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Neural Circuitry and Plasticity in the Adult Vertebrate Inner Retina

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\textbf{Abstract.} Glutamatergic synapses between retinal bipolar cells and amacrine cells code for transient and sustained events in the visual environment under widely varying conditions of background illumination; i.e. from very light to dark background conditions. The cell types and synaptic mechanisms responsible for coding the transient and sustained information are relatively well described. Recent studies suggest that these synapses are highly plastic in response to environmental stimulation, functionally and structurally remodeling during changes in the ambient lighting conditions bathing the retina. Our current studies of the zebrafish retina are investigating these plastic changes at the bipolar to amacrine cell synapses using a combination of techniques, including patch recording and 2-photon microscopy in the zebrafish retinal slice. We are particularly interested in how the newly discovered endogenous cannabionoid signaling system of the retina controls plasticity at these synapses.

1 Introduction

Glutamatergic synaptic transmission in the inner vertebrate retina occurs at specialized synapses that include synaptic ribbons [1] in the presynaptic bipolar cells, and a variety of glutamate receptors in the postsynaptic amacrine [2] and ganglion cells. In the fish retina, the release of glutamate from the presynaptic bipolar cells has been suggested to be sustained by some [3] and transient by others [4]. In tiger salamander retina, the presynaptic bipolar cells appear to only use sustained type voltage-gated calcium channels [5] that would support sustained calcium-dependent glutamate release. But in fish retina, the presynaptic bipolar cells come in two types with respect to voltage-gated calcium channels; one type expressing only sustained and the other type expressing only transient voltage-gated calcium channels [6], and thus calcium-dependent glutamate release could be brief at those synapses where the bipolar cell expresses transient calcium current, or prolonged where the bipolar cell expresses sustained calcium current.

Recent evidence suggests that these synapses structurally remodel themselves when they experience different lighting conditions. That is, when the retina experiences conditions where it goes from dark to light, or vice versa, the bipolar cell axon terminals rapidly remodel themselves [7]. In other parts of the brain, cannabinoids have been shown to regulate LTP [8], a form of synaptic plasticity, and to regulate focal adhesions, which control cellular shape and are thought to be involved in LTP [9].
2 Synaptic Transmission in Identified Retinal Neurons: Patch Clamp Electrophysiology

Retinal slices are made according to the procedures described by [6] as adapted from [10]. The physiologically recorded cells are also identified using either Lucifer yellow or Calcein dyes to fill the cell which is then made to fluoresce under appropriate illuminating conditions.

2.1 Bipolar Cell Voltage-Gated Calcium Currents

Voltage-gated calcium currents in bipolar cells of the tiger salamander are shown in Figure 1A, while those from the zebrafish retina are shown in Figure 1B. While all tiger salamander retinal bipolar cells exhibit sustained calcium currents, the zebrafish bipolar cells exhibit either transient (66%) or sustained (34%) calcium currents. Because glutamate release from these bipolar cells is calcium dependent, the kinetics of the calcium currents may very well control, at least partially, the kinetics of glutamate release from the bipolar cells.

![Graph showing calcium currents in bipolar cells](image)

**Figure 1.** A) Calcium currents in bipolar cells of the salamander retina are sustained, while those from the zebrafish retina can be either sustained or transient (B).

2.2 Release of Glutamate From Bipolar Cells

In salamander retina, the release of glutamate from bipolar cells appears to be sustained. This is inferred from measuring the excitatory postsynaptic currents (EPSCs) in the postsynaptic amacrine cells. Normally the EPSCs are very brief, but in the presence of drugs to remove rapid desensitization of the glutamate receptors in the amacrine cells, the EPSC is sustained (Figure 2). This suggests that the release of glutamate from bipolar cells onto amacrine cells is sustained in salamander. Similar experiments in the zebrafish retina are currently being performed.
2.3 Classification of Retinal Amacrine Cells

In salamander retina two basic types of amacrine cells have been observed: 1) cells responding with a burst of spikes at the onset and offset of a light stimulus, and 2) cells responding with continuous spiking during the presentation of the stimulus. Both cell types receive a sustained release of glutamate onto their processes, but the transient amacrine cells utilize rapidly desensitizing glutamate receptors to convert the sustained glutamate release into a transient EPSC. The transient amacrine cells also express NMDA type glutamate receptors, which may help to account for the bursting nature of their light responses [11].

Figure 2. The glutamatergic EPSCs in amacrine cells are sustained suggesting that the release of glutamate from bipolar cells onto amacrine cells is sustained.

Figure 3. A) Transient amacrine cell that responds to light stimulation at light-on and -off. B) Sustained amacrine cell that responds to light stimulation during the continued presence of the light stimulus.
3 Synaptic Remodeling: 2-Photon Laser Scanning Microscopy and Electron Microscopy

To measure light-evoked changes in the ultrastructure of zebrafish retinal bipolar cells, standard electron microscopy (EM) was performed on the retinas of zebrafish that were exposed to either bright light or total darkness, and then fixed for EM. For observing real-time or time-lapsed changes in the structure of retinal bipolar cell axon terminals, retinal slices were labeled

3.1 Spinule Formation in Zebrafish Retinal Bipolar Cells

Ultrastructural studies of the axon terminals of zebrafish bipolar cells show that ON type bipolar cell axon terminals are smoother in outline and do not show spinules in the light-adapted state, while the OFF type bipolar cells axons are more irregular and show spinules in the light-adapted state. These results are consistent with the report of [7] for the goldfish retina, and demonstrate that the inner retina is highly plastic, responding to environmental change by remodeling the structure of the synaptic contacts between retinal bipolar cells and amacrine cells.

![Figure 4. A) Electron micrograph showing that OFF bipolar cell axon terminals (Mb) are more irregular in shape and spinulated in the light than are (B) ON bipolar cell axon terminals (Ma), which are smoother and do not show spinules. Arrows indicate the spinules.](image)

3.2 Imaging of Living Bipolar Cell Axon Terminals

Imaging of living retinal bipolar cell axon terminals is now possible using either fluorescent laser scanning confocal microscopy or 2-photon fluorescent laser scanning microscopy. Figure 5 shows an example of a DiI labeled bipolar cell in the zebrafish retinal slice. The
area of interest is within the box and shows two bipolar cell axon terminals nestled together. A putative postsynaptic process impinges on the bipolar cell axon terminal that resides on the left. These are confocal images, and recently we have made similar observations with Drs. Fraser and Potter at CalTech using 2-photon laser scanning microscopy. Two-photon laser scanning microscopy has the advantages of allowing the tissue to be viewed for longer periods of time without bleaching and destruction of the tissue.

4 Cannabinoids in the Vertebrate Retina

Cannabinoids have been shown to regulate plasticity of the brain within the hippocampus [8]. we have therefore begun a search in the vertebrate retina to determine whether there is an endogenous cannabinoid signaling system in the retina, and whether cannabinoids regulate synaptic plasticity in the inner retina.
4.1 Endogenous Cannabinoids

Retinas were removed and prepared for biochemical analysis using mass spectrometry and gas chromatography [8]. Interestingly, 2-arachidonylglycerol and palmitoylethanolamide was found in the retina, but anandamide was not. The retinas were analyzed under light adapted conditions, and it is possible that anandamide production and/or release is regulated by the light adapted state of the retina such that anandamide is not released in the light. Thus, in light adapted retinas, two of the three endogenous cannabinoids found in other parts of the brain are found in the vertebrate retina.

![Figure 6. Identification of 2-AG and PEA in the vertebrate retina using gas chromatography and mass spectrometry.](image)

4.2 CB1 Cannabinoid Receptors

CB1 cannabinoid receptors were detected in the fish retina using fluorescent antibodies. Pronounced staining for CB1 was found in the inner plexiform layer of the retina, consistent with CB1 receptors being localized to the bipolar cell axon terminals. Future studies using double labeling techniques must demonstrate that CB1 receptors are expressed in the axon terminals.

4.3 CB2 Cannabinoid Receptors

We have also localized the mRNA for CB2 cannabinoid receptors in the retina. CB2 mRNA is present in the inner nuclear layer, but we don't yet know the expression pattern of the CB1 receptors. The functional role of CB2 in other parts of the nervous system is not well understood, although it does appear to regulate cAMP. Because cAMP is involved in synaptic plasticity in the retina, it will be of interest to determine whether CB2 is also involved in the remodeling of bipolar to amacrine cell synapses.
5 Conclusions

Glutamatergic synaptic transmission between retinal bipolar cell axon terminals and amacrine cells are capable of generating transient and sustained signals and operate over an extreme range of background lighting conditions with apparent gain changes during the changes in lighting conditions. Part of the process of adapting to the different lighting conditions involves a structural remodeling of these synapses which can now be viewed in living tissue using confocal and 2-photon imaging. We have discovered an endogenous cannabinoid signaling system in retina, thought to regulate synaptic plasticity in other parts of the brain, that we are investigating to determine whether synaptic plasticity at the bipolar to amacrine cell synapse is modulated by activation of CB1 and CB2 cannabinoid receptors.

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