

Introduction

The eye is the sensory organ that detects light and enables vision. The outermost layer includes the cornea, iris, pupil and lens which help to focus the light entering the eye and supplies the retinal cells with oxygen and nutrients. The middle of the eye is filled with a jelly like substance called the vitreous body that helps to preserve the shape of the eye and allows light to pass undisturbed to the retina. The innermost portion of the eye is a neural layer called the retina that sits at the back of eye and contains light sensitive photoreceptors. The job of the retina is to convert the image of the visual scene into signals that are transmitted to the brain where it interprets what we are seeing. This process occurs very rapidly, as we don't have to think very long about what we are looking at in order to identify its shape, or color, or tell whether an object is steady or in motion (Masland, 1986).

One may ask, how this thin structure is able to carry out such complex tasks. The retina is a thin translucent membrane approximately 100 μ m thick that contains three nuclear layers, the outer nuclear layer (ONL), the inner nuclear layer (INL), and the ganglion cell layer (GCL) in which the cell bodies of photoreceptors (rods and cones), horizontal cells, bipolar cells (BP), amacrine cells, and ganglion cells can be found. These neural layers are separated by two fiber layers, the inner plexiform (IPL), and outer plexiform layers (OPL) where synapses occur (Figures 1 and 2).

The outermost layer contains the rod and cone photoreceptors. In human retina, there are three types of cones and one type of rod photoreceptors (Curcio, 2001). The cones are active in daylight and respond to short, medium, and long wavelengths. Depending on the wavelength, the light will be absorbed by various combinations of

blue, green and red cones respectively. Rods come in a single variety and express the pigment rhodopsin. The rods are much more sensitive to light when compared with the cones. They become saturated in ambient light and active in dark of twilight. Once activated, rods and cones make synapses with horizontal and bipolar cells in the outer plexiform layer so that the signal can be transmitted to the inner retina.

The neurotransmitter glutamate is continuously released by all photoreceptors in the dark. In the presence of light however, glutamate release is inhibited, the ion channels close, and the photoreceptors go into a state of hyperpolarization. The rate at which hyperpolarization occurs differs due to the light intensity detected by the photoreceptor (Burns and Baylor, 2001).

The somas of bipolar cells are located in the inner nuclear layer (INL) and they have dendritic processes that extend into the OPL and axons that project to the IPL. Their dendrites receive input from rods and cones and their axons contact ganglion cell dendrites. This pathway provides a direct route for photoreceptors to activate ganglion cells. The bipolar population has ON and OFF types. By ON and OFF we mean that the cell is activated in the presence of light and inhibited in its absence (Kolb, 2003). The axons of these bipolar cells stratify in sublamina 'a', layers 1-2 of the IPL, or sublamina 'b', strata 3-5 depending on their type (Famiglietti and Kolb, 1976).

The OFF- bipolar cells contact all cones in the OPL and make synaptic connections with amacrine and OFF-center ganglion cells in sublamina 'a' of the IPL. In sublamina 'b', ON-bipolar cells synapse with ON-center ganglion cells and amacrine cells (Nelson et. al, 1978). These ON-bipolar cells could be one of two types BP cells that receive inputs from cones (Cone ON-BP) or BP cells that receive input from rods (Rod

ON-BP). The ON cone BP cells directly mediate excitatory signals, with the aid of any amacrine cell to the ganglion cells (Famiglietti and Kolb, 1976). The rod bipolar cells bear similar function to the cone-ON-BP cells but this cell type uses specifically AII and indoleamine-accumulating/A17 amacrine cells as its mediator. OFF-BP cells form flat contacts with cones while ON-BP cells form invaginating contacts (Boycott and Hopkins, 1993).

Ganglion cells have the largest somas and are located in the ganglion cell layer. Ganglion come in at least 12 varieties but the most numerous types are called alpha and beta (Rockhill, 2004; Kolb, 1981). Generally, alpha cells have larger somas and dendritic trees than beta cells. For instance, in central retina, and at 1mm eccentricity, the measured soma for beta cells were 12 and 25 μms compared to 20 and 35 μms for alpha cells. Their dendritic span measures between 20-25 μms and 150 μms or greater (Wässle and Boycott, 1974). The stratification of ganglion cell dendrites determines whether they are an ON or OFF type. Functionally, these cells encode light information gathered from bipolar and amacrine cells and this signal is projected to the brain via the optic nerve.

In addition to the direct pathway for information to travel from the photoreceptors to ganglion cells via the BP cells, there are two lateral pathways that modify ganglion cell responses. One involves the horizontal cells of the outer nuclear layer (ONL). There are at least two types of horizontal cells, HI and HII, in most animal species (Polyak, 1941). Recently a third type, HIII, has been identified in human retina (Kolb, 1992). The somas of these cells are dependent upon the type and their location in the retina. In the fovea, they are 15 μms and in the periphery, they measure between 80-100 μms . HI and HII respectively have dendritic fields between 150-250 μms and 75-150 μms , which extend

into pedicles. Unlike HI, HII also contain axons which extend greater than 300µms. These axons tend to extend into spherules (Kolb, 1974). HIII have dendrites that are significantly larger than HI and HII. HI and HIII both contact medium and long wavelength cones, while HII contact short wavelength cones (reviewed by Kolb, 1992). Regardless of the type, these cells function to provide light intensity between photoreceptors in close vicinity of each other. They provide feedback on to all rods or all cones.

The other lateral pathway is via the amacrine cells. Amacrine cells were first described by Cajal (1892) who noticed that the cells did not possess axons. Amacrine cells bodies are found at the innermost portion of the inner nuclear layer and their dendritic arbors extend into the IPL where they contact processes of bipolar and ganglion cells. Anatomical studies have revealed that there are approximately 22 types of amacrine cells (MacNeil and Masland, 1998) whose dendrites have a variety of shapes and stratification patterns within the inner plexiform layer. Amacrine cells can be characterized into three major groups which include narrow-field, medium-field and wide-field (Kolb et. all, 1981).

The narrow-field amacrine cells account for 28% (MacNeil et al, 1999) of all amacrine cells and have dendritic fields between 30-150 µm in diameter. These densely packed cells have a number of different morphologies, some of which have narrowly stratified and broadly stratified dendrites, while other narrow-field amacrine cells are more broadly stratified. The most common example of narrow-field amacrine cell is the AII amacrine cell. These cells have somas of 10-12µms in diameter with narrowly diffuse dendrites occupying both sublaminae. In sublamina 'a' the dendrites, which originate

from the cell body or primary dendrites, have lobular appendages of 2-3 μ ms in diameter that terminate in layer 2 of the IPL (Raviola and Dacheux, 1982). Those in sublamina 'b', which branch from the primary dendrites, have finer, spiny dendrites, and terminate in layer 5 of the IPL (MacNeil et al, 1999). The proximal and distal dendrites span of AII cells measure between 25-30 μ ms and 35-60 μ ms respectively. In peripheral retina, they have dendritic tree sizes between 75-80 μ ms (Kolb et al, 1981).

AII amacrine cells are glycinergic (Pourcho, 1981; Pourcho and Goebel, 1985, 187 a,b). This Rod-amacrine cell communicates with multiple Rod Bipolar cell, transmitting ON signals to ON ganglion cells (Kolb 2003). This narrow-field cell also transmits signals to OFF ganglion cells. In this same mechanism researchers also gathered information about the A17/indoleamine-accumulating amacrine cell. This wide-field cell has been found to form reciprocal synapse with rod bipolar cells (Ehinger and Holmgren, 1979; Holmegren-Taylor, 1982; Raviola and Dacheux, 1987; Sandell et al., 1989).

A8 is another example of narrow field type. Like AII amacrine cells, the A8 cell is also a narrowly bistratified cell with somas of equal diameter (Kolb et al, 1981). The difference lies in their dendritic morphology and arborization. Unlike the AII cell, A8 fine spidery dendrites in sublamina 'a' centrally stratifies layer 2 of the IPL. In sublamina 'b' the dendrites contain few varicosities and arborize at 50-60% of the IPL (MacNeil et al, 1999). Their dendritic tree span is 50 μ ms in both sublaminae (Kolb et. al, 1992). Like the AII cell, A8 cells are also glycinergic (Pourcho and Goebel, 1985; Crook and Kolb, 1992). Contrast to the AII cell, the A8 cell receives most of its input from OFF-center cone BP cells at gap junctions, and their output is to processes of beta

ganglion cells in sublamina 'a' of the IPL. Scientists suggest that they may function in disinhibiting ganglion cells (Kolb and Nelson, 1996).

Medium-field amacrine cells, have larger dendritic arbors of 170-500 μ m in diameter (MacNeil et al 1999) with thin processes that branch at multiple levels within the inner plexiform layer. The most common medium-field amacrine cell the is DAPI-3 cell. The DAPI-3 amacrine cell was first identified by Vaney (1990). Its cell body is round in shape and measures up to 8-9 μ m in diameter and contains a prominent nucleolus. DAPI-3 cells have fine dendrites with numerous, irregularly dispersed varicosities of approximately 1 μ m in diameter (Wright et. al, 1997). Their dendrites arborize in either sublamina 'a' or 'b', but not usually both, and are thus said to be regionally bistratified (Amthor et. al, 1983). DAPI-3 cells are glycinergic and have been said to play a role in ON-center responses (Bloomfield, 1992).

Another identified cell type belonging to this category is the fountain amacrine cell (Wright et al., 1999). The fountain amacrine cell was first identified by MacNeil and Masland (1998). Blue-violet excitation revealed that this cell stratified both layers of the IPL unlike the previously examined DAPI-3 cell. This cell has varicosed processes of approximately 2 μ ms in diameter. The thinner processes, which contain only varicosities and arborize in sublamina 'a', sometimes branched from their thicker counterparts. In addition to being varicosed, the thicker processes also contained spines and appendages (Wright et al., 1999). The dendritic field of fountain amacrine cell was measured between 70-90 μ ms in sublamina 'a' and 'b' respectively in the visual streak and between 200-350 μ ms in far peripheral retina. The function of this GABAergic cell remains unknown. However, its morphology suggest that it may function in the same manner as do AII

amacrine cells, receiving input from OFF-center cells (sublamina 'a') and making output to ON-center cells (sublamina 'b') (Famiglietti and Kolb, 1975; Strettoi et. Al, 1992; Vaney, 1997).

The third category comprises the wide field amacrine cells. These cells are the least densely packed of all amacrine cells and have very large dendritic arbors, with diameters greater than 500 μ m. They account for ~24% of amacrine cells (MacNeil et al 1999). Most wide-field cells possess thin varicosed, or spiny processes that are narrowly stratified within a single layer of the IPL. Since many of the cells look similar morphologically, these cells are classified very broadly according to the strata where their dendrites arborize. For each broad group there may be a single subgroup or multiple subgroups (MacNeil et. al, 1999).

The most common of all wide-field types is the starburst amacrine cell. As its name indicates, these cells when filled have the appearance of bursting stars. These cells were first clearly identified in rat retina by Walker and Perry (1980). The starburst amacrine cells occur as two mirror-symmetrical types. In rabbit retina they have been found to have dendritic span of 250-800 μ m stratifying layers 2 or 4 of the IPL (Famiglietti, 1983). Starburst amacrine cells release both GABA and acetylcholine (Ach) neurotransmitter (Famiglietti, 1983; Masland, 1988; Vaney, 1990; Ben-Ari, 2002; Sernagor et al., 2003) and are said to have synaptic connections with cone BP cells, and AII amacrine cells among other amacrine types. Some scientist believe that these cells may play a role in feed-back inhibition (Taylor et. al., 1995; Peters et al., 1996). Others believe that they play a role in generating direction selectivity of ganglion cells particularly in turtle and rabbit models (Kolb, 1997).

In addition to these conventionally placed amacrine cells about 1% of all non-starburst amacrine cells somas are displaced to the ganglion cell layer. There are amacrine cell types that do not fit into any of the three groups nor do they appear in their designated areas. (Masland et al, 1984; Vaney 1990). The somas of these displaced cells are located in the ganglion cell layer. Research has shown that the dendrites of certain displaced types such as the indoleamine-accumulating/A17 cell runs together with the conventional types in layer 5 (Sandell and Masland, 1986). In addition to the location of their cell bodies, the only other difference is that they also arborize in the INL (Sandell and Masland, 1989). It might be difficult to conceptualize that cells which have such developmental errors do function normally. However, studies show, at least in mouse retina that these cells, like other amacrine cells that aren't displaced, do in fact play a critical role in ganglion cell activities in response of light stimuli.

Overall amacrine cells account for approximately 40% of all neurons in rabbit retina (Strettoi & Masland, 1995; Jeon et. al, 1998), and are involved in 64-84% of cell communication in the IPL (Dubin, 1970; Raviola, 1982). The multiple shapes of amacrine cells with dendrites in different portions of the IPL suggest they have different functions. The exact functions of most of these amacrine cell types are still unknown. However, for each cell with an identified function such as AII and the starburst cell, which have very different functions, also have very different shapes.

Generally, amacrine cells function as interneurons, intermediating with the bipolar cells which convey messages between the outer plexiform layer and the inner plexiform layer. Amacrine cells can also inhibit neurotransmitters at the axon terminals of BP cells. Since they conduct messages from the bipolar cells to the ganglion cells, the

response of the ganglion cells can be manipulated or altered by amacrine cells. If the amacrine cells are of the wide-field type, they can cause inhibition over longer ranges.

Immunocytochemical staining has revealed that amacrine cells possess many neurotransmitters. However, two main inhibitory neurotransmitters, GABA and glycine, have been found in the majority of amacrine cells in models such as monkey, cats, and rabbits. Cells can possess one or both of these chemical messengers but in general, glycinergic cells have been found to be those with narrow-fields and cells with larger dendritic field tend to be GABAergic. Research has also shown that cells which release the GABA neurotransmitter are also likely to release other substances such as substance P, somatostatin, vasointestinal peptide, and cholecystokin, which collectively fall under the category neuromodulators (Kolb, 2003).

The ability to selectively label the different populations of cells is somewhat limited. Currently cell morphology can be revealed using Golgi staining, immunohistochemistry, or photofilling techniques. Golgi staining enables us to label a variety of cell types in great detail. However, this method is limited because it does not allow for selective labeling. Immunohistochemistry allows one to selectively label all cells of a single type, but not all types can yet be recognized. Photofilling is a quantitative photochemical technique used to allow a single cell to be labeled with a fluorescent product. This method allows us to selectively label specific cells, and with great skills one can successfully label 94% of all targeted cells (Masland, 2001).

However, this method has some serious disadvantages. The most limiting is that the labeled cells cannot be fixed for permanent preservation. We have tried another way to

label cells using selective uptake of biocytin. When biocytin is injected into the vitreous of the eye, some cells take it up to reveal their morphology (Jeon and Masland, 1995).

My primary interest is on a wide-field amacrine cell type stratifying layer four of the IPL that selectively takes up biocytin and has never been described as a population. For this cell I will describe its morphology.

Materials and Methods

Tissue preparation. Wide field amacrine cells were labeled by selective uptake of biocytin (Sigma, St. Louis, MO) that was injected into the vitreous of the eye. Adult New Zealand rabbits of either sex were anesthetized with an intramuscular injection of ketamine (30-60 mg/kg) and xylazine (mg/kg) and 20ul of 5% biocytin (Sigma, St. Louis, MO) in ddH₂O and 0.5% DMSO were injected in each eye. After a 28, 32, 41, 44, and 48 hr incubation period, the rabbits were re-anesthetized with the same mixture. The corneas were anesthetized with 2 drops of proparacaine into each eye before the eyes were enucleated and hemisected. The retinas were carefully teased from the eyecup while completely submerged in with Ames medium. The rabbit was then euthanized with Ethanol (100mg/kg, iv). All procedures using animals were in accordance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee at York College, CUNY. Free floating retinas were fixed in 4% formaldehyde (Ted Pella, Redding, CA) for approximately 60-90 minutes, after which they were thoroughly rinsed 3x in sodium phosphate buffer (0.1M, PH 7.4) for a total time of 30 minutes. Retinal wholemounts were reacted immediately with antibodies. Sections were made by either embedding a piece of retina in agarose (4%, Sigma, low melting point) and at cutting

60µm using on a vibratome or cryoprotecting the retina in 30% sucrose and cutting 30µms thick frozen sections using a cryostat.

Biocytin Staining. Retinas labeled with biocytin were permeabilized with 0.5% Triton-X 100 in 0.1M sodium phosphate buffer for 2hrs with rocking. This was followed by rinsing with 3 changes of buffer over 30 minutes. The tissue was then incubated in Avidin DN [1:50] overnight in the cold room. The following day, the tissue was rinsed 3x for 10 mins each in buffer with rocking. We then incubated the tissue with Florescein Anti-avidin [1:200] for 2 hours while rocking. The final wash was done with tris buffer for the same duration. The tissue was mounted flat on a slide and coverslipped in vectashield.

Antibody Staining. After incubating for 28, 32, 41, 44, and 48 hours, the only incubating time when our cell was optimally stained was during the 41hour time frame. Therefore, we used 41hr biocytin cryostat or 60µms sections that were fixed in 4% paraformaldehyde for one hour. These sections were made permeable by being placed in 0.5% Triton-X 100 in 0.1M sodium phosphate, pH 7.4 (immunobuffer) then blocked in 4% normal goat serum for 60 seconds each. It was then rinsed in immunobuffer for three changes of immunobuffer for a period of 60 seconds each. Retinas were incubated in the primary anti-body, mouse anti-tyrosine hydroxylase (1:50, Sigma) in immunobuffer with 2% normal donkey serum while irradiating the tissue in the microwave for 13 minutes (3minutes ON, 2 minutes OFF, 3 minutes ON, 2 minutes OFF, 3 minutes ON). After three changes of immunobuffer at 60 seconds duration each, the retina was incubated for

10 minutes in the secondary antibody, Alexa Fluor 594 donkey anti-mouse IgG (1:200, Molecular Probes). This was followed by rinses in phosphate buffer (3 x 60 sec).

Similar protocol was followed using as primary, goat anti-ChAt (Chemicon) and rabbit anti-recoverin (Chemicon) with Alexa Fluor 647 donkey anti-goat (Molecular Probes) and Alexa Fluor 594 donkey anti-rabbit (Molecular Probes) as their respective corresponding secondary antibodies.

Imaging. Labeled retinas were imaged with an Olympus Fluoview 300 Confocal microscope equipped with helium, argon and Neon lasers with an Olympus 20X air, and 60X water immersion lenses at resolutions of 1024 x 1024. A series of stacked images of 0.5 μ m or 1.0 μ m were collected from the inner plexiform to the outer plexiform layers of the retina to show the cell bodies and dendritic processes of wide-field amacrine cells. We were also observing levels of stratification within the IPL. Using Metavue, all excessive planes were removed leaving only those in which the processes of the cells of interest were in focus. Adobe Photoshop was used to magnify certain portions of emphasis.

Data Analysis. We montaged stacks of images to view a field whose area measured approximately 0.06mm² to see the extent of the cell dendrites in NeuroLucida. This was done primarily so as to obtain cells of a single wide-field type since it can be difficult to distinguish between wide-field cell types. With this method, we were able to use markers to label the population. The designated areas were sampled and using another program, neuroexplorer, analysis showed us the distance one cell was to its closes and farthest neighbor. Knowing the number of cells in the field and obtaining the

areas of the selected field, we were able to compute the density from the whole-mount tissue, at varying eccentricities.

Results

A number of cell types take up biocytin when it is injected into the vitreous of the eye. The specific type however, is time dependent. At an incubation time of 48hrs, a wide-field bipolar cell type is optimally stained (Jeon and Masland, 1995). We noticed that with shorter incubation times other cell types are preferentially labeled. One such type was a previously undescribed population of wide-field amacrine cells (Figure 3). For the most part, the cell body of this cell appeared to be fairly round in shape. The cells bodies were measured between 10-12 μms . Its proximal dendrites extend into the inner plexiform layer, approximately 60-80% of its depth (Figure 3). From there it bifurcates and the varicosed dendrites ramify continuously laterally and in a symmetrical manner, throughout layer 4.

This is best observed in a through-focus series of optical planes in the IPL. As one focuses through the tissue, one can see how the processes come in and out of focus (Figure 4). At approximately 60-80% of the IPL, the proximal dendrites branch and extend outward for hundreds of microns. The processes of similar cells which also branch at these same depths, suggest they are of similar type. A careful tracing of a single cell clearly shows that these cells have a wide dendritic arbor that extends for more than a millimeter in every direction (Figure 5). All labeled cells with processes at this depth appeared similar in morphology (Figure 6). The processes are studded with numerous varicosities, which suggest the presence of synapses (Figure 7). The cells have a uniform

coverage of the IPL as seen by the meshwork created by wide interwoven dendritic processes. We traced a single cell from a vertical section and assessing its dendritic arbor relative to the cell bodies of the innerplexiform layer and the ganglion cell layer suggests that this amacrine cell type stratify at approximately 80% of the IPL (Figure 8).

Determination of arborization

To ensure that our cell was different from other wide-field types that have been previously described, we double-labeled the wide-field biocytin amacrine cell with various other antibodies that have been shown to selectively label other retinal populations. One such reaction involve tyrosine hydroxylase (TH), which labels a population of amacrine cells that stratifies at approximately 20% of the IPL (Figure 9). We also labeled for Choline Acetyl Transferase (ChAT). ChAT, which labels starburst amacrine cells that stratifies at approximately 20% and 70% of the IPL (MacNeil et al, 1999) (Figure 10), was conducted so as to ensure that our target cell was still unknown. To obtain legitimate results, we scoped the sections for an area where we would presumably see our cell and another amacrine type in the same field. We know that the dendritic processes of the TH amacrine cells span layer 1. Thus double labeling would allow us to see if the same soma reacted with both TH and Biocytin antibodies. Colocalizing with the TH cell and not colocalizing with our cell would indicate that our cell and the TH cell were not of the same type. Thus we would rule out the option that the cell in question is a TH amacrine cell. A similar pattern of elimination was followed for other experiments, namely ChAT, to rule out any misleading results. The results from ChAT experiment showed both cells were not colocalized which suggests different types.

The information we gathered from this result showed that the processes of our cell runs slightly below the ChAT bands. This ChAT experiment was conducted to give precise location of the cell process within the IPL.

Cell densities and distribution.

To determine the density of the wide-field amacrine cell population we sampled fields of labeled cells at 20x magnification in the midline along the dorsal-ventral extent. Based on the size of the area, and the number of cells within that field the density was determined to be approximately 20 cells/mm². In thorough focus series, the cell bodies are in focus together as are their dendrites which indicate that they were the same type.

When the biocytin wide-field density was plotted as a function of its retinal eccentricity, we observed that the cell was predominantly localized in ventral-peripheral retina. The overall wide-field cell distribution observed showed a peak in density between V2-V4 (2-4mm ventral to the optic nerve). From V4-V10, there was a steady decrease to about 17 cells/mm² (Figure 11). This result is consistent with the density of other wide-field types such as indoleamine-accumulating cell, which also has low densities (Sandell and Masland, 1986).

Discussion

We have shown that biocytin injected into the eye selectively labels a population of wide-field amacrine cells. These cells which stratify layer 4 of the IPL have somas of 10-12µms (Figure 8) and varicosed dendritic arbors greater than 1mm (Figure 5). Their density ranges from 16-24 cells/mm² (Figure 11). This sparsely populated amacrine type

uniformly covers ventral retina as indicated by the meshwork it creates in the IPL. In addition to this type, other previous wide-field amacrine cell types that have been identified includes A17, A18, and A22.

The A17 amacrine cell measures between 9-10 μ m and contains very thin processes along which are evenly spaced varicosities. These cells according to research has been found to stratify layer 5 or 80-100% of the IPL (Ehinger and Floren, 1976). The A17 cell releases both GABA and serotonin and feedback onto rod BP cells (Ehinger and Holmgren, 1979; Holmgren, 1982; Raviola and Dacheux, 1987; Sandell et al., 1989). Research suggests that these cells might play a role in enhancing center responses by amplifying signals (Their and Wässle, 1984; Brunken and Daw, 1987, 1988). We do not believe that this biocytin amacrine cell is our type because its processes stratify in a different layer.

The A18/ Dopaminergic cell is another wide-field type whose function is known. This cell has a large cell body of 15 μ m in diameter from which five primary dendrites extend and stratify the layer directly below amacrine cell somas (Kolb, 1981). This dopaminergic amacrine cell makes major synaptic connections with other amacrine cells and has minor arrangements with cone BP cells in stratum 1 of the IPL (Pourcho, 1982; Voigt and Wässle, 1987; Hokoc and Mariani, 1987; Kolb, 1997). Scientist believe that these cells play a role in adjusting our vision when we make transitions between a dark to a light environment by modulating the adaptational state of the retina (Kolb, 1997). A18 is likely to be the TH amacrine cell. We have shown that it and our cell of interest did not overlap (Figure 9), and therefore conclude that they cannot be the same type.

The A22 cell is yet another wide-field cell whose function has been postulated. This cell which stratify layer 3 and 4 of the IPL contain axon-like processes. The A22 cell receives major input from OFF-cone BP cells and other BP types, as well as input from unknown amacrine types. These cells are said to supply nerve fibers with blood. They are also involved in the rapid coding of visual information on the part of ganglion cells (Kolb, 1997).

Using other known wide-field cell types such as the tyrosine hydroxylase or TH cell (Brecha et. al, 1984) and ChAT, we were able show differences in wide-field cell types and narrow down the layers in which our cell stratified. For instance, double labeling with TH and Biocytin showed no colocalization as was expected since this cell stratifies in layer one (Figure 9). Analysis of a piece of biocytin labeled ventral retina showed that the processes of our cell appeared directly above a wide-field bipolar cell population (Figure 12). Furthermore, a ChAT experiment using sections suggest that the processes of our WF4 amacrine cell run slightly below the labeled bands (Figure 10). This merely suggest that the cell stratifies somewhat centrally within the layer 4 region.

Interestingly, other researchers have also found another wide-field cell type stratifying layer 4. Golgi staining of vertical sections revealed two stout primary dendrites branch from the cell body, each of which continuously branches laterally for several millimeters in opposite direction (MacNeil et al., 1999). Compared to this cell, immunofluorescence staining reveals a single primary stout which submerges into layer 4, bifurcates then monostratifies laterally in opposite directions. In addition to varicosities found on our cell, the previously described cell also had spines. These slight differences

suggest one of two things. Either the cells are of different subtypes of the WF4 group, or they are physiologically the same type.

The position of the previously identified WF4 amacrine cell appears at 4.4mm from the fiber bundles (MacNeil et al, 1999) which suggest that they were localized in central retina. Our cell type on the other hand was found exclusively in peripheral retina. The soma of this previously stained WF4 amacrine cell measured 12.1x9.5µms (MacNeil et al, 1999) compared to our type which had a diameter of 10-12µms. If they were indeed the same type, this would explain the slight discrepancy in the sizes of their cell bodies.

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