Chapter 2

Multi-Electrode Stimulation and Recording In the Isolated Retina

2.1 Introduction

This chapter describes the methods used to stimulate and record from neurons in isolated retinas using a planar, photo-lithographically patterned multi-electrode array. The methods are adapted primarily from two sources: Ames' *in vitro* rabbit retina preparation (Ames III and Nesbett, 1981), and the techniques of Meister, Pine, and Baylor for multi-electrode ganglion cell recording (Meister et al., 1994). These methods were used to conduct studies of ganglion cell axon (and possibly dendrite) excitation thresholds as a function of monopolar electrode position and bipolar electrode orientation, the results of which are described in Chapter 3.

The experimental methods were developed specifically for this thesis, in an effort to improve upon techniques used by a colleague, Ralph Jensen, for similar work (Jensen et al., 1996; Rizzo et al., 1997). Jensen measured thresholds for eliciting single ganglion cell spikes with a sharp-ended stimulating electrode placed at various points on and above the retinal surface, to provide a quantitative description of the spatial extent of electric stimulation. This technique offered a fairly limited choice of stimulating electrode geometries, typically a central point or disk, optionally surrounded by a concentric ring. Furthermore, it was necessary to raise and re-lower the stimulating electrode when changing the position of on-surface threshold measurements to reduce the likelihood of dragging the retina. Dragging was undesirable because it could introduce electrode placement imprecision or "loss" of a neuron which had been inadvertently moved away from the recording electrode. The possibility of dragging could not be completely eliminated, however, since the stimulating electrode had to be moved between threshold measurements.

Mechanical disruption of the retina may be avoided by using a multi-electrode array instead of a single electrode (Greenberg, 1998c; Kuras and Gutmanienė, 1997). Stimulus positions and geometries are controlled by the choice of stimulator con-

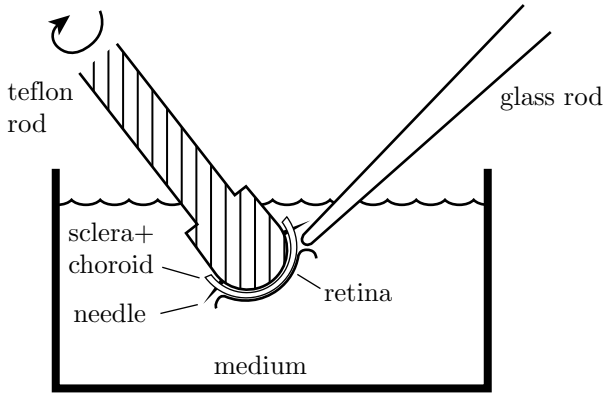

Figure 2.1: Method for isolating retina from the pigment epithelium.

nections to the array, allowing for rapid switching among a large number of configurations. Photolithographic techniques, which have been used in electrophysiology research since the 1970's (Pickard, 1979), make it possible to pattern these arrays with essentially arbitrary electrode geometries and distributions.

2.2 *In vitro* preparation

Retinas were prepared for study as follows. Female Dutch Belted rabbits, weighing 2-2.5kg, were sedated by intramuscular injection of ketamine (35mg/kg) and xylazine (5mg/kg). The rabbits were then sacrificed by an intravenous overdose of sodium pentobarbitol. Immediately following death, one of the eyes was removed and a small cut made near its equator using a sharp blade. The cut was advanced completely around the eyeball using miniature surgical scissors. The front portion of the eye and the vitreous were then gently pulled away with forceps as Ames' medium (Sigma Chemical Co., St. Louis, MO, USA) was poured into the eyecup. The Ames' medium was buffered with sodium bicarbonate (per manufacturer's instructions) and equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide, bringing the solution pH to 7.3-7.4. The interval between death and introduction of medium was five to seven minutes.

To facilitate separation of the retina from the pigment epithelium, the eyecup was turned inside-out and mounted under medium on a rounded Teflon rod as illustrated in Figure 2.1. A plane of cleavage was developed between the retina and pigment epithelium using a round-tipped glass rod, and the retina progressively separated from the pigment epithelium by advancing the cleavage plane toward the optic disk while rotating the Teflon rod about its central axis. When only the attachment at the optic disk remained, the sclera and choroid were cut from around the disk leaving

just the retina and small segment of optic nerve.

The retina was transferred to a shallow dish filled with medium, using the nerve stump as a handle. The stump was then cut away so that the retina could be laid flat against the bottom of the dish and a small trapezoid-shaped patch cut from the central portion, a few millimeters below the optic disk.

The patch and a small volume of medium were transferred to a fluid-tight chamber created by sealing a plastic frame to an electrode array which formed the floor. The patch was positioned at the center of the array with the ganglion cells facing the exposed electrodes, and oriented so that the cluster of stimulating electrodes was between the optic disk and the cluster of recording electrodes (see Figure 2.2). The optic disk was not actually contained within the patch area—rather, it's location in an intact retina could be deduced from the shape of the patch. Medium was then removed from the chamber to the fix the retina in place, and a castle-shaped brace lowered onto the retina to hold it against the array. The assembly consisting of brace, retina, frame, and array is illustrated in Figure 2.3a. The center of the brace was hollow and covered with dialysis membrane (Spectrum, Laguna Hills, CA, USA) so as to provide light force on the retina while allowing for exchange of Ames' medium. Spacers (approximately 100μm thick) were glued to the bottom of the brace to prevent excessive force on the retina. A detailed view of the brace is shown in Figure 2.3b. The chamber was immediately refilled with fluid once the brace was in place.

Medium was brought to one side of the chamber from a drip-bottle hanging above the preparation. A valve limited the inflow rate to approximately one milliliter per minute. Fluid was removed from the opposite end of the chamber by a peristaltic pump. The array rested on a metallic base which was warmed to 32-34 degrees Celsius using DC ohmic heaters. This slightly cool temperature was used because at higher temperatures the retina left behind sticky residues which reduced the lifetime of the arrays. Though some measurements were made at 37-38 degrees Celsius, these were too few in number to support any general statements about the possible effects of temperature on thresholds.

All manipulations and measurements were carried out in dim white light. Physiologic responses were studied under these conditions for a period of four to nine hours.

2.3 Electrode array design

The electrode arrays were formed by patterning a series of conducting and insulating layers on a rigid glass substrate measuring 0.8mm $\times$ 24.4mm $\times$ 40.9mm. Each electrode was a 10μm-diameter disk, and each could be used for either stimulation or recording. In most cases, half of the electrodes were used for stimulation and the other half for recording.

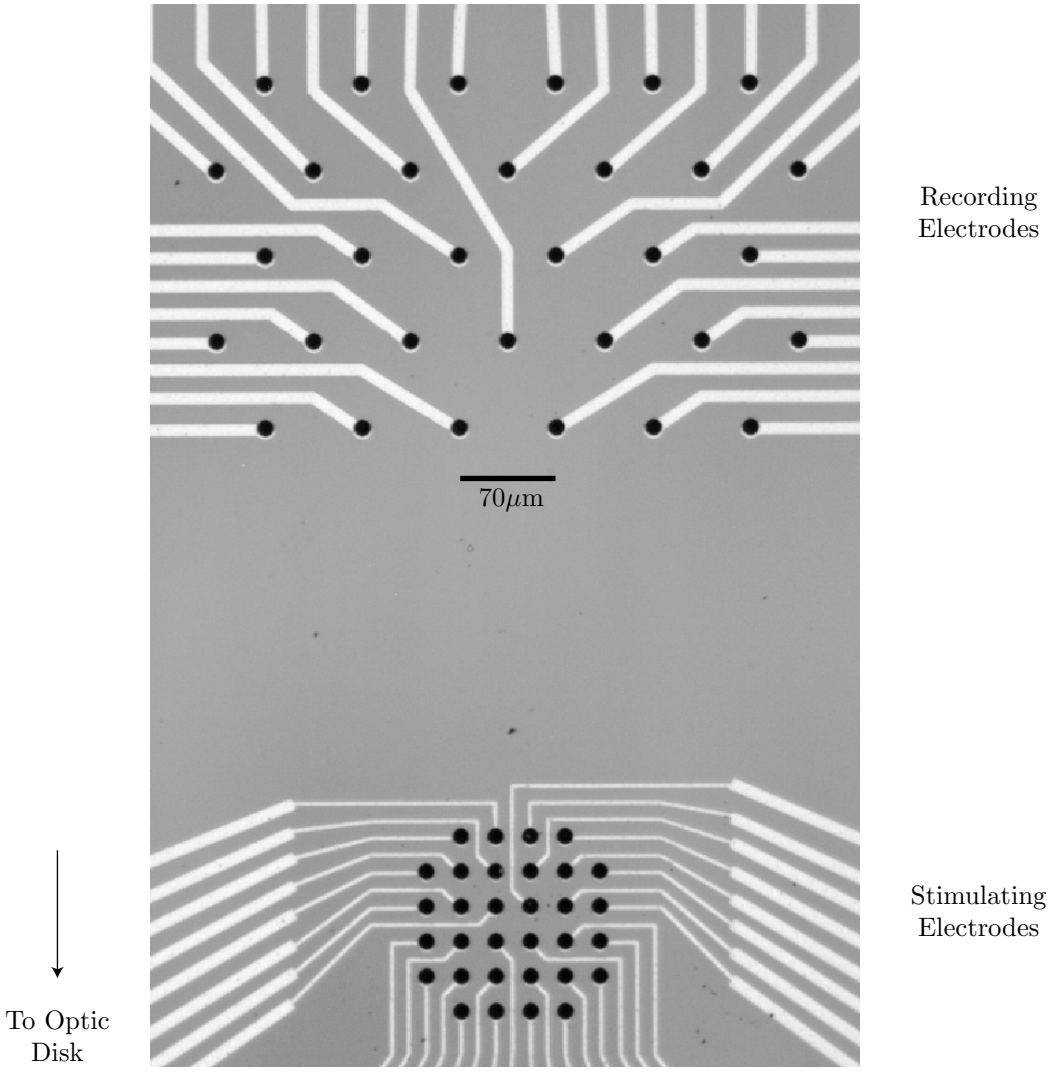

Figure 2.2: Head on view of the electrode array. During an experiment the retina patch was oriented so that in an intact retina the optic disk would be located below the stimulating electrodes.