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NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH
Section A

The retinal readout array

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We have fabricated and tested a set of electrode arrays for the study of information processing in the retina. Live retinal tissue is placed on top of an array with the output neurons directly above the electrodes. Absorption of light by the photoreceptor cells leads to the generation of electrical pulses in the output neurons. These pulses, in turn, produce voltage signals on the electrodes which are recorded simultaneously by external electronics. Thus, for the first time, the spatial and temporal firing patterns of a large set of retinal nerve cells can be studied.

The arrays are fabricated on quartz wafers coated with a transparent conducting layer of indium tin oxide. The electrodes are electroplated with platinum black. Polyimide is used for insulation. The fabrication and properties of these arrays, and illustrative results with retinal tissue, are described.

1. Introduction

The retina is a superb two-dimensional position sensitive detector. It is a thin tissue, ≈ 200 μm thick, which lines the back half of the eye and which converts a visual image into processed electrical signals which travel up the optic nerve to the brain. As shown in fig. 1, the retina consists of input cells (the photoreceptors, rods and cones) which generate electrical signals when photons are absorbed, three layers of cells (the horizontal, bipolar, and amacrine cells) which perform intermediate processing, and the output cells (ganglion cells) whose axons form the optic nerve. In humans, the retina has 10^8 photoreceptors covering an area of about 10 cm^2. The output signals are carried by about 10^6 ganglion cells [1].

The traditional method for the study of information processing in the retina has been in use for more than 40 years: a pattern of light is focussed on the photoreceptors while the signal from an individual retinal cell is monitored with a single microelectrode. Studying one cell at a time in this way is clearly an inefficient and time-consuming process, and makes it very difficult or impossible to investigate the correlations in the signals from multiple cells and the resulting spatial and temporal firing patterns. Fortunately, modern integrated circuit (IC) technology provides an alternative technique which allows the neurobiologist to simultaneously record the signals from tens (now), hundreds (soon), and eventually thousands of retinal output cells.

This alternative technique uses an array of electrodes, fabricated with IC technology, to read out the output signals from the retina. As illustrated in fig. 1, retinal tissue is placed on top of a planar electrode array consisting of a transparent substrate (quartz),

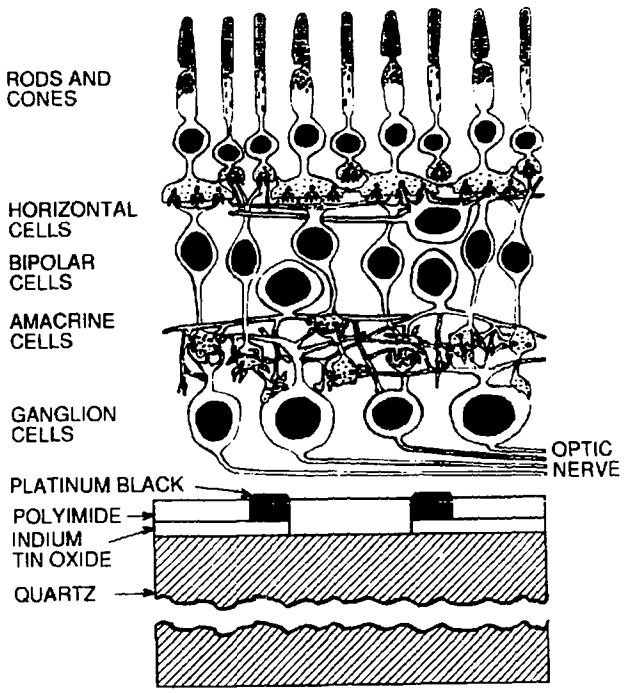

Fig. 1. A schematic view of the retina placed on top of an electrode array. The vertical dimensions of the electrode array as drawn are not to scale; the correct dimensions are given in the text.

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platinized electrodes connected to external circuitry with transparent conductors (indium tin oxide), and a transparent insulating layer (polyimide). The few micron feature size readily available with IC photolithography is a good match to the density of the ganglion cells. For example, the spacing between ganglion cells in the tiger salamander (one of the animals commonly used in studies of the retina) is about 30 μm, and it is straightforward to fabricate electrodes with this same spacing.

Electrode arrays, fabricated with IC technology, have been used in physiology since 1970 [2]. The specific fabrication technique we have used for our arrays follows closely the work of Pine and collaborators [3], who made arrays to study cultured neurons. The results reported here represent the first use of electrode arrays to study the retina.

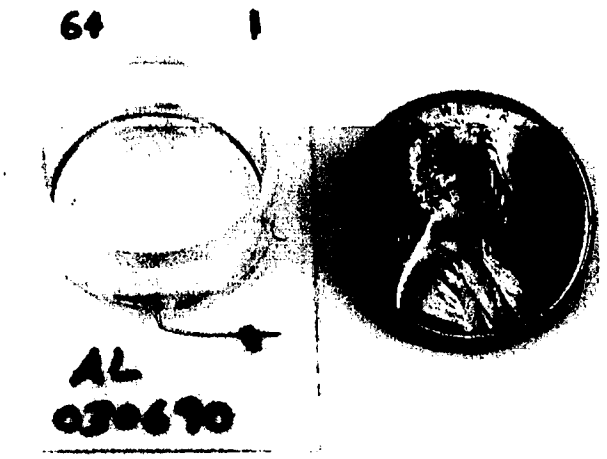

Fig. 2. A photograph of a retinal readout array. The quartz rectangle has the dimensions 24×40.4 mm^2.

2. Electrode arrays: application to the retina

2.1. The retina

We would like first to answer the question: "Why study the retina?". There are a number of characteristics that make the retina an excellent system for investigating the computational properties of neural circuits: (a) the retina is part of the brain with a well-known functional role; (b) its input is known (namely the light pattern on the photoreceptors), and easily controlled; (c) it performs sophisticated processing operations on the input signals; and (d) it has been widely studied with single electrodes. The neural processing performed by the retina is complex enough to be interesting, but is not so complicated to exclude the possibility of detailed analysis and understanding. Hence the study of the retina may aid in understanding the operation of other, more mysterious parts of the brain.

The retina is also quite suitable for study with the electrode array technique. Specifically, retinal tissue (a) is easily separated intact from the animal; (b) can be kept alive for several hours; (c) lies flat; and (d) has a layered structure (as illustrated in fig. 1). Hence, if retinal tissue is placed on top of a planar electrode array, extracellular signals can be recorded from the entire population of output neurons.

2.2. The retinal readout array

A photograph of a retinal readout array is shown in fig. 2. Live retinal tissue is placed in the cylindrical well with the output neurons directly above the electrodes. (The electrodes are too small to be seen in fig. 2, but sections of the conducting traces that connect the electrodes with external electronics are visible.) The well is filled with a saline solution that has been aerated with oxygen, and that contains the chemicals necessary to keep the tissue alive. The tissue is pressed against the array surface with a transparent membrane. The wire which is placed around the inside of the well, and then connected externally, is made from platinum and serves as a reference electrode.

In the experiments, an input pattern of light (for example, from a computer display monitor) is focussed on the photoreceptor cells. This pattern can be a function of both space and time. It can be in color, and the intensity of the light can be varied. The pattern is under the control of the computer used for data acquisition.

In response to the input light pattern, the output neurons fire and generate local currents in the saline solution; these lead to voltage drops (relative to the reference electrode voltage) at the positions of the electrodes. These extracellular signals are recorded as a function of time. With this experimental technique, it is possible to rapidly study a large number of output cells. Measurements can be performed over a period of several hours, with a stable response from the cells. Moreover, the spatial location of each neuron can be calculated from the pulse amplitudes it produces simultaneously on several electrodes; the cell location is given approximately by the pulse-amplitude-weighted electrode positions. These positions can be confirmed by microphotography of the tissue through the bottom of the transparent electrode array. Hence, for a given input, one can study the spatial and temporal firing pattern of a large set of output cells.

2.3. Array fabrication

The starting point for fabrication of an electrode array is a 400 μm thick, 3 in. diameter quartz wafer

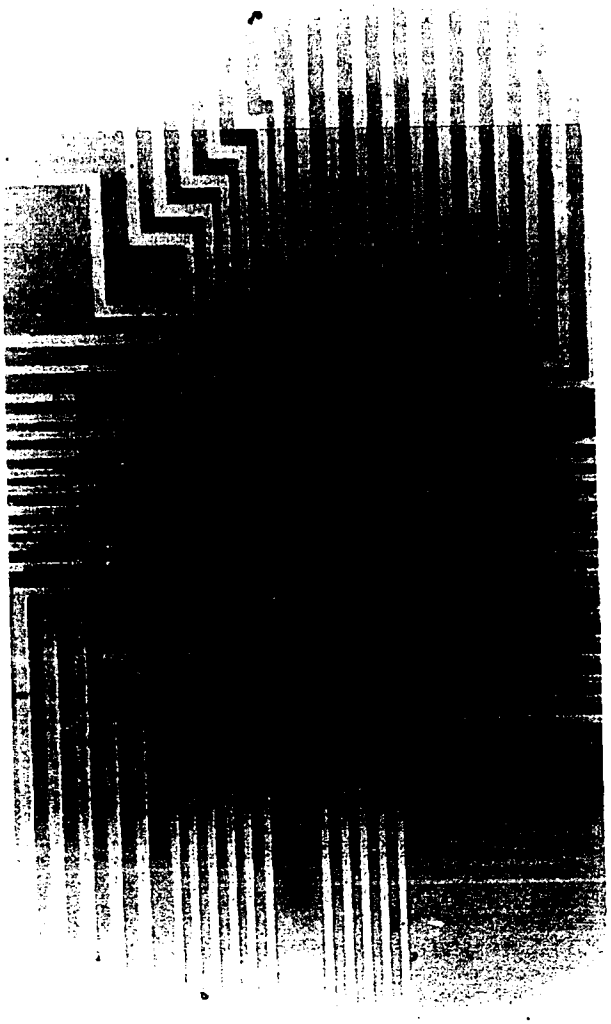

Fig. 3. A photograph of the electrode region of a retinal readout array. The 61 dark squares are the platinized electrodes, each 15×15 μm^2. The light traces are the transparent conductors made of indium tin oxide.

coated with 2000 Å thick indium tin oxide (ITO) [4]. The sheet resistance of the ITO is 10 $\Omega/\square$. After application of photoresist, first mask exposure, and photoresist development, the ITO is etched to form the electrode sites with their connecting traces. After removal of the photoresist, 25 Å of aluminum is evaporated onto the wafer. In air, the aluminum rapidly oxidizes; the aluminum oxide serves as an adhesion promoter for the polyimide. A polyimide coat is followed by the second mask exposure, polyimide development, and a bake. The result is a 3 μm thick polyimide insulating layer with openings for the electrodes and the connections to the external electronics. The wafers are sawed, giving two electrode arrays per wafer. The electrodes are then electroplated with platinum, forming a dark, cauliflower-like region of "platinum black". The large surface area of this structure leads to a dramatic reduction in the impedance of the saline-solution/electrode interface which is crucial for obtaining adequate signal-to-noise [5]. Fabrication of these arrays has been carried out at the Center for Integrated Systems, Stanford University.

A photograph of the electrode region of an array after platinization is shown in fig. 3. There are 61 electrodes, each 15×15 μm^2. The spacing between columns is 60 μm, and the spacing between electrodes in the same column is also 60 μm. An enlarged view of some of the electrodes is shown in fig. 4. The cauliflower-like appearance of the platinum black is clearly visible.

Of course, an unlimited variety of electrode geometries is possible. For example, fig. 5 shows the layout for a quasi-one-dimensional array. This array can be used to study, over a long (1.8 mm) linear dimension, the response of the retina to a one-dimensional pattern, such as a moving edge.

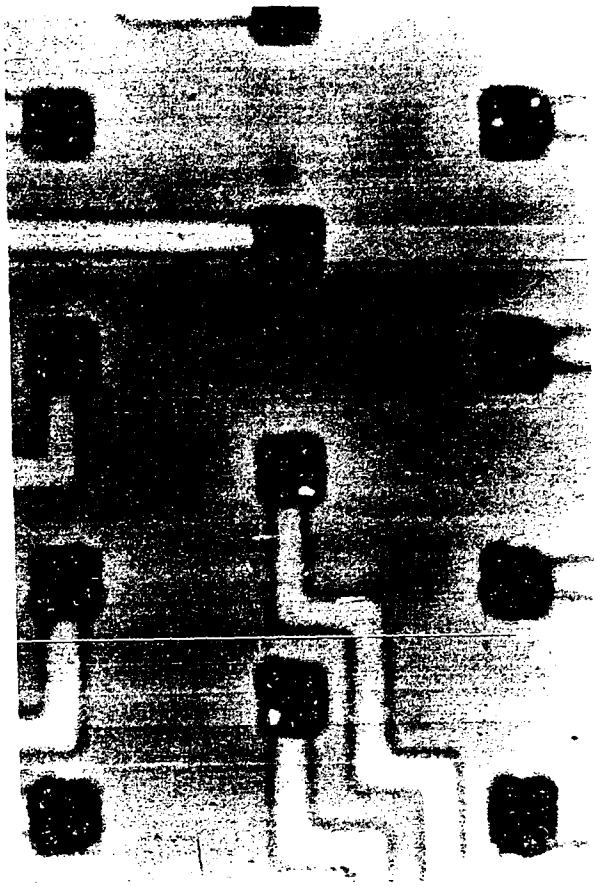

Fig. 4. An enlarged view of a subset of the 15×15 μm^2 platinized electrodes shown in fig. 3.

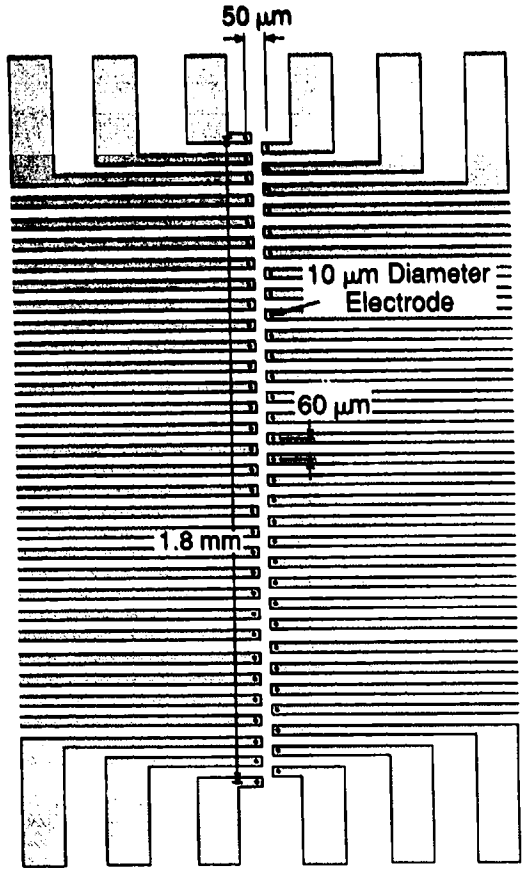

Fig. 5. The layout of the 61 electrodes for a quasi-one-dimensional retinal readout array.

2.4. Signal processing and data acquisition

The signals from each electrode are amplified and filtered in the passband of 20 to 2000 Hz. For each signal above a set threshold, the pulse width, time, and amplitude are recorded. The amplitudes are in the range 50 to 250 μV, and the widths are 1 to 2 ms. The threshold is set typically at about 15 μV, and the rms noise on each channel (σ_{noise}) is on the order of 5 μV.

2.5. Illustrative results

In an initial experiment, a dark-adapted retina from a tiger salamander was exposed in darkness to a dim, diffuse light which stayed on for 2 s. This was repeated 100 times with a period of about 11 s. The signals were recorded with an electrode array of the geometry shown in fig. 3. The outputs from 10 of the electrodes are displayed in fig. 6. One observes that a number of electrodes show signals about 1/4 s after the light is turned on. On trace #1, the two spikes labelled A have very similar amplitudes, but this amplitude is very different from that of spike B. This indicates that this single electrode is recording signals from two separate

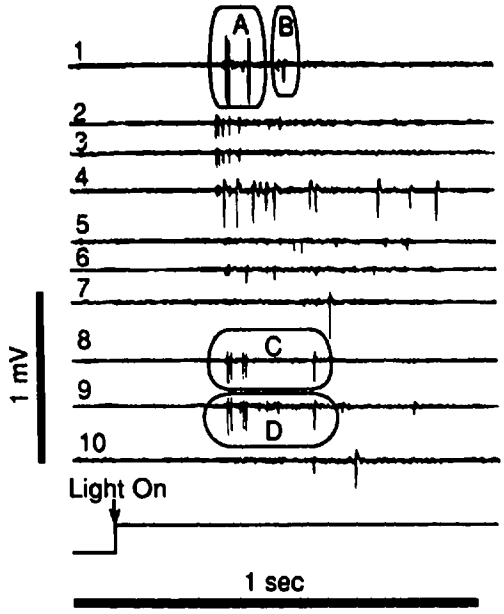

Fig. 6. The signals from tiger salamander retinal tissue recorded on 10 separate electrodes. The onset of a 2-s long light signal is indicated.

cells (more on this topic later). On trace #8, the spikes labelled C are in time-coincidence with the corresponding spikes labelled D in trace #9. This indicates that the same cell is being recorded on the two electrodes.

Fig. 7 shows a scatter plot of amplitude versus width for signals recorded on a single electrode. One sees a set of low-amplitude signals, just above threshold. These most likely arise from electrical noise and from cells that are far from the electrode. One also sees two distinct clusters of signals at greater amplitudes which

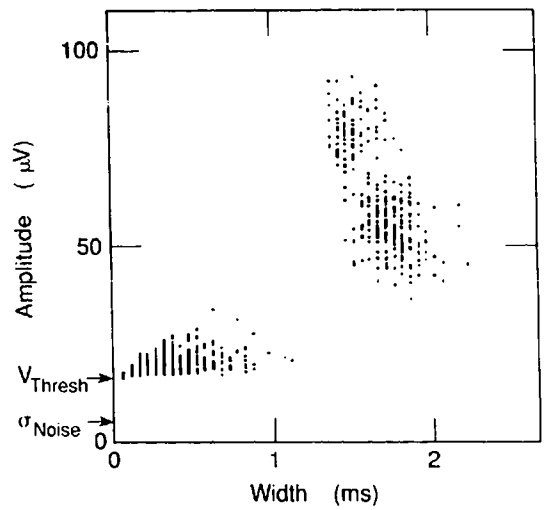

Fig. 7. A scatter plot of amplitude vs width for signals from tiger salamander retinal tissue recorded on a single electrode. The threshold voltage and σ_{noise} are also shown.

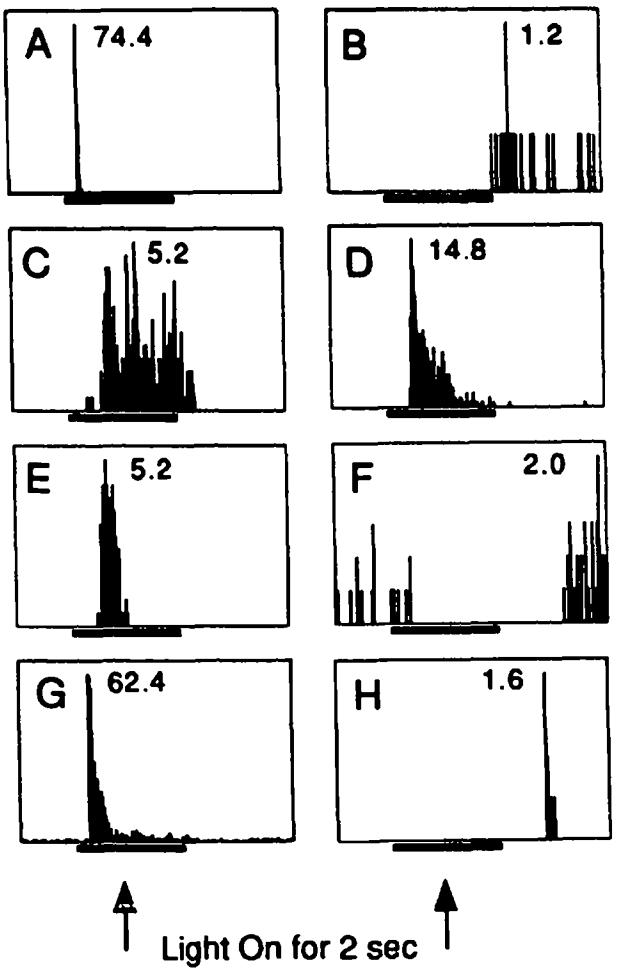

Fig. 8. The spike rate vs time (relative to the onset of the light) for 8 identified cells. The 2-s on period for the light is indicated by the horizontal bars. The number in each histogram gives the maximum spike rate value in Hz for that histogram. The bin width is 25 ms.

differ in both amplitude and (to a lesser extent) width. Each of these clusters represents signals generated by a different cell.

With the amplitude vs width scatter plots, and through tagging time-coincident signals, it is possible to identify the signals from a large number of individual cells. Fig. 8 shows data from 8 identified cells (this is a sample from a total of 50 identified cells). For each cell, the average spike rate is plotted as a function of time, relative to the onset of the 2-s long light signal. A rich diversity of cell responses is indicated by these results. For example, cell A fires very rapidly (spike rate over 74 Hz) just after the onset of the light, but stops firing 50 ms later. Cell B begins to fire after the light goes off. Cell C fires during the entire 2 s period the light is on (with some time delay). Cell D also fires during the entire 2 s light-on period (again, after a delay), but more rapidly just after onset.

3. Applications

With the retina, one will be able to use this electrode array technique to study the patterns of firing of the retinal output cells when different patterns of light are focussed on the photoreceptors. This light pattern can be in black-and-white or color. It can be a random, white-noise pattern, an edge sweeping across the visual field, a simulated bug moving about, etc. In short, the information processing performed by the retina in converting an input visual image into the coded output signals sent to the brain, can be studied in detail.

Also, developmental studies can be undertaken. The response of retinal tissue (including fetal tissue) taken from animals at different stages of development can be investigated. Hence one can study how the properties of the retinal circuits change as their internal and external interconnections are established.

These electrode arrays can be used to study other neural systems, such as cultured neurons and slices of brain tissue. In addition to recording neural activity, the electrodes can be employed to stimulate specific neurons by the injection of current.

4. Summary

Using integrated circuit technology, we have fabricated a set of electrode arrays which are able to read out the signals from a large number of retinal output neurons. This provides a new technique for the study of the retina.

Acknowledgements

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