthalmology Vis. Sci.* **58**(9), 3770.
- Halupka, K. J., Shivdasani, M., Cloherty, S. L., Grayden, D. B., Burkitt, A. N. & Meffin, H. (2014), ‘A linear-nonlinear model accurately predicts cortical responses to simultaneous electrical stimulation with a retinal implant’, *BMC Neurosci.* **15**(Suppl 1), P95.
- Halupka, K. J., Shivdasani, M. N., Cloherty, S. L., Grayden, D. B., Wong, Y. T., Burkitt, A. N. & Meffin, H. (2016), ‘Prediction of cortical responses to simultaneous electrical stimulation of the retina’, *J. Neural Eng.* **14**, 016006–016022.
- Hartong, D. T., Berson, E. L. & Dryja, T. P. (2006), ‘Retinitis pigmentosa’, *Lancet* **368**, 1795–1809.
- Hayes, J. S., Yin, V. T., Piyathaisere, D., Weiland, J. D., Humayun, M. S. & Dagnelie, G. (2003), ‘Visually Guided Performance of Simple Tasks Using Simulated Prosthetic Vision’, *Artif. Organs* **27**(11), 1016–1028.

- Heffer, L. F. & Fallon, J. B. (2008), ‘A novel stimulus artifact removal technique for high-rate electrical stimulation.’, *J. Neurosci. Methods* **170**(2), 277–84.
- Hoffmann, K. P., Stone, J. & Sherman, S. M. (1972), ‘Relay of receptive-field properties in dorsal lateral geniculate nucleus of the cat.’, *Journal of neurophysiology* **35**(4), 518–31.
URL: <http://www.ncbi.nlm.nih.gov/pubmed/4338566>
- Hornig, R., Dapper, M., Le Joliff, E., Hill, R., Ishaque, K., Posch, C., Benosman, R., LeMer, Y., Sahel, J.-A. & Picaud, S. (2017), Pixium Vision: First Clinical Results and Innovative Developments, *in* ‘Artif. Vis.’, Springer, pp. 99–113.
- Horsager, A., Boynton, G. M., Greenberg, R. J. & Fine, I. (2011), ‘Temporal interactions during paired-electrode stimulation in two retinal prosthesis subjects’, *Investig. Ophthalmol. Vis. Sci.* **52**(1), 549–557.
- Horsager, A., Greenberg, R. J. & Fine, I. (2010), ‘Spatiotemporal interactions in retinal prosthesis subjects.’, *Invest. Ophthalmol. Vis. Sci.* **51**(2), 1223–33.
- Horton, J. C. & Hoyt, W. F. (1991), ‘The representation of the visual field in human striate cortex. A revision of the classic Holmes map.’, *Arch. Ophthalmol.* **109**(6), 816–824.
- Humayun, M. S. (2001), ‘Intraocular retinal prosthesis.’, *Trans. Am. Ophthalmol. Soc.* **99**(October), 271–300.
- Humayun, M. S. (2017), Argus II Clinical Trial Update, Annual Meeting of the Association for Research in Vision and Ophthalmology.
- Humayun, M. S., Dorn, J. D., da Cruz, L., Dagnelie, G., Sahel, J.-A., Stanga, P. E., Cideciyan, A. V., Duncan, J. L., Elliott, D., Filley, E., Ho, A. C., Santos, A., Safran, A. B., Arditi, A., Del Priore, L. V. & Greenberg, R. J. (2012), ‘Interim results from the international trial of Second Sight’s visual prosthesis.’, *Ophthalmology* **119**(4), 779–88.
- Humayun, M. S., Juan, E. D., Dagnelie, G., Greenberg, R. J., Propst, R. H., Phillips, D. H., MS, H., E, d. J., Jr, E. D. J., RJ, G. & RH, P. (1996), ‘Visual Perception Elicited by Electrical Stimulation of Retina in Blind Humans’, *Arch Ophthalmol.* **114**(1), 40–46.
- Humayun, M. S., Prince, M., De Juan, E., Barron, Y., Moskowitz, M., Klock, I. B. & Milam, a. H. (1999), ‘Morphometric analysis of the extramacular retina

- from postmortem eyes with retinitis pigmentosa.’, *Invest. Ophthalmol. Vis. Sci.* **40**(1), 143–8.
- Humayun, M. S., Weiland, J. D., Fujii, G. Y., Greenberg, R., Williamson, R. P., Little, J., Mech, B., Cimmarrusti, V., Van Boemel, G., Dagnelie, G. & De Juan, E. (2003), ‘Visual perception in a blind subject with a chronic microelectronic retinal prosthesis’, *Vision Res.* **43**(24), 2573–2581.
- Illing, R. & Wässle, H. (1981), ‘The retinal projection to the thalamus in the cat: a quantitative investigation and a comparison with the retinotectal pathway’, *J. Comp. Neurol.* **202**(2), 265–285.
- Jager, R. D., Mieler, W. F. & Miller, J. W. (2008), ‘Age-Related Macular Degeneration’, *N. Engl. J. Med.* **358**(24), 2606–2617.
- Jensen, R. J., Iii, J. F. R., Ziv, O. R., Grumet, A., Wyatt, J. L. & Rizzo, J. F. (2003), ‘Thresholds for activation of rabbit retinal ganglion cells with an ultrafine, extracellular microelectrode’, *Investig. Ophthalmol. Vis. Sci.* **44**(8), 3533–3543.
- Jensen, R. J., Ziv, O. R. & Rizzo, J. F. (2005), ‘Responses of rabbit retinal ganglion cells to electrical stimulation with an epiretinal electrode.’, *J. Neural Eng.* **2**(1), S16–S21.
- Jepson, L. H., Hottowy, P., Mathieson, K., Gunning, D. E., Dąbrowski, W., Litke, A. M. & Chichilnisky, E. J. (2014), ‘Spatially patterned electrical stimulation to enhance resolution of retinal prostheses.’, *J. Neurosci.* **34**(14), 4871–81.
- John, S. S. E., Shivdasani, M. N. M., Leuenberger, J., Fallon, J. B. J., Shepherd, R. K. R. R. K., Millard, R. E. R., Rathbone, G. D. G. & Williams, C. E. (2011), ‘An automated system for rapid evaluation of high-density electrode arrays in neural prostheses.’, *J. Neural Eng.* **8**(3), 036011.
- Jones, B. W. & Marc, R. E. (2005), ‘Retinal remodeling during retinal degeneration.’, *Exp. Eye Res.* **81**(2), 123–37.
- Jones, B. W., Pfeiffer, R. L., Ferrell, W. D., Watt, C. B., Marmor, M. & Marc, R. E. (2016), ‘Retinal remodeling in human retinitis pigmentosa’, *Exp. Eye Res.* **150**, 149–165.
- Jones, B. W., Watt, C. B., Frederick, J. M., Baehr, W., Chen, C. K., Levine, E. M., Milam, A. H., Lavail, M. M. & Marc, R. E. (2003), ‘Retinal remodeling triggered by photoreceptor degenerations’, *J. Comp. Neurol.* **464**(1), 1–16.

- Julien, S., Peters, T., Ziemssen, F., Arango-Gonzalez, B., Beck, S., Thielecke, H., Büth, H., Van Vlierberghe, S., Sirova, M., Rossmann, P., Rihova, B., Schacht, E., Dubruel, P., Zrenner, E. & Schraermeyer, U. (2011), 'Implantation of ultra-thin, biofunctionalized polyimide membranes into the subretinal space of rats', *Biomaterials* **32**(16), 3890–3898.
- Kanda, H., Morimoto, T., Fujikado, T., Tano, Y., Fukuda, Y. & Sawai, H. (2004), 'Electrophysiological Studies of the Feasibility of Suprachoroidal-Transretinal Stimulation for Artificial Vision in Normal and RCS Rats', *Investigative Ophthalmology and Visual Science* **45**(2), 560–566.
- Kasi, H., Bertsch, A., Guyomard, J. L., Kolomiets, B., Picaud, S., Pelizzone, M. & Renaud, P. (2011), 'Simulations to study spatial extent of stimulation and effect of electrode-tissue gap in subretinal implants', *Med. Eng. Phys.* **33**(6), 755–763.
- Kathawate, J. & Acharya, S. (2008), 'Computational modeling of intravitreal drug delivery in the vitreous chamber with different vitreous substitutes', *Int. J. Heat Mass Transf.* **51**(23-24), 5598–5609.
- Khalili Moghaddam, G., Lovell, N. H., Wilke, R. G. H., Suaning, G. J. & Dokos, S. (2014), 'Performance optimization of current focusing and virtual electrode strategies in retinal implants.', *Comput. Methods Programs Biomed.* **117**(2), 334–42.
- Klauke, S., Goertz, M., Rein, S., Hoehl, D., Thomas, U., Eckhorn, R., Bremmer, F. & Wachtler, T. (2011), 'Stimulation with a wireless intraocular epiretinal implant elicits visual percepts in blind humans', *Investig. Ophthalmol. Vis. Sci.* **52**(1), 449–455.
- Koch, D. B., Downing, M., Osberger, M. J. & Litvak, L. (2007), 'Using current steering to increase spectral resolution in CII and HiRes 90K users.', *Ear Hear.* **28**(2 Suppl), 38S–41S.
- Kolb, H. (1974), 'The connections between horizontal cells and photoreceptors in the retina of the cat: Electron microscopy of Golgi preparations', *Journal of Comparative Neurology* **155**(1), 1–14.
URL: <https://doi.org/10.1002/cne.901550102>
- Kolb, H. (2003), 'How the Retina Works', *Am. Sci.* **91**(1), 28–35.
- Kondo, M., Sakai, T., Komeima, K., Kurimoto, Y., Ueno, S., Nishizawa, Y., Usukura, J., Fujikado, T., Tano, Y. & Terasaki, H. (2009), 'Generation of a

- transgenic rabbit model of retinal degeneration', *Investigative Ophthalmology and Visual Science* **50**(3), 1371–1377.
- Krause, F. & Schum, H. (1931), 'Die epileptischen Erkrankungen', *Neue Dtsch. Chir.* **49**, 482–486.
- Landsberger, D. M. & Srinivasan, A. G. (2009), 'Virtual channel discrimination is improved by current focusing in cochlear implant recipients', *Hear. Res.* **254**(1-2), 34–41.
- Leung, R. T., Shivdasani, M. N., Nayagam, D. A. X. & Shepherd, R. K. (2015), 'In vivo and in vitro comparison of the charge injection capacity of platinum macroelectrodes', *IEEE Trans. Biomed. Eng.* **62**(3), 849–857.
- Lewis, P. M., Ackland, H. M., Lowery, A. J. & Rosenfeld, J. V. (2015), 'Restoration of vision in blind individuals using bionic devices: A review with a focus on cortical visual prostheses', *Brain Res.* **1595**(2015), 51–73.
- Li, Z. Y., Wong, F., Chang, J. H., Possin, D. E., Hao, Y., Petters, R. M. & Milam, A. H. (1998), 'Rhodopsin transgenic pigs as a model for human retinitis pigmentosa', *Investig. Ophthalmol. Vis. Sci.* **39**(5), 808–819.
- Lilly, J. C., Hughes, J. R., Alvord, E. C. & Galkin, T. W. (1955), 'Brief, Noninjurious Electric Waveform for Stimulation of the Brain', *Science (80-.)*. **121**(3144), 468–469.
- Lovell, N. H., Dokos, S., Cheng, E. & Suaning, G. J. (2005), 'Simulation of parallel current injection for use in a vision prosthesis', *2nd Int. IEEE EMBS Conf. Neural Eng.* **2005**(APRIL), 458–461.
- Lowery, A. J., Rosenfeld, J. V., Lewis, P. M., Browne, D., Mohan, A., Brunton, E., Yan, E., Maller, J., Mann, C., Rajan, R., Rosa, M. & Pritchard, J. (2015), 'Restoration of vision using wireless cortical implants: The Monash Vision Group project', *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS 2015-Novem*, 1041–1044.
- Luo, X., Landsberger, D. M., Padilla, M. & Srinivasan, A. G. (2010), 'Encoding pitch contours using current steering.', *J. Acoust. Soc. Am.* **128**(3), 1215–23.
- MacNeil MA (1998), 'Extreme diversity among amacrine cells: implications ... [Neuron. 1998] - PubMed - NCBI', **20**, 971–982.
URL: [http://www.ncbi.nlm.nih.gov/pubmed?term=Extreme diversity among amacrine cells%3A implications for function%5C](http://www.ncbi.nlm.nih.gov/pubmed?term=Extreme+diversity+among+amacrine+cells%3A+implications+for+function%5C)

- Majji, A. B., Humayun, M. S., Weiland, J. D., Suzuki, S., D’Anna, S. A., De Juan, E. & Anna, S. a. D. (2015), ‘Long-Term Histological and Electrophysiological Results of an Inactive Epiretinal Electrode Array Implantation in Dogs’, *Invest. Ophthalmol.* **40**(9), 2073–2081.
- Marc, R. E., Jones, B. W., Watt, C. B. & Strettoi, E. (2003), ‘Neural remodeling in retinal degeneration’, *Prog. Retin. Eye Res.* **22**(5), 607–655.
- Marc, R. E., Jones, B. W., Watt, C. B., Vazquez-Chona, F., Vaughan, D. K. & Organisciak, D. T. (2008), ‘Extreme retinal remodeling triggered by light damage: implications for age related macular degeneration.’, *Mol. Vis.* **14**(February 2016), 782–806.
- Marozeau, J., McDermott, H. J., Swanson, B. a. & McKay, C. M. (2015), ‘Perceptual Interactions Between Electrodes Using Focused and Monopolar Cochlear Stimulation’, *J. Assoc. Res. Otolaryngol.* .
- Martens, H. C. F., Toader, E., Decré, M. M. J., Anderson, D. J., Vetter, R., Kipke, D. R., Baker, K. B., Johnson, M. D. & Vitek, J. L. (2011), ‘Spatial steering of deep brain stimulation volumes using a novel lead design’, *Clin. Neurophysiol.* **122**(3), 558–566.
- Matteucci, P. B., Barriga-Rivera, A., Eiber, C. D., Lovell, N. H., Morley, J. W. & Suaning, G. J. (2016), ‘The Effect of Electric Cross-Talk in Retinal Neurostimulation’, *Investig. Ophthalmology Vis. Sci.* **57**(3), 1031.
- Matteucci, P. B., Chen, S. C., Tsai, D., Dodds, C. W. D., Dokos, S., Morley, J. W., Lovell, N. H. & Suaning, G. J. (2013), ‘Current steering in retinal stimulation via a quasimonopolar stimulation paradigm.’, *Invest. Ophthalmol. Vis. Sci.* **54**(6), 4307–20.
- Maturana, M. I., Apollo, N. V., Hadjinicolaou, A. E., Garrett, D. J., Cloherty, S. L., Kameneva, T., Grayden, D. B., Ibbotson, M. R. & Meffin, H. (2016), ‘A Simple and Accurate Model to Predict Responses to Multi-electrode Stimulation in the Retina’, *PLoS Comput. Biol.* **12**(4), 1–27.
- Michelson, R. P. (1986), ‘METHOD AND APPARATUS FOR VISUAL PROSTHE-SIS’.
- Missel, P. J. (2002), ‘Hydraulic flow and vascular clearance influences on intravitreal drug delivery’, *Pharm. Res.* **19**(11), 1636–1647.

- Mitchell, C. H. & Reigada, D. (2008), ‘Purinergic signalling in the subretinal space: A role in the communication between the retina and the RPE’, *Purinergic Signal.* **4**(2), 101–107.
- Montezuma, S. R., Loewenstein, J., Scholz, C. & Rizzo, J. F. (2006), ‘Biocompatibility of materials implanted into the subretinal space of Yucatan pigs’, *Investig. Ophthalmol. Vis. Sci.* **47**(8), 3514–3522.
- Musarella, M. A. & Macdonald, I. M. (2011), ‘Current concepts in the treatment of retinitis pigmentosa.’, *J. Ophthalmol.* **2011**, 753547.
- Mut, F., Lago, G., Silveira, A., Bellis, C. D. E., Velluti, R. & Pedemonte, M. (1999), ‘Changes in the Cerebral Blood Flow in Postlingual Cochlear Implant Users’, pp. 239–243.
- Nakajima, M., Yuge, K., Senzaki, H., Shikata, N., Miki, H., Uyama, M. & Tsubura, a. (1996), ‘Photoreceptor apoptosis induced by a single systemic administration of N-methyl-N-nitrosourea in the rat retina.’, *Am. J. Pathol.* **148**(2), 631–641.
- Nanduri, D., Fine, I., Horsager, A., Boynton, G. M., Humayun, M. S., Greenberg, R. J. & Weiland, J. D. (2012), ‘Frequency and amplitude modulation have different effects on the percepts elicited by retinal stimulation’, *Investig. Ophthalmol. Vis. Sci.* **53**(1), 205–214.
- Nanduri, D., Humayun, M., Greenberg, R., McMahon, M. & Weiland, J. (2008), ‘Retinal prosthesis phosphene shape analysis’, *2008 30th Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.* pp. 1785–1788.
- Naumann, J. (2012), *Search for Paradise: A Patient’s Account of the Artificial Vision Experiment*, Xlibris corporation.
- Nayagam, D. A. X., McGowan, C., Villalobos, J., Williams, R. A., Salinas-LaRosa, C., McKelvie, P., Lo, I., Basa, M., Tan, J. & Illiams, C. E. (2013), ‘Techniques for Processing Eyes Implanted With a Retinal Prosthesis for Localized Histopathological Analysis’, *JoVE* (78), e50411.
- Nayagam, D. a. X., Williams, R. A., Allen, P. J., Shivdasani, M. N., Luu, C. D., Salinas-LaRosa, C. M., Finch, S., Ayton, L. N., Saunders, A. L., McPhedran, M., McGowan, C., Villalobos, J., Fallon, J. B., Wise, A. K., Yeoh, J., Xu, J., Feng, H., Millard, R., McWade, M., Thien, P. C., Williams, C. E. & Shepherd, R. K. (2014), ‘Chronic electrical stimulation with a suprachoroidal retinal prosthesis: a preclinical safety and efficacy study.’, *PLoS One* **9**(5), e97182.

- Newbold, C., Richardson, R., Huang, C. Q., Milojevic, D., Cowan, R. & Shepherd, R. (2004), ‘An in vitro model for investigating impedance changes with cell growth and electrical stimulation: Implications for cochlear implants’, *J. Neural Eng.* **1**(4), 218–227.
- Nirenberg, S. & Pandarinath, C. (2012), ‘Retinal prosthetic strategy with the capacity to restore normal vision.’, *Proc. Natl. Acad. Sci. U. S. A.* **109**(37), 15012–7.
- Palanker, D., Vankov, A., Huie, P. & Baccus, S. (2005), ‘Design of a high-resolution optoelectronic retinal prosthesis.’, *J. Neural Eng.* **2**(1), S105–20.
- Pardue, M. T., Stubbs, E. B., Perlman, J. I., Narfström, K., Chow, a. Y. & Peachey, N. S. (2001), ‘Immunohistochemical studies of the retina following long-term implantation with subretinal microphotodiode arrays.’, *Exp. Eye Res.* **73**(3), 333–343.
- Payne, B. R. (1994), ‘Neuronal interactions in cat visual cortex mediated by the corpus callosum’, *Behav. Brain Res.* **64**(1-2), 55–64.
- Pezaris, J. S. & Reid, R. C. (2007), ‘Demonstration of artificial visual percepts generated through thalamic microstimulation’, *Proceedings of the National Academy of Sciences* **104**(18), 7670–7675.
- Pfingst, B. E., Zwolan, T. A. & Holloway, L. A. (1997), ‘Effects of stimulus configuration on psychophysical operating levels and on speech recognition with cochlear implants’, *Hear. Res.* **112**(1-2), 247–260.
- Pollen, D. A. (1975), ‘Some perceptual effects of electrical stimulation of the visual cortex in man’, *Nerv. Syst.* **2**, 519–528.
- Portera-Cailliau, C., Sung, C. H., Nathans, J. & Adler, R. (1994), ‘Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa.’, *Proc. Natl. Acad. Sci. U. S. A.* **91**(3), 974–978.
- Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W. C., LaMantia, A.-S., McNamara, J. O. & White, L. E. (2004), Vision: The Eye, *in* ‘Neuroscience’, 3rd edn, Sinauer Associates, Sunderland.
- Puthussery, T., Yee, P., Vingrys, A. J. & Fletcher, E. L. (2006), ‘Evidence for the involvement of purinergic P2X7 receptors in outer retinal processing’, *Eur. J. Neurosci.* **24**(1), 7–19.

- Rizzo, J. F., Wyatt, J. L., Loewenstein, J., Kelly, S. & Shire, D. (2003), ‘Perceptual Efficacy of Electrical Stimulation of Human Retina with a Microelectrode Array during Short-Term Surgical Trials’, *Investig. Ophthalmol. Vis. Sci.* **44**(12), 5362–5369.
- Rizzo, S., Belting, C., Cinelli, L., Allegrini, L., Genovesi-Ebert, F., Barca, F. & Di Bartolo, E. (2014), ‘The Argus II retinal prosthesis: 12-month outcomes from a single-study center’, *Am. J. Ophthalmol.* **157**(6), 1282–1290.
- Roessler, G., Laube, T., Brockmann, C., Kirschkamp, T., Mazinani, B., Goertz, M., Koch, C., Krisch, I., Sellhaus, B., Trieu, H. K., Weis, J., Bornfeld, N., R  thgen, H., Messner, A., Mokwa, W. & Walter, P. (2009), ‘Implantation and explantation of a wireless epiretinal retina implant device: Observations during the EPIRET3 prospective clinical trial’, *Investig. Ophthalmol. Vis. Sci.* **50**(6), 3003–3008.
- Sakaguchi, H., Fujikado, T., Fang, X., Kanda, H., Osanai, M., Nakauchi, K., Ikuno, Y., Kamei, M., Yagi, T., Nishimura, S., Ohji, M., Yagi, T. & Tano, Y. (2004), ‘Transretinal electrical stimulation with a suprachoroidal multichannel electrode in rabbit eyes’, *Jpn. J. Ophthalmol.* **48**(3), 256–261.
- Santos, A., Humayun, M. S., Juan, E. D., Marsh, M. J., Klock, I. B. & Milam, A. H. (1997), ‘Preservation of the Inner Retina in Retinitis Pigmentosa’, *Arch Ophthalmol.* **115**, 511–515.
- Saoji, A. a. & Litvak, L. M. (2010), ‘Use of ”phantom electrode” technique to extend the range of pitches available through a cochlear implant.’, *Ear Hear.* **31**(5), 693–701.
- Saunders, A. L., Williams, C. E., Heriot, W., Briggs, R. J., Yeoh, J., Nayagam, D. A., Mccombe, M., Villalobos, J., Burns, O., Luu, C. D., Ayton, L. N., Mcphedran, M., Opie, N. L., McGowan, C., Shepherd, R. K., Guymer, R. & Allen, P. J. (2014), ‘Development of a surgical procedure for implantation of a prototype suprachoroidal retinal prosthesis’, *Clin. Exp. Ophthalmol.* **42**(7), 665–674.
- Savage, C. O., Grayden, D. B., Meffin, H. & Burkitt, A. N. (2013), ‘Optimized single pulse stimulation strategy for retinal implants.’, *J. Neural Eng.* **10**(1), 016003.
- Schiefer, M. A. & Grill, W. M. (2006), ‘Sites of neuronal excitation by epiretinal electrical stimulation’, *IEEE Trans. Neural Syst. Rehabil. Eng.* **14**(1), 5–13.
- Schnapf, J. L., Kraft, T. W. & Baylor, D. A. (1987), ‘Spectral sensitivity of human cone photoreceptors’, *Nature* **325**(6103), 439.

- Schwartz, E. (1977), ‘Spatial mapping in the primate sensory projection: analytic structure and relevance to perception’, *Biol. Cybern.* .
- Senn, P. (2014), ‘Peripheral nerve stimulation for the treatment of chronic neuropathic pain’.
- Senn, P., Shepherd, R. K. & Fallon, J. B. (2018), ‘Focused electrical stimulation using a single current source’, *J. Neural Eng.* **in press**.
- Sharma, A., Dorman, C. A. M. F. & Spahr, A. J. (2002), ‘Rapid development of cortical auditory evoked potentials after early cochlear implantation’, **13**(10), 17–20.
- Shepherd, R. K. & Clark, G. M. (1990), ‘Scanning electron microscopy of platinum scala tympani electrodes following chronic stimulation in patients’, *Biometaterials* **12**(1991), 417–423.
- Shepherd, R. K. & Hardie, N. A. (2001), ‘Deafness-Induced Changes in the Auditory Pathway : Implications for Cochlear Implants’, *Audiol. Neurotol.* **6**(6), 305–318.
- Shepherd, R. K., Linahan, N., Xu, J., Clark, G. M. & Araki, S. (1999), ‘Chronic electrical stimulation of the auditory nerve using non-charge-balanced stimuli.’, *Acta Otolaryngol.* **119**(6), 674–684.
- Shepherd, R. K., Matsushima, J., Millard, R. E. & Clark, G. M. (1991), ‘Cochlear pathology following chronic electrical stimulation using non charge balanced stimuli.’, *Acta Otolaryngol.* **111**(5), 848–860.
- Shepherd, R. K., Shivdasani, M. N., Nayagam, D. a. X., Williams, C. E. & Blamey, P. J. (2013), ‘Visual prostheses for the blind.’, *Trends Biotechnol.* **31**(10), 562–71.
- Shepherd, R. K., Wise, A. K., Enke, Y. L., Carter, P. M. & Fallon, J. B. (2017), ‘Evaluation of focused multipolar stimulation for cochlear implants: A preclinical safety study’, *Journal of Neural Engineering* **14**(4).
- Sherman, S. M. & Koch, C. (1986), ‘The control of retinogeniculate transmission in the mammalian lateral geniculate nucleus’, *Experimental Brain Research* **63**(1), 1–20.
- Shivdasani, M. N., Fallon, J. B., Luu, C. D., Cicione, R., Allen, P. J., Morley, J. W. & Williams, C. E. (2012), ‘Visual cortex responses to single- and simultaneous multiple-electrode stimulation of the retina: implications for retinal prostheses.’, *Invest. Ophthalmol. Vis. Sci.* **53**(10), 6291–300.

- Shivdasani, M. N., Luu, C. D., Cicione, R., Fallon, J. B., Allen, P. J., Leuenberger, J., Suaning, G. J., Lovell, N. H., Shepherd, R. K. & Williams, C. E. (2010), 'Evaluation of stimulus parameters and electrode geometry for an effective suprachoroidal retinal prosthesis.', *J. Neural Eng.* **7**(3), 036008.
- Shivdasani, M. N., Sinclair, N. C., Dimitrov, P. N., Varsamidis, M., Ayton, L. N., Luu, C. D., Perera, T., McDermott, H. J. & Blamey, P. J. (2014), 'Factors Affecting Perceptual Thresholds in a Suprachoroidal Retinal Prosthesis', *Invest. Ophthalmol. Vis. Sci.* **55**, 6467–6481.
- Sinclair, N. C., Shivdasani, M. N., Perera, T., Gillespie, L. N., McDermott, H. J., Ayton, L. N. & Blamey, P. J. (2016), 'The appearance of phosphenes elicited using a suprachoroidal retinal prosthesis', *Investig. Ophthalmol. Vis. Sci.* **57**(11), 4948–4961.
- Smalley, E. (2017), 'First AAV gene therapy poised for landmark approval', *Nat. Biotechnol.* **35**, 998.
- Smith, Z. M., Parkinson, W. S. & Long, C. J. (2013), 'Multipolar current focusing increases spectral resolution in cochlear implants', *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS* pp. 2796–2799.
- Spencer, T. C., Fallon, J. B. & Shivdasani, M. N. (2018), 'Creating virtual electrodes with 2D current steering', *J. Neural Eng.* **15**(3), 35002.
- Spencer, T. C., Fallon, J. B., Thien, P. C. & Shivdasani, M. N. (2016), 'Spatial restriction of neural activation using focused multipolar stimulation with a retinal prosthesis', *Investig. Ophthalmology Vis. Sci.* **57**(7), 3181.
- Stickney, G. S., Loizou, P. C., Mishra, L. N., Assmann, P. F., Shannon, R. V. & Opie, J. M. (2006), 'Effects of electrode design and configuration on channel interactions', *Hear. Res.* **211**(1-2), 33–45.
- Stingl, K., Bartz-Schmidt, K. U., Besch, D., Chee, C. K., Cotttriall, C. L., Gekeler, F., Groppe, M., Jackson, T. L., MacLaren, R. E., Koitschev, A., Kusnyerik, A., Neffendorf, J., Nemeth, J., Naeem, M. A. N., Peters, T., Ramsden, J. D., Sachs, H., Simpson, A., Singh, M. S., Wilhelm, B., Wong, D. & Zrenner, E. (2015), 'Subretinal Visual Implant Alpha IMS - Clinical trial interim report', *Vision Res.* **111**, 149–160.
- Stingl, K. K. T., Bartz-Schmidt, K. U., Besch, D., Braun, A., Bruckmann, A., Gekeler, F., Greppmaier, U., Hipp, S., Hörtdörfer, G., Kernstock, C., Koitschev,

- A., Kusnyerik, A., Sachs, H., Schatz, A., Stingl, K. K. T., Peters, T., Wilhelm, B. & Zrenner, E. (2013), ‘Artificial vision with wirelessly powered subretinal electronic implant alpha-IMS.’, *Proc. Biol. Sci.* **280**(1757), 20130077.
- Stosiek, C., Garaschuk, O., Holthoff, K. & Konnerth, A. (2003), ‘In vivo two-photon calcium imaging of neuronal networks.’, *Proc. Natl. Acad. Sci. U. S. A.* **100**(12), 7319–24.
- Stronks, H. C. & Dagnelie, G. (2014), ‘The functional performance of the Argus II retinal prosthesis’, *Expert Rev. Med. Devices* **11**(1), 23–30.
- Talalla, A., Bullara, L. & Pudenz, R. (1974), ‘Electrical stimulation of the human visual cortex; preliminary report.’, *Can. J. Neurol. Sci.* **1**(4), 236–238.
- Tassicker, G. E. (1956), ‘Retinal stimulator’.
- Tassinari, G., Bentivoglio, M., Chen, S. & Campara, D. (1997), ‘Overlapping Ipsilateral and Contralateral Retinal Projections to the Lateral Geniculate-Nucleus and Superior Colliculus in the Cat - A Retrograde Triple Labeling Study’, *Brain Res. Bull.* **43**(2), 127–139.
- Thompson, R. W., Barnett, G. D., Humayun, M. S. & Dagnelie, G. (2003), ‘Facial Recognition Using Simulated Prosthetic Pixelized Vision’, *Investig. Ophthalmol. Vis. Sci.* **44**(11), 5035–5042.
- Tootell, R. B. H., Born, R. T. & Ash-Bernal, R. (1993), Columnar Organization in Visual Cortex in Non-Human Primates and Man, *in* B. GULYAS, D. OTTOSON & P. E. R. E. B. T. F. O. o. t. H. V. C. ROLAND, eds, ‘Wenner–Gren International Series’, Vol. 61, Pergamon, pp. 59–73.
URL: <http://www.sciencedirect.com/science/article/pii/B9780080420042500095>
- Tusa, R. (1978), ‘The retinotopic organization of area 17 (striate cortex) in the cat’, *J. Comp. Neurol.* **177**, 213–235.
- Tusa, R. J., Palmer, L. A., Rosenquist, A. C. & Palmer, L. A. (1979), ‘Retinotopic organization of area 18 and 19 in the cat’, *J. Comp. Neurol.* **185**, 213–236.
- Valente, V., Demosthenous, A. & Bayford, R. (2012), ‘A tripolar current-steering stimulator ASIC for field shaping in deep brain stimulation’, *IEEE Trans. Biomed. Circuits Syst.* **6**(3), 197–207.
- van den Honert, C., Carlyon, R. P., Long, C. J., Smith, Z. M., Shelton, C. & Kelsall, D. C. (2007), Perceptual evidence of spatially-restricted excitation with focused

- stimulation, *in* J. Middlebrooks & Q. Summerfield, eds, ‘CIAP Conf. Implant. Audit. prostheses, Granlibakken, CA’, University of Southern California, p. 58.
- van den Honert, C. & Kelsall, D. C. (2007), ‘Focused intracochlear electric stimulation with phased array channels.’, *J. Acoust. Soc. Am.* **121**(6), 3703–16.
- Veraart, C., Raftopoulos, C., Mortimer, J. T., Delbeke, J., Pins, D., Michaux, G., Vanlierde, A., Parrini, S. & Wanet-Defalque, M. C. (1998), ‘Visual sensations produced by optic nerve stimulation using an implanted self-sizing spiral cuff electrode’, *Brain Research* **813**(1), 181–186.
- Vessey, K. A., Greferath, U., Aplin, F. P., Jobling, A. I., Phipps, J. A., Ho, T., De Iongh, R. U. & Fletcher, E. L. (2014), ‘Adenosine triphosphate-induced photoreceptor death and retinal remodeling in rats’, *J. Comp. Neurol.* **522**(13), 2928–2950.
- Villalobos, J., Allen, P. J., McCombe, M. F., Ulaganathan, M., Zamir, E., Ng, D. C., Shepherd, R. K. & Williams, C. E. (2012), ‘Development of a surgical approach for a wide-view suprachoroidal retinal prosthesis: evaluation of implantation trauma.’, *Graefes Arch. Clin. Exp. Ophthalmol.* **250**(3), 399–407.
- Villalobos, J., Nayagam, D. a. X., Allen, P. J., McKelvie, P., Luu, C. D., Ayton, L. N., Freemantle, A. L., McPhedran, M., Basa, M., McGowan, C. C., Shepherd, R. K. & Williams, C. E. (2013), ‘A wide-field suprachoroidal retinal prosthesis is stable and well tolerated following chronic implantation.’, *Invest. Ophthalmol. Vis. Sci.* **54**(5), 3751–62.
- Wald, G. (1964), ‘The Receptors of Human Color Vision’, *Science* **145**(3636), 1007–1016.
URL: <http://www.jstor.org/stable/1714070>
- Wang, L., Mathieson, K., Kamins, T. I., Loudin, J. D., Galambos, L., Goetz, G., Sher, A., Mandel, Y., Huie, P., Lavinsky, D., Harris, J. S. & Palanker, D. V. (2012), ‘Photovoltaic retinal prosthesis: Implant fabrication and performance’, *J. Neural Eng.* **9**(4).
- Warren, D. J., Fernandez, E. & Normann, R. a. (2001), ‘High-resolution two-dimensional spatial mapping of Cat striate cortex using A100-Microelectrode array’, *Neuroscience* **105**(1), 19–31.
- Wässle, H. (2004), ‘Parallel processing in the mammalian retina.’, *Nat. Rev. Neurosci.* **5**(10), 747–757.

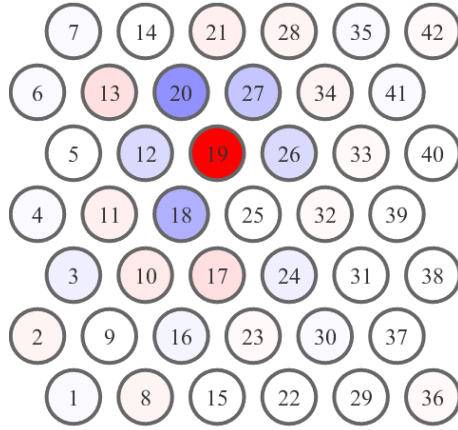
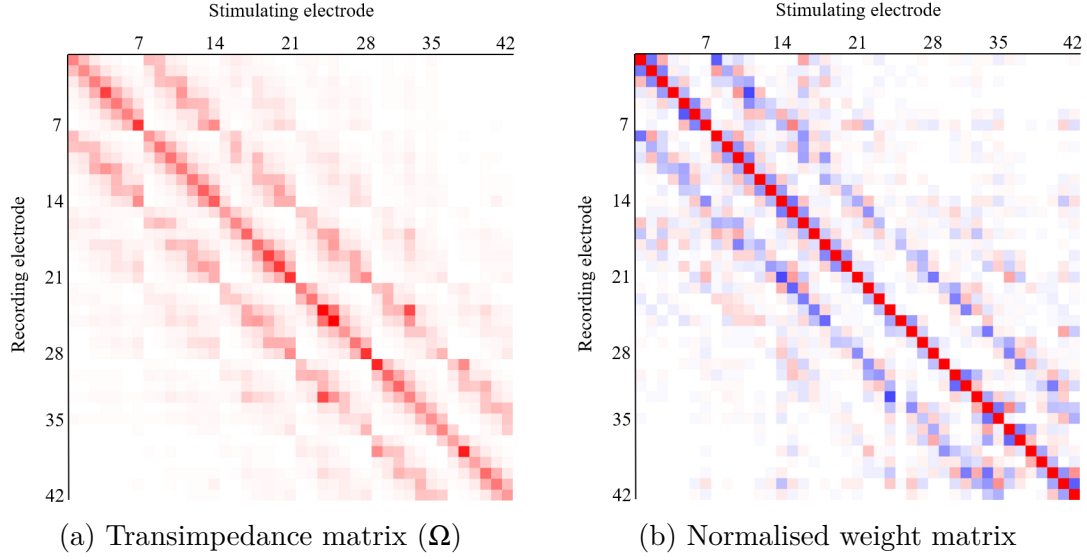
- Wässle, H., Boycott, B. B. & Röhrenbeck, J. (1989), ‘Horizontal Cells in the Monkey Retina: Cone connections and dendritic network’, *European Journal of Neuroscience* **1**(5), 421–435.
- Wässle, H. & Illing, R. (1980), ‘The retinal projection to the superior colliculus in the cat: a quantitative study with HRP’, *J. Comp. Neurol.* **190**(2), 333–356.
- Weiland, J. D., Liu, W. & Humayun, M. S. (2005), ‘Retinal prosthesis.’, *Annu. Rev. Biomed. Eng.* **7**, 361–401.
- Weitz, A. C., Behrend, M. R., Lee, N. S., Klein, R. L., Chiodo, V. A., Hauswirth, W. W., Humayun, M. S., Weiland, J. D. & Chow, R. H. (2013), ‘Imaging the response of the retina to electrical stimulation with genetically encoded calcium indicators’, *J. Neurophysiol.* **109**(7), 1979–1988.
- West, B. T., Welch, K. B. & Galecki, A. T. (2014), *Linear mixed models: a practical guide using statistical software*, Chapman and Hall/CRC.
- Wilke, R. G. H., Gabel, V. P., Sachs, H., Schmidt, K. U. B., Gekeler, F., Besch, D., Szurman, P., Stett, A., Wilhelm, B., Peters, T., Harscher, A., Greppmaier, U., Kibbel, S., Benav, H., Bruckmann, A., Stingl, K., Kusnyerik, A. & Zrenner, E. (2011a), ‘Spatial resolution and perception of patterns mediated by a subretinal 16-electrode array in patients blinded by hereditary retinal dystrophies’, *Investig. Ophthalmol. Vis. Sci.* **52**(8), 5995–6003.
- Wilke, R. G. H., Greppmaier, U., Stingl, K. & Zrenner, E. (2011c), ‘Fading Of Perception In Retinal Implants Is A Function Of Time And Space Between Sites Of Stimulation’, *Invest. Ophthalmol. Vis. Sci.* **52**(14), 458.
- Wilke, R. G. H., Moghadam, G. K., Lovell, N. H., Suaning, G. J. & Dokos, S. (2011b), ‘Electric crosstalk impairs spatial resolution of multi-electrode arrays in retinal implants.’, *J. Neural Eng.* **8**(4), 046016.
- Wong, Y. T., Chen, S. C., Kerdraon, Y. A., Allen, P. J., McCombe, M. F., Morley, J. W., Lovell, N. H. & Suaning, G. J. (2008), ‘Efficacy of supra-choroidal, bipolar, electrical stimulation in a vision prosthesis.’, *Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.* **2008**, 1789–92.
- Wong, Y. T., Chen, S. C., Seo, J. M., Morley, J. W., Lovell, N. H. & Suaning, G. J. (2009), ‘Focal activation of the feline retina via a suprachoroidal electrode array’, *Vision Res.* **49**(8), 825–833.

- Wu, C.-C. & Luo, X. (2013), ‘Current steering with partial tripolar stimulation mode in cochlear implants.’, *J. Assoc. Res. Otolaryngol.* **14**(2), 213–31.
- Xu, J., Heys, J. J., Barocas, V. H. & Randolph, T. W. (2000), ‘Permeability and diffusion in vitreous humor: Implications for drug delivery’, *Pharm. Res.* **17**(6), 664–669.
- Yamauchi, Y., Franco, L. M., Jackson, D. J., Naber, J. F., Ziv, R. O., Iii, J. F. R., Kaplan, H. J., Enzmann, V., Rizzo, J. F., Kaplan, H. J., Enzmann, V., Iii, J. F. R., Kaplan, H. J. & Enzmann, V. (2005), ‘Comparison of electrically evoked cortical potential thresholds generated with subretinal or suprachoroidal placement of a microelectrode array in the rabbit.’, *J. Neural Eng.* **2**(1), S48–S56.
- Yoshizawa, K., Yang, J., Senzaki, H., Uemura, Y., Kiyozuka, Y., Shikata, N., Oishi, Y., Miki, H. & Tsubura, A. (2000), ‘Caspase-3 inhibitor rescues N-methyl-N-nitrosourea-induced retinal degeneration in Sprague-Dawley rats’, *Exp. Eye Res.* **71**(6), 629–635.
- Zeki, S. M. (1978), ‘Functional specialisation in the visual cortex of the rhesus monkey’, *Nature* **274**, 423–428.
- Zrenner, E. (2002), ‘Will retinal implants restore vision?’, *Science* (80-.). **295**(5557), 1022–1025.
- Zrenner, E., Bartz-Schmidt, K. U., Benav, H., Besch, D., Bruckmann, A., Gabel, V.-P., Gekeler, F., Greppmaier, U., Harscher, A., Kibbel, S., Koch, J., Kusnyerik, A., Peters, T., Stingl, K., Sachs, H., Stett, A., Szurman, P., Wilhelm, B., Wilke, R. G. H. & Ulrich, K. (2011), ‘Subretinal electronic chips allow blind patients to read letters and combine them to words.’, *Proc. Biol. Sci.* **278**(1711), 1489–97.
- Zrenner, E., Benav, H., Bruckmann, A., Greppmaier, U., Kusnyerik, A., Stett, A., Stingl, K. & Wilke, R. G. H. (2010), ‘Electronic implants provide continuous stable percepts in blind volunteers only if the image receiver is directly linked to eye movement’, *Doc. Ophthalmol.* **121**(1), 18–19.

Appendices

Appendix A

Transimpedance measurements

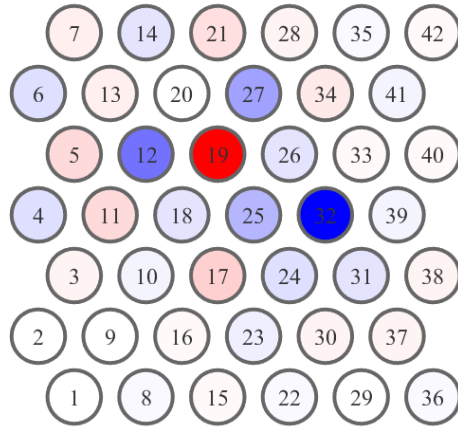
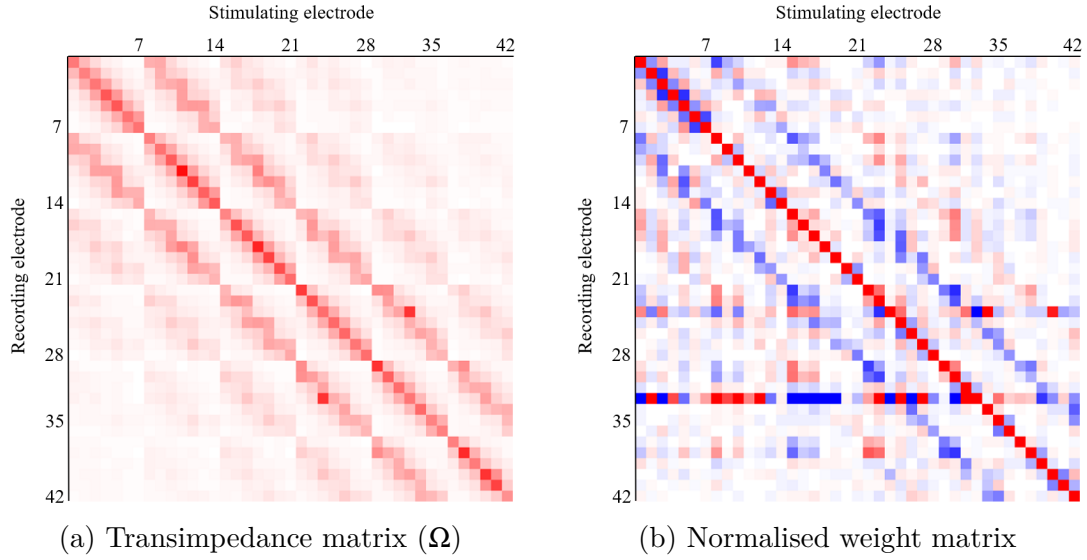


(c) Example weights for electrode 19



(d) Normalised units

Figure A.1: Example of when a transimpedance matrix produced expected FMP weightings without any modification. The weights shown in (c) where electrode 19 is the primary stimulating electrode (PSE) are taken from column 19 of the normalised weight matrix in (b).



(c) Example weights for electrode 19



(d) Normalised units

Figure A.2: Example of when a transimpedance matrix produced unusable FMP weightings. Note that most of the outlying weightings are exhibited on electrodes 24 and 32.

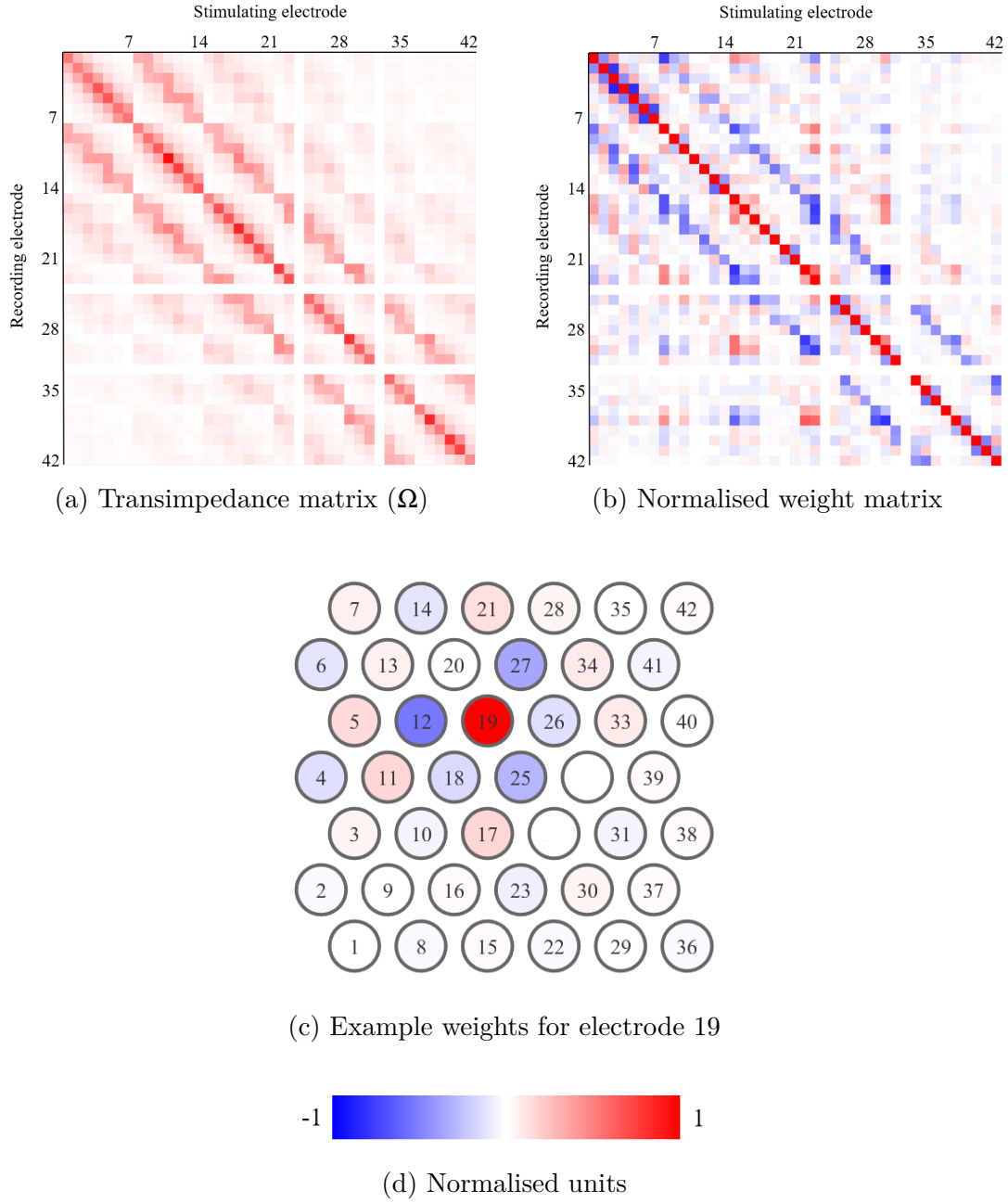
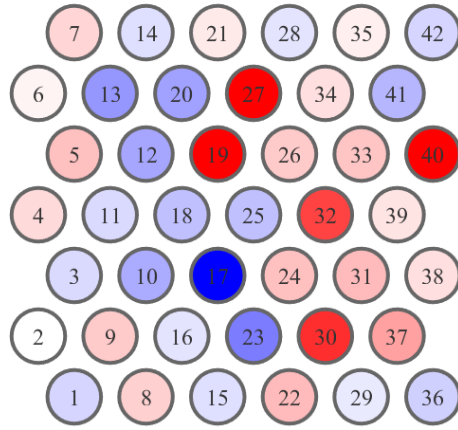
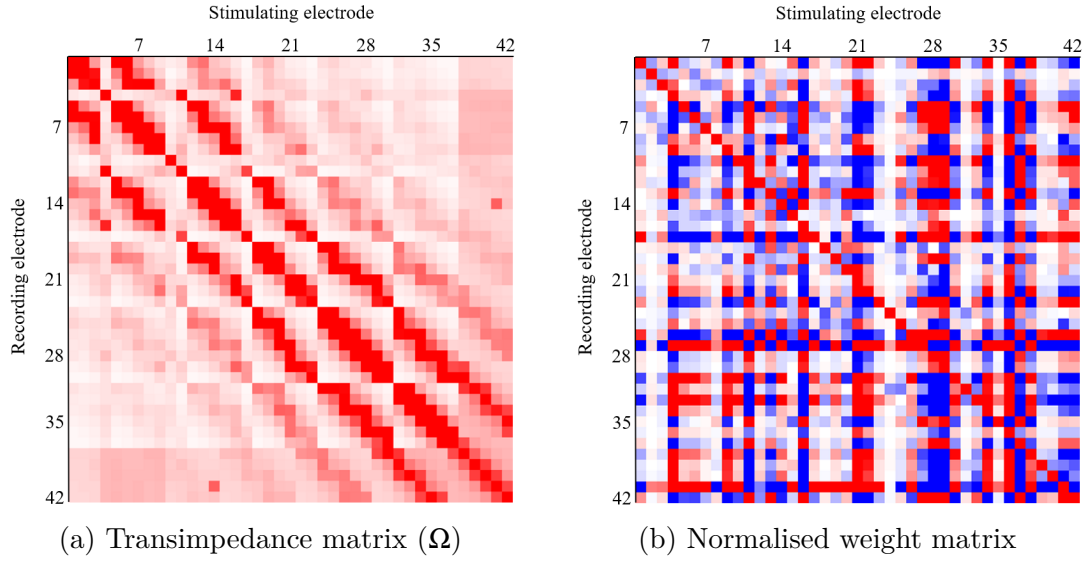


Figure A.3: Example of how removing electrodes from a transimpedance matrix can result in the calculation of more useable FMP weights. Electrodes 24 and 32 were removed from the transimpedance array shown in Figure A.2 prior to inversion, resulting in more expected FMP weights. Note that electrodes 24 and 32 are close to each other on the array, suggesting that the cause of high transimpedance values may have been similar for both electrodes. The downside of this modification is that these electrodes cannot be stimulated as part of FMP array.



(c) Example weights for electrode 19



(d) Normalised units

Figure A.4: Example of when a transimpedance matrix produced unusable FMP weightings. This example was not fixable by modifying the transimpedance matrix due to the high number of affected electrodes. In this case, it appears that outlying transimpedance values were observed for the entire fourth row and last two columns of the array. It is unclear what caused this issue.