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RETINAL ELECTROPHYSIOLOGY

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THIS CHAPTER WILL INCLUDE A REVIEW OF:

- Extracellular recording
- Intracellular recording
- Ganglion cells
- Photoreceptors and phototransduction
- Other retinal neurons

The next section involves the study of the electrical response of individual retinal neurons. Much of what we know about retinal electrophysiology comes from laboratory research using animals such as mudpuppies, cats or monkeys. Two important techniques have been used: extracellular and intracellular recording.

EXTRACELLULAR RECORDING

- Anesthetize the laboratory animal
- Insert an electrode into the retina near the cell you wish to study, for example, near a ganglion cell. You do not actually penetrate the neuron you are studying, but the electrode is nearby
- Stimulate the cell by shining light on that part of the retina and record the electrical response from that cell
- Used to study neurons that respond with **action potentials** (sudden electrical impulses) – amacrine cells or ganglion cells

INTRACELLULAR RECORDING

- Insert a microelectrode into the neuron
- Can record from cells that respond with **graded potentials**. They respond by a slower, sustained change in electrical charge
- These include photoreceptors, horizontal cells, bipolar cells
- After recording, a dye can be injected into the neuron so the cell can later be identified and studied histologically

GANGLION CELLS

ELECTRICAL RESPONSE

Ganglion cells respond to stimulation with **action potentials**. This may be described as a sudden burst, or spike-like burst of electrical activity. Even when no stimulus is present, these neurons produce occasional random bursts. The resting level of firing is called the **spontaneous activity** or **maintained discharge** of the ganglion cell. Ganglion cells were designed to use action potentials, rather than the graded potential seen in the photoreceptors, since action potentials can be relayed across long distances.

Depending on the nature of the stimulating light (white or black spot), its location, and the type of ganglion cell, it may respond with either **excitation** or **inhibition**. In excitation, the action potential frequency increases. In inhibition, the rate of firing decreases below the maintained discharge. This is illustrated by the spikes in Schwartz, 2004 Fig. 12-3.

SPATIAL SUMMATION

Schwartz, 2004 Fig. 12-4 illustrates the principle of spatial summation in a ganglion cell receptive field. If a small spot is projected onto the center of this receptive field (an on-center field), it will cause excitation. If the spot size is gradually increased, the frequency of the action potentials (rate of firing) will increase. This is illustrated by section B in the graph. This demonstrates that spatial summation occurs within the receptive field. A maximal response occurs when the spot fills the central excitatory region of the receptive field (C on the graph).

SPATIAL ANTAGONISM (LATERAL INHIBITION)

If the spot size is further increased (segment D on the graph), it will begin to fall onto the surrounding inhibitory region. The rate of neuronal firing will decrease.

When the spot is as large as the entire receptive field, the frequency of the action potentials falls to the maintained-discharge rate. The annular inhibitory region also shows spatial summation, and it offsets the response of the excitatory center.

Ganglion cell receptive fields are circular and display **spatial antagonism**; that is, center and surround regions that show opposite responses to a stimulus. This is also referred to as **lateral inhibition**.

CONTRAST VERSUS ILLUMINATION

When light illuminates the entire receptive field, the response is the same as if there was no light at all. Therefore ganglion cells do not respond well to diffuse illumination of the visual field (Schwartz, 2004 Fig. 12-5). Instead ganglion cells respond most strongly to stimuli that create a pattern with **contrast** within their receptive fields. Schwartz, 2004 Fig.12-6 shows that a black and white grating stimulates the ganglion cell strongly since the center is strongly stimulated by light and the black bars don't cause inhibition in the surrounding region.

This feature of the ganglion cell response shows that the visual system has been designed to respond more strongly to contrast rather than to diffuse illumination.

GANGLION CELL TYPES BY RESPONSE

In addition, the excitation response of a ganglion cell can differ depending on whether it is a **sustained** or **transient-type cell**. In a sustained response, excitation continues as long as the light is on. In the transient response, the cell responds briefly only when the light is turned on or off.

On-center cells respond with excitation when the receptive field center is illuminated, but with inhibition when the annular surround is illuminated. **Off-center cells** respond with inhibition when the center is illuminated but excitation when the surround is illuminated. A dark spot causes the opposite response to that caused by a white spot. So, an on-center cell will respond with excitation when a light spot falls on the center, but with inhibition if a dark spot falls on the center.

GANGLION CELLS (CONT.)

In the cat retina, certain neurons, known as **X cells** or **Beta cells** show the following features:

- Sustained response
- Small receptive fields
- Prevalent in the fovea
- Specialize in fine detail
- Associated with the **parvocellular system**.

They can be either on-center or off-center.

Other neurons in the cat retina are classified as **Y-cells or Alpha cells**. They show:

- Transient response
- Large receptive fields
- Found mostly in the periphery
- Specialize in motion detection
- Associated with the **magnocellular system**.

They can be either on-center or off-center.

Based on research with cats, retinal ganglion cells can be classified into four types:

- X-type on-center
- X-type off-center
- Y-type on-center
- Y-type off-center

The X and Y classification is based on the distinct physiological response of these two neurons. The Beta and Alpha categories are based on anatomical distinctions.

The computer program, **MacRetina** (http://www.oercommons.org/libraries/macretina/view), nicely demonstrates the receptive fields characteristics of these four kinds of cat ganglion cells.

MORE ON GANGLION CELLS

In monkeys, ganglion cells corresponding to the cat X- (beta) and Y- (alpha) cells have been found. Some, known as midget cells, are connected to the parvocellular tract, while others, known as parasol cells, which are found mostly in the periphery, are connected to the magnocellular tract. In addition other ganglion cell types have been found.

Quoting from Web Vision, the excellent website with a wealth of information about the retina, created by Dr. Helga Kolb (University of Utah, School of Medicine):

> *There are at least 18 different morphological types of ganglion cell in the human retina. All but three of these correspond to cells in the cat retina and have, where possible, been given the same G number. Some of the cat types have not been seen in human retina but undoubtedly they are there. Special names are reserved for P and M ganglion cells that are specific to the human high visual acuity and colour processing systems. They are thought to be specialized […] correlates of beta and alpha cells respectively (Leventhal et al., 1981) and comprise the major input to the geniculo-striate areas (Perry, 1981; 1984a, 1984b; Shapley, 1986). Thus P cells are considered to be mostly midget ganglion cells and equivalent to beta cells of cat, and project to the Parvocellular layers of the LGN. M cells, on the other hand, probably equivalent to alpha cells of cat and project to the Magnocellular layers of the LGN. (http://webvision.med.utah.edu/book)*

Summary of two major ganglion cell classifications and comparative names:

- Parvo: X-cells, Beta cells, P cells, midget cells
- Magno: Y-cells, Alpha cells, M cells, parasol cells

PHOTORECEPTORS AND PHOTOTRANSDUCTION

Photoreceptors do not respond with action potentials but with **graded potentials**. In graded potentials the electrical charge within the cell varies depending on the strength of stimulus.

Recall that photoreceptors have been designed with an outer segment and inner segment (Schwartz, 2004 Fig. 3-1). The disks containing the photopigment are in the outer segments, so the outer segments are the "business end" of the photoreceptors. At rest, photoreceptors have a negative electrical charge within the outer segment equal to about -50 mV, which is lower than most other neurons. This is caused, in part, by the active transport of Na+ ions from the outer segment to the inner segment. Other ions such as K+ and Ca++ are also pumped out of the outer segment. There is a net deficit of Na+ and other cations (positively charged ions) inside the outer segment, and this causes a net negative charge inside the cell. This is partially offset by slow diffusion of Na+ ions back into the outer segment, but it normally reaches an equilibrium of about –50 mV in the photoreceptor's outer segment. When stimulated by light, the photoreceptor **hyperpolarizes**. The electrical charge changes from -50 mV to -70 mV. Most other neurons depolarize when stimulated.

SITUATION PRIOR TO EXPOSURE TO LIGHT

The photoreceptor membrane contains tiny pores that allow slow passive diffusion of some Na+ back into the outer segment. The diffusion of Na+, however, is not enough to completely balance the cation deficit, therefore the outer segment maintains a slight negative charge inside the cell.

A biochemical known as cyclic guanosine monophosphate (cGMP) accumulates near the membrane pores, and cGMP clusters help keep the pores (Na+ gates) partially open. The limited diffusion of Na+ back into the outer segment keeps the resting potential at -50 mV.

SITUATION AFTER EXPOSURE TO LIGHT

A complex biochemical chain reaction, known as **phototransduction**, begins with the absorption of a photon and ends with the **hyperpolarization** within the outer segment.

A detailed explanation of **phototransduction** may be found in the following article: Shnapf JL, Baylor DA. How photoreceptors respond to light. Scientific American 1987; 256:40-47.

Photons are absorbed by molecules of rhodopsin, which is the photopigment contained in the disks in the rod outer segments (ROS). Grossly simplified, the process of phototransduction goes through the following steps:

1. A rhodopsin molecule is composed of an optically inert opsin and a light absorbing chromophore portion. The chromophore consists of 11-cis retinal, which absorbs a photon and undergoes a chemical change to 11-trans retinal
2. This activates a chemical called transducin that causes the release of enzymes (phosphodiesterase) which, ...
3. Cleaves small clusters of cGMP. In the resting state, cGMP clusters stand guard at the pores and keep them open so Na+ ions can diffuse back into the cells
4. When the cGMP clusters are broken up, the Na+ gates slam shut. Na+ can no longer diffuse in, and the outer segment rapidly increases its negative charge—it hyperpolarizes

Schwartz, 2004 Fig. 12-8 and Adler's Fig. 14-12, 14-14 summarize the process of photo-transduction.

The flow of sodium and other cations into and out of the cell while it is in the dark is referred to as the **dark current** (Adlers, 1992 Fig. 14-12). The dark current is blocked following exposure to light and the cell hyperpolarizes.

Maximum hyperpolarization occurs when about 10% of the rhodopsin is bleached. Therefore rod saturation is complete after only about 10% of the rhodopsin is bleached.

OTHER RETINAL NEURONS

HORIZONTAL CELLS

See Schwartz, 2004 Fig. 12-9. Horizontal cells contribute to spatial summation within the retina by synapsing with a large number of photoreceptors. When stimulated, their electrical response is similar to photoreceptors – they hyperpolarize (graded potentials). Schwartz (2004) mentions two kinds of horizontal cells, H1 and H2 cells.

The electrical sign of the response is the same as the photoreceptors, so the horizontal cell-photoreceptor synapse is called a **sign-conserving synapse**.

Like photoreceptors, horizontal cell receptive fields do not show spatial antagonism (center-surround organization with opposite responses in each). See Schwartz, 2004 Fig. 12-7.

BIPOLAR CELLS

See Schwartz Fig. 12-10. Bipolar cells show spatial antagonism. There are at least 9 different types of bipolar cells that differ in terms of whether they are on-center, off-center, which photoreceptors they connect with, and their connections with ganglion cells. Schwartz Fig. 12-11 illustrates five different kinds of bipolar cells that he discusses in his chapter. Read Schwartz for more details on the bipolar cells.

AMACRINE CELLS

- Spatial antagonism (like bipolar and ganglion cells)
- Some respond with sustained action potentials
- Some with transient action potentials. Two kind of transient action potentials:
  - Brief increase in action potentials when the light turns on
  - Brief increase in action potential when the light turns off
- Transient response may contribute to motion perception

SELECTED READING/REFERENCES

- Schwartz SH. **Visual Perception - A Clinical Orientation, 3rd Edition**. Appleton & Lange, Stamford, Connecticut, 2004
- Adler FH and Hart WH. **Adler's Physiology of the eye: Clinical application**. 9th Edition. Mosby, USA. 1992.
- Shnapf JL, Baylor DA. How photoreceptors respond to light. Scientific American 1987; 256:40-47