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**GLYCINE RECEPTOR SUBUNITS $\alpha 2$ AND $\alpha 3$ PARTICIPATE IN DIFFERENT
INHIBITORY CIRCUITS THAT ALTER THE RECEPTIVE FIELD
ORGANIZATION OF ON- AND OFF-CENTER RETINAL GANGLION CELLS**

By

Regina D. Nobles

B. A. Psychology, M. A. Psychology, University of Louisville, 2000, 2005

A Dissertation

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University of Louisville

Louisville, Kentucky

August 2010

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Dissertation Approved on

June 30, 2010

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Dissertation Director

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ABSTRACT

GLYCINE RECEPTOR SUBUNITS $\alpha 2$ AND $\alpha 3$ PARTICIPATE IN DIFFERENT INHIBITORY CIRCUITS THAT ALTER THE RECEPTIVE FIELD ORGANIZATION OF ON- AND OFF-CENTER RETINAL GANGLION CELLS

Regina D. Nobles

June 30, 2010

In the retina, the receptive fields (RFs) of most neurons are comprised of an excitatory center and a suppressive surround. Retinal ganglion cell (RGC) RF center excitatory input arises from bipolar cell (BC) inputs, while their surround arises from lateral inhibitory inputs. Because of the availability of selective antagonists the role of GABAergic inputs has been well defined. In contrast, the role of individual glycine receptor (GlyR) subunit inhibition is less clear because the antagonist, strychnine, blocks all GlyR subunit combinations. To define individual retinal circuits that utilize specific glycinergic subunits, I examined maintained and visually-evoked responses of ON- and OFF-center GCs from mice lacking expression of the GlyR $\alpha 2$ (*Gla2*^{-/-}) or GlyR $\alpha 3$ (*Gla3*^{-/-}) subunits to those of *C57Bl/6J* (WT) RGCs using an *in vivo* extracellular approach. Previous observations have defined glycine and GABA inputs across BC classes and in a variety of amacrine and RGCs. Using this information and by comparing the responses of WT vs. *Gla2*^{-/-} and *Gla3*^{-/-} RGCs; I conclude that both subunits modulate local RF interactions. Within the On pathway, GlyR $\alpha 2$ and GlyR $\alpha 3$ inputs play similar roles. Their responses predict that they participate in serial inhibitory circuits that

decrease a direct GABAergic inhibition that modulates maintained, but not peak firing rates. In contrast within the Off pathway, GlyR α 2 and GlyR α 3 inputs define two populations of RGCs. In one, GlyR α 2 participates in a serial inhibitory circuit that modulates maintained firing, whereas in the other, GlyR α 3 mediates direct inhibition that controls the peak firing rate. Only GlyR α 2 modulates lateral interactions to the RF surround where it mediates a direct inhibitory input to all OFF-center RGCs. My results suggest that GlyR α 2 and GlyR α 3 inputs define two populations of OFF-center RGCs. In addition, both subunits participate in retinal circuits that can be distinguished not only by the RGC RF center type, but also by the type of inhibitory circuit. These results are the first demonstration of subunit specific control of RGC visual responses and, are the first evidence of serial glycine to GABA as well as glycine to glycine circuits in the retina.

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CHAPTER 1

INTRODUCTION TO THE RETINA AND ITS CIRCUITRY

The Retina is a Laminar Structure

The specialized neural circuitry of the retina forms the initial basis for what we see. The retina extracts spatiotemporal information from the environment and relays that information to more central visual processing areas of the brain. The retina is a laminar structure organized into three nuclear and two synaptic layers that contain five basic cell types (Figure 1-1). The outer nuclear layer (ONL) contains the cell bodies of the photoreceptors (PRs). There are two types of PRs, rods and cones. In the mouse, rods comprise around 97% of the total PR population (Jeon et al., 1998), are responsible for vision under low or scotopic light levels and are so sensitive they can detect a single photon (Hecht et al., 1941). Cones are less sensitive and operate under bright or photopic light levels. In the mouse retina, there are two types of cones that contain photopigments sensitive to UV- (~360 nm) or middle-wavelengths (~509-512 nm) of the visible spectrum (Jacobs et al., 2004). The PRs contact the dendrites of bipolar (BCs) and horizontal (HCs) cells at the first synaptic layer, the outer plexiform layer (OPL). The cell bodies of BCs, HCs and amacrine cells (ACs) make up the inner nuclear layer (INL). Excitatory information from the outer retina is conveyed to the retinal ganglion cells

(RGCs) by BCs and this signal is modulated by inhibitory HCs and ACs. The BC excitatory inputs to the RGCs as well as the modulation by AC inhibition occur in the second synaptic layer, the inner plexiform layer (IPL). RGC somas make up the final layer and their axons form the optic nerve, which conveys retinal information to higher areas of the brain for further visual processing.

Figure 1-1. A cross-section of mouse retina immunostained to show the retinal layers and major cell classes. The PRs are stained with anti-cone arrestin (purple). The BCs are reacted for green fluorescent protein (GFP, green). The HCs, ACs and RGCs (red) are immunostained for calbindin, a calcium binding protein. The PR cell bodies make up the outer nuclear layer (ONL). The outer plexiform layer (OPL, white) is the first synaptic layer between the PRs and BCs and HCs. The BC, HC and AC bodies make up the inner nuclear layer (INL). The inner plexiform layer (IPL) contains the synaptic contacts of the BCs and ACs to the RGCs (GCL). (Source: www.wonglab.biostr.washington.edu)

Light is Transduced in the Photoreceptors

The PRs transduce light energy in the form of photons into a biochemical message that changes the membrane potential of the PRs and alters the release of glutamate signaling to secondary neurons (Yau, 1994). In the dark rods and cones continually

release glutamate and light stimulation hyperpolarizes these cells, thus reducing glutamate release. A reduction in glutamate release hyperpolarizes the postsynaptic HCs which create an inhibitory feedback signal to both rods and cones in order to modulate both the gain of the PR response and their output to BCs (Oyster, 1999). HCs have large dendritic arbors that are coupled together by a syncytium of gap junction connections (Mills and Massey, 1994). This provides local and long range interactions with PRs over a wide range of light intensities and contributes to the receptive field (RF) center/surround organization in BCs (Werblin and Dowling, 1969).

Parallel Divergence at the Synapse between Photoreceptors and Bipolar Cells

Following the initial hyperpolarization by light of PRs, distinct visual and parallel pathways are created by two classes of BCs to signal light onset and offset. These BCs either depolarize (DBC) or hyperpolarize (HBC) in response to reduced glutamate release and establish the On and Off pathways, respectively (Werblin and Dowling, 1969; Werblin, 1991). The depolarizing and hyperpolarizing responses of BCs result from the type of postsynaptic receptor that binds glutamate. Depolarizing BCs express metabotropic glutamate receptors (mGluR6 or Grm6; Masu et al, 1995) and a light-induced reduction in glutamate initiates a G protein signaling cascade that opens the cation channel, Trpm1, and depolarizes the cell (Bellone et al, 2008; Morgans et al, 2009; Shen et al, 2009). In contrast, HBCs express ionotropic glutamate receptors (AMPA/Kainate) and a light-induced reduction in glutamate creates a hyperpolarization (Saito and Kaneko, 1983). Depolarizing BCs receive inputs from both rod and cone PRs, whereas cone HBCs only receive direct inputs from cone PRs. The rod and cone DBCs

and cone HBCs also differ in the stratification patterns of their axon terminals, which creates the On and Off sublamina within the IPL, respectively. To maintain the segregation of ON and OFF information from the retina to the brain, the IPL is subdivided into five layers: two Off sublaminae and three On sublaminae. The dendrites of morphologically distinct RGCs also stratify within the specific layers of the IPL and the stratification patterns of RGCs correlates to their physiological responses to light (Famiglietti and Kolb, 1976). ON-center RGCs respond to light onset, or increment, and ramify in the On sublamina of the IPL, whereas OFF-center RGCs respond to light offset, or decrement, and ramify in the Off sublamina of the IPL (Famiglietti and Kolb, 1976).

The structure of the retina and the interactions between the neuronal subtypes gives rise to distinct functional pathways through which information is transmitted (Figure 1-2). The excitatory vertical transmission from PRs to BCs to RGCs is modulated by inhibitory input from HCs in the OPL and ACs in the IPL. The inhibitory processes are mediated by the neurotransmitters, GABA and glycine and provide the basis of receptive field spatial organization in BCs, ACs and RGCs. The transfer of the signal from the outer to the inner retina is further divided into parallel Cone/Rod and On/Off pathways which are described in detail in the following sections.

Figure 1-2. The functional pathways of the retina. The vertical pathway of excitatory transmission begins with the synapse between PRs and BCs, which in turn synapse onto RGCs. The signal is modulated by the inhibitory inputs of HCs in the OPL and by ACs in the IPL. Cone PRs contact cone DBCs and cone HBCs, which in turn synapse directly onto their respective ON or OFF RGCs. The rod PR contacts a single rod DBC (RBC), which uses the AII AC interneuron to relay information to the ON and OFF cone pathways. The AII uses a sign-inverting, glycinergic synapse (—) to relay information to the cone HBCs and OFF RGCs, and a sign-conserving, electrical synapse (ΛΛ) to relay to the cone DBCs and ON RGCs.

Parallel ON and OFF Cone Pathways

Cone photoreceptors function under bright, photopic light levels, mediate spatial acuity, and color vision. Each cone terminal, known as a pedicle, contains between 20-50 pre-synaptic ribbons, each flanked by synaptic vesicles (Wässle, 2004). In the mouse and the peripheral retina of human, the pedicle is innervated by the processes of HCs and the dendrites of at least 8-10 BCs (Wässle, 2004). Thus, each cone pedicle makes hundreds of synaptic contacts making this initial synapse in the retina one of the most complex synapses in the central nervous system (Figure 1-3; Wässle, 2004).

Figure 1-3. The cone pedicle and its post-synaptic components. (A) In the schematic diagram the cone pedicle is apposed to the triad formed by HCs and cone DBCs (ON) as a synaptic ribbon is flanked by synaptic vesicles (arrowhead) and cone HBCs (OFF) (Hack et al, 2001). (B) An enlarged view of the post-synaptic components of the cone terminal. Cone DBCs (ON BC) make invaginating contacts with the cone pedicle and are flanked by two HC processes. Cone HBCs (OFF BC) make only basal contacts. The dendrites of cone DBCs express metabotropic glutamate receptors (**mGluR6**) whereas HCs and cone HBC dendrites contain ionotropic glutamate receptors (iGluRs) (Source: www.webvision.com).

Cone DBCs make invaginating contacts with cone pedicles and express metabotropic glutamate receptors, specifically mGluR6, on their dendrites (Figure 1-3; Vardi and Morigawa, 1997). In the dark, the mGluR6 receptor binds glutamate that is tonically released from the PRs. This keeps a G-protein gated cation channel, TrpM1, closed and keeps the cone DBC hyperpolarized (Nawy and Jahr, 1990). Light stimulation decreases glutamate release from the PRs resulting in fewer mGluR6 receptors bound by glutamate. This causes a reduction in the cellular signal which allows the cation channel to open and depolarize the cone DBC membrane.

Cone HBCs make flat contacts at the base of the cone pedicle and express ionotropic glutamate receptors (iGluRs), specifically AMPA/kainate receptors (Figure 1-3; Slaughter and Miller, 1983). In the dark, the AMPA/kainate receptors conserve the polarity of the cone signal and maintain the depolarization of cone HBCs (Saito and Kaneko, 1983). Thus, the decrease in glutamate release at light onset results in the hyperpolarization of cone HBCs. The ionotropic AMPA/kainate receptors have distinct temporal properties that further diversify signaling in the Off pathway (Devries, 2000). AMPA receptors generate phasic synaptic transmission and produce a transient response to light stimulation, whereas kainate receptors generate tonic synaptic transmission and produce a sustained response to light (Wässle, 2004; Devries, 2000).

In the mouse retina there are ten different morphological types of BCs: 5 types of cone DBCs, one type of rod DBC and 4 types of cone HBCs (Figure 1-4; Ghosh et al, 2004). Rod DBCs do not directly contact RGCs; instead they synapse upon AII amacrine cells (AII ACs). AII ACs relay information to the RGCs via a gap junction connection with cone DBCs and a glycinergic synapse with cone HBCs (see Figure 1-2; Famiglietti and Kolb, 1975). The stratification patterns of BC axon terminals within the IPL correlates with visual function: cone HBCs ramify in the Off substrata (1-2) and cone and rod DBCs ramify within the On substrata (3-5) of the IPL (Ghosh et al, 2004). Each BC class releases glutamate onto its postsynaptic cells. Thus, light onset causes cone DBCs to increase glutamate release onto ON-center RGCs; whereas cone HBCs decrease glutamate release onto OFF-center RGCs to the same stimulus. The time course of the cone BC output is shaped by several factors: 1) its glutamate receptors in the OPL; 2) the different types of voltage-gated Na^+ , Ca^{2+} and K^+ channels that they express; 3) the

modulation of the inhibitory feedback inputs that they mediate due to AC inputs; and 4) the complements of GABA and glycine receptors on their axon terminals (Eggers et al, 2007; Euler and Masland, 2000; Dowling and Boycott, 1969). Although the diversity of cone BCs would suggest a variety of parallel functions involved in relaying the visual world to the inner retina, their individual roles are not yet understood (Strettoi, 2008).

Figure 1-4. Confocal micrographs of the different BC types in the mouse retina taken from a vertical retinal slice after the injection of Lucifer Yellow. There are four morphological types of cone HBCs (1-4) whose axon terminals stratify in the Off layer of the IPL (strata 1-2). There are five morphological types of cone DBCs (5-9) and one morphological type of rod DBC whose axon terminals stratify within the On layer of the IPL (strata 3-5). BCs that ramify in the On and Off sublayers contact ON- and OFF-center RGCs, respectively. (Source: Ghosh et al, 2004)

Parallel ON and OFF Rod Pathways

Rod PRs are very sensitive and convey vision under dim or scotopic conditions. The synapse at the rod terminal, also called the spherule, consists of invaginating contacts by the processes of two HCs and the dendrites of rod DBCs (Figure 1-5; Dowling and Boycott, 1969). To increase sensitivity under low light levels, signals from multiple rod PRs converge onto a single rod DBC which then pools and spreads the signal among the ACs in the IPL, specifically the A17 and AII ACs (Sterling et al, 1988; Kolb and Nelson, 1993). The A17 AC collects information over a large region and provides reciprocal

feedback inhibition to the rod DBC terminal (Nelson and Kolb, 1985) whereas the AII AC relays information from the rod DBC to the On and Off cone pathways.

Figure 1-5. The rod spherule and its post-synaptic components. (A) The schematic diagram of a rod spherule shows the invaginating processes of HCs and cone DBCs (ON), which are apposed to a ribbon synapse that is flanked by synaptic vesicles (Hack et al, 2001). (B) An enlarged view of the post-synaptic components of the rod spherule. The invaginating contacts of HCs and cone DBCs have ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (**mGluRs**), respectively. (Source: www.wwebvision.com)

The rod signal reaches ON- and OFF-center RGCs through three morphologically and functionally distinct pathways (Volgyi et al, 2004; Tsukamoto et al, 2001; Soucy et al, 1998). The primary mammalian rod pathway relays rod PR information to rod DBCs, which transmits an excitatory signal to AII ACs (Figure 1-6A; Kolb and Famiglietti, 1974). The excitatory signal from rod DBCs is relayed to the Off pathway through a sign-inverting glycinergic inhibitory synapse between the AII AC and cone HBCs. Thus depolarization of the AII AC, resulting from light onset, increases glycinergic transmission and hyperpolarizes post-synaptic cone HBCs. Within the On pathway, rod

DBC signaling is relayed through a sign-conserving gap junction connection from AII AC to cone DBCs. Here, depolarization of the AII AC will depolarize cone DBC terminals. The cone DBCs and HBCs then transmit their signals to their respective ON- and OFF-center RGCs (Famiglietti and Kolb, 1975).

A secondary rod pathway is formed by gap junctions between rod spherules and cone pedicles (Figure 1-6B). In this pathway, a hyperpolarization in rods would directly hyperpolarize cones and this light evoked reduction in glutamate will be then transmitted via the parallel cone DBC and HBC pathways to the RGCs (Schneeweis and Schnapf, 1995; Raviola and Gilula, 1973). Lastly, a tertiary rod pathway is thought to occur by syncytia of electrically coupled rod PRs making a direct, sign-conserving chemical synapse with cone HBCs which then transmit information directly to OFF RGCs (Figure 1-6C; Tsukamoto et al, 2001; Soucy et al, 1998). This novel pathway was discovered when light evoked responses in OFF RGCs persisted in a coneless transgenic mouse and in WT retina in the presence of the glutamate agonist APB, suggesting a direct glutamatergic input from rod PRs to cone HBCs (Soucy et al, 1998). Hypothetically, the latter two pathways are independent of the rod DBC signaling pathway (van Genderen et al, 2009). RGCs receive the separate or convergent inputs from one or more of these pathways to broaden the RGC operating range in the intensity domain (Volgyi et al, 2004).

Figure 1-6. Three rod signaling pathways in the mammalian retina. (A) The primary rod pathway. Rod PRs release glutamate to rod DBCs (rod ON BC) which then relay information to AII AC. The AII AC provides an excitatory signal through gap junctions with cone DBCs (cone ON BC) and an inhibitory signal through a glycinergic synapse with cone HBCs (cone OFF BC). (B) The secondary rod pathway. Rod PRs are electrically coupled to cone PRs and the glutamatergic signal flows through gap junctions with cone PRs to cone DBC and HBC pathways to the RGCs. (C) The tertiary rod pathway. Rod PRs provide a direct glutamatergic input to cone HBCs to OFF RGCs. (Source: van Genderen et al, 2009)

Retinal Ganglion Cells and Receptive Field Spatial Organization

RGCs convert chemical and electrical signals into action potentials that are required to carry retinal output to higher visual processing areas in the brain. There are 15-20 different morphological types of RGCs in the mammalian retina, which are characterized by axon diameter, soma size and shape, and dendritic arborization (Sun et al, 2002). RGCs also have unique intrinsic membrane properties that shape synaptic input and further diversify their function (O'Brien et al, 2002). RGCs have spatially organized receptive fields (RFs) that are composed of an excitatory "center" and a co-extensive antagonistic "surround" (Rodieck and Stone, 1965a; Kuffler, 1953). The differences in

synaptic input to the RFs of RGCs along with their dendritic stratification in the IPL are well correlated with their functional responses. ON- center RGCs increase their spike frequency in response to a light increment and have an OFF surround; whereas OFF-center RGCs increase their spike frequency in response to a light decrement and have an ON surround. ON- and OFF-center RGCs stratify in the On and Off sublamina where they connect to their respective pre-synaptic BC partners. ON-OFF-center RGCs produce a response to both light onset and offset. Their dendrites are bi-stratified and contact both cone DBC and HBCs (Boycott and Wassle, 1974; Famiglietti and Kolb, 1976; Nelson et al, 1978).

The functional implications of the RF center/surround spatial organization are to produce equal yet opposite effects on RGC output when each mechanism is independently stimulated (Enroth-Cugell and Robson, 1984). Secondly, although the RF center/surround mechanisms have different spatial distributions in the retina, the sensitivity profiles of the two mechanisms are well balanced so that RGCs are less sensitive to changes in stimulus luminance and more sensitive to changes in stimulus contrast (Enroth-Cugell and Robson, 1984). The net effect of the center and surround summation to changes in contrast, luminance, stimulus size and intensity results in the spatial tuning of RGCs to stimuli in the visual scene.

Receptive Field Center/Surround Interactions

The primary feature of RGCs is spatial tuning; where some RGCs are sensitive to higher spatial frequencies and others are more sensitive to lower spatial frequencies (Enroth-Cugell and Robson, 1966). The sensitivity of RGCs to spatial patterns is

dependent on the size and strength of their RF components (Rodieck and Stone, 1965b). The RF center mechanism extends over a narrow region of visual space and has a greater effect on the firing rate of a RGC compared to the surround (Rodieck and Stone, 1965b). The RF center mechanism is formed by the direct recruitment of excitatory inputs from pre-synaptic BCs. The recruitment of BCs is limited spatially by the span of the RGC dendritic arbor (Lukasiewicz and Werblin, 1990) where stimulation of RF center responses are summed as function of spot size (Figure 1-7; Sagdullaev and McCall, 2005). For example, a small spot presented directly to the RF center will elicit a high frequency excitatory response and will reach a maximum response to a spot comparable to the RF center size. A decrease in firing frequency occurs as the stimulus is either spatially displaced from the RF center or increases in outer diameter. When a RGC's RF center and surround are simultaneously stimulated, the antagonistic surround reduces the excitatory center response. It is this spatial disparity in the excitatory and inhibitory inputs to RGCs that produces the surround mechanism (Rodieck and Stone, 1965a).

Figure 1-7. ON-center RGC RF center summation and surround antagonism. (A) Spot sizes of varying diameter elicit an excitatory response from the RF center. When the RF center and surround are stimulated simultaneously by large spots or full field stimuli, the excitatory response is reduced. (B) An area response function demonstrates center summation in the RF center until a stimulus matches the RF center size. The larger spots and full field stimuli elicit inhibitory inputs from the RF surround that attenuates the RF center response. (Source: Sagdullaev and McCall, 2005).

Generation of Receptive Field Surround

Spatial Opponency in the Outer Retina

Surround inhibition in the outer retina was first identified at the level of the BC in mudpuppy retina (Werblin and Dowling, 1969). Since then BC RF organization has been explored in other vertebrates such as *Xenopus*, goldfish and turtle (Stone and Witkovsky, 1987; Yazulla, 1976) and also identified in primate (Dacey et al, 2000). The HCs generate the RF surround of BCs through two pathways: feedback inhibition to cone photoreceptors and feedforward inhibition onto BCs (Werblin and Dowling, 1969; Dowling, 1970; Fahey and Burkhardt, 2003). The BC RF spatial organization also forms the basic structure for center/surround antagonism in RGCs and therefore, mechanisms that contribute to BC RF spatial organization contributes to those of RGCs. For example in rabbit, it has been shown that feedback inhibition from HCs in the outer retina contributes to the antagonistic local RF surround response in RGCs (Mangel, 1991). The exact synaptic mechanism that govern surround antagonism in the outer retina is still unclear, but proposed mechanisms include GABAergic, pH-sensitive feedback and hemichannel-mediated feedback inhibition to cone photoreceptors (Kamermans et al, 2001a, 2001b; Dimetrev and Mangel, 2004; Kamermans et al, 2004; Fahrenfort et al, 2010). Overall, spatial opponency in the outer retina governs global changes in intensity and initiates contrast enhancement of the retina image through the formation of center/surround antagonism at the level of the BCs (Dowling, 1970; Kamermans and Spekrijse, 1999).

Spatial Opponency in the Inner Retina

The lateral inhibitory interactions of ACs in the inner retina are thought to mediate more complex processes such as contrast enhancement, spatial tuning and motion detection (Kamermans and Spekreijse, 1999; Werblin, 1991; Cook and McReynolds, 1998a). The ACs comprise a morphologically diverse group of cells (>20 types) and are characterized by the vertical and horizontal stratification patterns of their dendritic trees and type of neurotransmitters they use (MacNeil and Masland, 1998; Pérez de Sevilla Müller et al, 2007). Narrow-field ACs (NF-ACs) have small dendritic arbors (<125 μ m) and stratify within one or more layers of the IPL (MacNeil and Masland, 1998). The spatial extent of NF-AC dendrites is restricted to the areas of BC terminals and RGC RF centers (MacNeil and Masland, 1998). Assuming they make synaptic contacts with these cells they could modify the vertical transmission of information between the ON and OFF layers of the IPL (MacNeil & Masland, 1998; MacNeil et al, 1999). NF-ACs have been shown to mediate glycinergic inhibition to the RF center of RGCs and are thought to modulate the temporal properties of these cells (O'Brien et al, 2003). In contrast, wide-field ACs (WF-ACs) have very large dendritic arbors ($\geq$ 500 μ m) and are narrowly stratified within a single layer of the IPL (MacNeil & Masland, 1998; Pérez de Sevilla Müller et al, 2007). The expansive dendritic field of WF-ACs provides lateral communication over large areas of the retina (MacNeil et al, 1999). In general, WF-ACs are GABAergic and their synaptic inputs contribute to RGC RF surround inhibition in RGCs and are thought to modify their spatial properties (Cook & McReynolds, 1998; Flores-Herr et al, 2001). Other types of ACs include medium-field, polyaxonal and starburst ACs. My focus is on glycine receptor inhibition and therefore, I

will focus on NF- and WF-ACs. In conclusion, ACs receive glutamatergic signaling from BCs and GABAergic and glycinergic inputs from other ACs. In turn, ACs provide inhibitory inputs to BCs, RGCs and other ACs (Lukasiewicz and Shields, 1998; Euler and Wässle, 1998; Wässle et al, 1998; Zhang et al, 1997).

Inhibition in the Retina

In the mammalian retina the distribution of inhibitory receptors and their pre-synaptic counterparts have been well characterized using both immunohistochemistry and electrophysiology techniques (Koulen et al, 1996; Euler and Wässle, 1998; Ivanova et al, 2006; Eggers et al, 2007; Heinze et al, 2007; Majumdar et al, 2007; Weiss et al, 2008). Although both GABA and glycine are inhibitory, it is the complexity of their receptor composition, the kinetic properties of the receptor, and the localization to neuronal subtypes that is hypothesized to give rise to their distinct inhibitory functions. Glycine and GABA receptors are heteromeric, composed of different subunits that form chloride ion channels with unique current kinetics. GABA_A receptors (GABA_ARs) confer fast, transient inhibition to RGCs (Lukasiewicz and Shields, 1998; Pan and Lipton, 1995); whereas the GABA_C receptors (GABA_CRs) produce an inhibitory current with significantly slower kinetics (Lukasiewicz, 2004; Eggers et al, 2007). Glycine receptors (GlyRs) mediate a fast, strychnine-sensitive current and a slow, strychnine-insensitive current that can suppress fast and slow excitatory currents in postsynaptic RGCs (Han et al, 1997). The unique kinetic profiles of GABA_ARs and GlyRs not only shape excitation but also temporally tune inhibition through a variety of inhibitory circuits that include

feedback, feedforward, serial and cross-over inhibition (Zhang et al, 1997; Roska et al, 2006; Molnar and Werblin, 2007; Hsueh et al, 2008; Eggers and Lukasiewicz, 2010; Werblin, 2010).

Two well-characterized reciprocal feedback inhibitory circuits include GABAergic AC feedback to cone DBCs and A17 AC feedback to rod DBCs, both mediated mainly by GABA_C Rs and less by GABA_ARs (Shields et al, 2000; Eggers and Lukasiewicz, 2006, 2010). Glycinergic inputs dominate feedforward inhibition to cone HBCs, particularly under dark adapted conditions (Famiglietti and Kolb, 1976; Ivanova et al, 2006; Eggers et al, 2007). AC feedback and feedforward inhibition are modulated by local and lateral serial inhibitory synapses from other ACs (Zhang et al, 1997; Eggers and Lukasiewicz, 2010). GABA_AR-mediated inhibition suppresses GABA_CR pathways to delay feedback inhibition to BCs (Zhang et al, 1997; Eggers and Lukasiewicz, 2010), and interactions between GABA_AR-mediated inhibition to GlyR pathways, or vice versa, enhance feedforward inhibition to RGCs (Zhang et al, 1997, Roska et al, 2006). More recently cross-over inhibition has been reported as a common interaction among ACs where ON ACs inhibit OFF ACs and OFF ACs inhibit ON ACs (Hsueh et al, 2008). A well-characterized cross-over inhibitory circuit is the rod DBC → AII AC → cone HBCs. In this circuit, rod DBCs depolarize AII ACs in the On sublamina and AII ACs release glycine to hyperpolarize cone HBCs in the Off sublamina. Cross-over inhibition is hypothesized to enhance, not oppose, excitation in RGCs (Werblin, 2010). Taken together, GABA_AR and GlyR kinetics modulate the time course of pre-synaptic inhibition to regulate excitation in RGCs, while their local and lateral inhibitory circuits modulate overall spatial processing in RGCs (Eggers and Lukasiewicz, 2010).

While the overall inhibitory interactions and circuits within the OPL and the IPL are well understood, the specific synaptic mechanisms within the intricate network between retinal neurons remains largely unknown. To further investigate inhibition in the retina, I will use two separate GlyR subunit knockout mice to evaluate the specific contribution of subunit-mediated glycinergic inputs to the RF center/surround organization and visual responses of RGCs.

CHAPTER 2

GLYCINE IN THE RETINA

General Structure and Function of the Glycine Receptor

The amino acid glycine is one of the two most common inhibitory neurotransmitters found in the central nervous system (CNS). Glycine receptor (GlyR)-mediated inhibition is crucial for the control of muscle movements, the coordination of reflexes and sensory perception (Grudzinska et al, 2005). The GlyR is a ligand gated chloride (Cl^-) channel with similar structural organization and sequence homology to nicotinic acetylcholine (nAChR) and GABA_A receptors (Matzenbach et al, 1994). GlyRs are antagonized by the alkaloid, strychnine (Webb and Lynch, 2007). The GlyR is a pentameric structure with five subunits that are symmetrically arranged around a central pore (Webb and Lynch, 2007). In mammals there are four genes that encode alpha subunit isoforms ($\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$) and one gene that encodes the beta (β) subunit (Malosio et al, 1991; Harvey et al, 2004). Both the α and β subunits participate in ligand binding and channel gating of the receptor (Grenningloh et 1988; Grudzinska et al, 2005). The β subunit also is necessary to bind with the structural protein, gephyrin, which is essential for postsynaptic clustering of the GlyRs to the cell membrane (Kirsch and Betz, 1995; Meyer et al, 1995).

Each subunit of the GlyR shares similar membrane topology containing a large, extracellular ligand binding site at the N-terminus (M1), portions of a transmembrane domain (M2) form the central pore and a phosphorylation site within the intracellular loop between M3-M4 (Figure 2-1; Legendre, 2001; Webb and Lynch, 2007). The ligand binding domain (M1) is composed of an α -helix and multiple β strands. The β strands form a twisted structure with two hydrophobic cores and a binding pocket in the position where adjacent subunits interface (Grudzinska et al, 2005; Webb and Lynch, 2007). The conserved cystein loop within the receptor and the β sheet loop protrude from the bottom of the M1 and relay information from the ligand binding pocket to the channel pore activation gate (M2). Conformational changes of the β sheets upon ligand binding are thought to be the gating mechanism of the GlyR although this hypothesis, among others, is still under debate (Webb and Lynch, 2007).

Figure 2-1. Structure of the glycine receptor. The receptor configuration includes 2 α :3 β subunits with extracellular N- and C-termini and four membrane spanning domains (M1-M4). The transmembrane domain M2 (pink) forms the channel pore (Source: Modified from the Australian Society for Biophysics website, www.biophysics.org.au).

The GlyR is an ionotropic receptor that mediates fast inhibition via increased Cl^- conductance. The pre-synaptic release of glycine is detected by a diverse population of post-synaptic homomeric (α) or heteromeric (α/β) receptors that are composed of the various subunits ($\alpha 1-4$ and β). Each α subunit can form functional homomeric receptors however, those receptors are found primarily during embryonic development (Takahashi et al, 1992; Singer and Berger, 2000) or in the extrasynaptic regions of the adult CNS (Shen and Jiang, 2007). The homomeric GlyRs are not thought to participate in effective synaptic transmission between cells due to their inability to insert into the cell membrane because of the absence of a structural β subunit (Webb and Lynch, 2007).

GlyRs undergo a series of developmentally dependent changes in their physiological function. For example, the GlyR is best known for inhibitory neurotransmission within mature motor circuits of the spinal cord and brainstem. During embryonic development however, its regulatory role is excitatory (Webb and Lynch 2007). The developmental switch is dependent on the intracellular chloride (Cl^-) concentration, which is high during development and lower in mature neurons (Webb and Lynch, 2007). The regional expression patterns of the individual GlyR subtypes throughout the CNS also are developmentally regulated (Aguayo et al, 2004). The $\alpha 2$ and β subunits are highly expressed during embryonic development followed by a switch to $\alpha 1/\beta$ receptors around postnatal week three (Malosio et al, 1991). The various subunit combinations of postsynaptic α/β receptors give rise to distinct physiological properties and diversify the strength of the glycinergic synapse (Legendre, 2001; Aguayo et al, 2004).

Localization of Glycine Receptor Subunits within the Retina

The five GlyR subunits ($\alpha 1$ -4 and β) expressed in the retina have been cloned and antibodies have been produced to identify their expression pattern within the different strata of the inner plexiform layer (IPL) (Figure 2-2; Haverkamp et al, 2003; Haverkamp et al, 2004; Heinze et al, 2007). Immunoreactivity for the $\alpha 1$ subunit shows sparse punctate labeling in the outer plexiform layer (OPL) and is thought to represent glycinergic synapses with interplexiform cells and BCs (Jiang and Shen, 2010). This however, has not been functionally verified in the mammalian retina (Jiang and Shen, 2010). The GlyR $\alpha 1$ predominantly labels clusters in the Off sublamina of the IPL with faint labeling in the On sublamina (Sassoé-Pognetto, et al, 1994). GlyR $\alpha 2$ is diffusely expressed throughout all layers of the IPL with no differential label in a particular sublamina and is expressed in the highest density compared to all other α subunits (Haverkamp et al, 2004). GlyR $\alpha 3$ is expressed in four distinct bands within the IPL, with a high density in the Off sublamina and reduced labeling in the outer two bands of the On sublamina (Haverkamp et al, 2003). Lastly, GlyR $\alpha 4$ shows a low intensity and diffuse labeling throughout the IPL with a high density band through sublamina 3/4 of the On-cholinergic stratum (Heinze et al, 2007).

Figure 2-2. Photomicrographs of vertical sections through the mouse retina show the localization of the four GlyR α subunits in the IPL. (A&D) Normarski images of the five retinal layers: ONL= outer nuclear layer; OPL= outer plexiform layer; INL= inner nuclear layer; IPL= inner plexiform layer; GCL= ganglion cell layer. **(B)** GlyR α 1 is predominantly located in the Off sublamina of the IPL with reduced labeling in the OPL. **(C)** GlyR α 2 is evenly distributed throughout the IPL. **(E)** GlyR α 3 is localized to four distinct bands with the densest label in the Off sublamina. **(F)** GlyR α 4 has a high intensity label between strata 3/4 of the IPL. Scale bar = 50 μ m (Source: Heinze et al 2007).

Glycine Receptor Kinetics and Currents in Retinal Cells

The different isoforms of the GlyR α subunits (α 1-4) have unique kinetic properties that control the time course of inhibition. The kinetic properties have been characterized for cultured homomeric and for heteromeric GlyRs (Harvey et al, 2000; reviewed in Aguayo et al, 2004). With the exception of the α 4 subunit, the kinetic profiles and the localization of heteromeric GlyRs to specific cell types in the retina are summarized in Table 1. The fastest conducting GlyR expresses the α 1 subunit ($\tau_{\text{rise}} \sim$

1.1ms, $\tau_{\text{decay}} \sim 5.9\text{ms}$) and represents the glycinergic synapse between AII ACs and cone HBCs (Sasso-Pognetto et al, 1994). GlyR α 1 also has been shown to mediate glycinergic currents in cone HBCs (Ivanova et al, 2006) and in A-type ganglion cells in mouse (Majumdar et al, 2007). The slowest conducting GlyRs express the α 2 subunit ($\tau_{\text{rise}} \sim 1.5\text{ms}$, $\tau_{\text{decay}} \sim 27\text{ms}$) and has been shown to mediate glycinergic currents in type 5/6 and 7 NF-ACs (Weiss et al, 2008) and displaced GABAergic WF-ACs (Majumdar et al, 2009). The medium-fast kinetics of the GlyR α 3 subunit ($\tau_{\text{rise}} \sim 1.4\text{ms}$, $\tau_{\text{decay}} \sim 11\text{ms}$) has been shown to mediate glycinergic currents in AII ACs (Weiss et al, 2008). There is very little electrophysiological data on GlyR α 4 as it has only recently been localized in the IPL of mouse retina (Heinze et al, 2007). According to Harvey et al (2000), the kinetics of homomeric mouse α 4 subunits expressed in *Xenopus* oocytes resembles those of the GlyR α 1. However, Majumdar et al (2009) reported that glycine currents in ON starburst ACs had prolonged decay time constants (~ 50 to 70ms), which are too slow for the other GlyR subunits and are most likely mediated by GlyR α 4. Although a functional role for this particular subunit has not yet been determined, the ON starburst AC is known to play a role in the responses of direction selective (DS) neurons (Taylor and Vaney, 2003) and it is possible the GlyR α 4 subunit may influence DS light responses in RGCs (Heinze et al, 2007). The diversity of GlyR subunit configuration and their different kinetic profiles suggests they are involved in different retinal circuits that carry out specific roles in visual processing (Wssle et al, 2009).

Table 1. The diverse populations of GlyR subunits have very different kinetic profiles.

Subunit	$\tau_{\text{rise time}}(\text{ms})$	$\tau_{\text{decay time}}(\text{ms})$	Kinetics	Cell Type
GlyRα1	1.1 ± 0.3	5.9 ± 1.4	Fast	All AC $\rightarrow$ cone HBC synapse; cone HBC; rod DBC axon
	2.3 ± 1.6	3.9 ± 2.5	Fast	A-type RGCs
GlyRα2	1.5 ± 0.6	27 ± 6.8	Slow	NF-ACs; WF GABAergic ACs
GlyRα3	1.4 ± 0.7	11.2 ± 0.2	Med-fast	All ACs
GlyRα4	2 - 10	66.2 ± 90	Very slow	ON starburst ACs

Functional Assessment of Glycinergic Inhibition in vitro

The heterogeneity of pre- and post-synaptic GlyRs and the variability in their kinetics gives rise to multiple microcircuitries within the retina (Sassoè-Pognetto et al, 1994). Numerous *in vitro* studies have been done in an attempt to elucidate the contribution of GlyR-mediated inhibition and how it shapes the visual responses of RGCs. These studies will be described in the following sections.

Localization of Glycine Receptors and Amacrine Cells

The glycine transporter-1 (GlyT1) is a membrane marker of retinal neurons that release glycine as their neurotransmitter and is found predominantly in amacrine cells (ACs) (Pow, 1998; Pow and Hendrickson, 2000). The uptake of tritiated glycine and the expression of GlyT1 in the rat retina indicated that half of the ACs are glycinergic (Menger et al, 1998). There are around 8-10 known types of glycinergic ACs and they

are typically NF-ACs (MacNeil and Masland, 1998; Menger et al, 1998). Figure 2-3 shows an example of some of the glycinergic NF-ACs in the mouse retina.

Figure 2-3. The different morphological types of glycinergic ACs in the mouse retina. Five examples of glycinergic ACs from a transgenic mouse (GFP-O) which expresses green fluorescent protein (GFP) under the control of a thy-1 promoter (Feng et al, 2000; Heinze et al, 2007). The retina is double labeled with calretinin (red) to show the different layers of the IPL. **(A)** The most common glycinergic AC is the AII AC; **(B)** Type 2; **(C)** Type 3; **(D)** Type 4; **(E)** Type 7 (all according to the classification by Menger et al, 1998); **(F)** A8 (according to the classification by Kolb et al, 1998). INL= inner nuclear layer; IPL= inner plexiform layer; GCL= ganglion cell layer; S1-S5= substrata of the IPL (Source: Modified from Wässle et al, 2009).

The most commonly studied glycinergic AC is the AII AC, which plays a crucial role in the signaling of information under scotopic conditions. Present at the glycinergic chemical synapse between AII ACs and the cone HBCs is a fast conducting GlyR α 1 (Sassoè-Pognetto et al, 1994). Gill et al (2006) observed that the glycinergic spontaneous inhibitory postsynaptic currents (sIPSCs) of AII ACs in the rat display fast kinetics and a deactivation time course composed of a fast and a slow component. In addition, single-channel analysis yielded conductance states characteristic for α 1 β heteromeric and α 1 homomeric receptors, suggesting both types of receptors exist in AII ACs. In contrast, Weiss et al (2008) did not observe the fast kinetics of glycine sIPSCs in AII ACs, but

rather observed medium fast kinetics more like the $\alpha 3$ subunit. Weiss et al (2008) compared glycine activated currents and sIPSCs of AII ACs in retinal slices from WT mice and mice deficient for the $\alpha 1$ (*Glr1^{spd-ot}*), $\alpha 2$ (*Glr2^{-/-}*) and $\alpha 3$ (*Glr3^{-/-}*) subunits. There were no differences between WT and *Glr1^{spd-ot}* and *Glr2^{-/-}* AII ACs (Figure 2-4A), however no glycinergic currents could be elicited from AII ACs in *Glr3^{-/-}*. These results suggest that the $\alpha 3$ subunit is an integral component of the GlyRs in AII ACs in the mouse retina. Discrepancies between the two studies are possibly due to differences between albino rats (Gill et al, 2006), WT, and GlyR KO mice (Weiss et al, 2008). In addition, outside-out somatic patches (Gill et al, 2006) versus a whole cell patch clamp technique (Weiss et al, 2008) also may account for the different results.

Figure 2-4. Cumulative frequency plots show the decay time constants of glycineric sIPSCs differ among the AC population. (A) The glycineric decay time constant did not differ between WT, *Glr1^{spd-ot}*, *Glr2^{-/-}* AII ACs. No glycinergic sIPSCs could be elicited from the *Glr3^{-/-}* mouse suggesting the $\alpha 3$ subunit is a necessary component of the synaptic GlyRs in AII ACs. **(B)** The decay time constants of glycineric sIPSCs were significantly longer in the NF-ACs in *Glr2^{-/-}* compared to WT, *Glr1^{spd-ot}* and *Glr3^{-/-}*. These results suggest that NF-ACs, type 5/6 and 7 receive glycinergic inputs via the $\alpha 2$ subunit (Source: Weiss et al, 2008).

Weiss et al (2008) also examined the contribution of GlyR subunit-specific mediated inhibition in types 5/6 and 7 glycinergic NF-ACs (Figure 2-3E). In the WT mouse retina, the average decay time constant for glycinergic sIPSCs in NF-ACs is ~27 ms, which is considerably slower than the faster conducting $\alpha 3$ subunit ($\tau = \sim 11$ ms). In the *Gla2*^{-/-} NF-ACs few sIPSCs had decay time constants <20ms, however the majority of glycinergic sIPSCs had prolonged decay time constants ($\tau = \sim 69$ ms). The absence of decay time constants with kinetics characteristic of GlyR $\alpha 2$ may simply be due to the lack of $\alpha 2$ subunit expression; whereas the prolonged decay time constants may be due to an up-regulation of the $\alpha 4$ subunit. Previous immunocytochemical studies have shown that about a third of GlyR $\alpha 2$ post-synaptic clusters are co-localized with the $\alpha 4$ subunit (Heinze et al, 2007). Weiss et al (2008) concluded it is possible that the results for *Gla2*^{-/-} NF-ACs are due to a serial inhibitory or network effects, where the absence of GlyR $\alpha 2$ expression disinhibits a neighboring AC with very slow response kinetics, thus causing prolonged decay time constants. Regardless, in the mouse retina type 5/6 and 7 NF-ACs receive glycinergic inputs via the GlyR $\alpha 2$ and are not dependent on the $\alpha 1$ or $\alpha 3$ subunits (Figure 2-4B).

Although the majority of WF-ACs are GABAergic (MacNeil and Masland, 1998; Menger et al, 1998), Veruki et al (2007) explored the functional properties of glycine receptors in a population of WF-ACs using whole-cell and outside-out patch recording techniques. The WF-ACs were identified by a medium sized soma and long, thin processes that stratify within a single layer (S2, S3 or S4) of the IPL. The kinetic properties of the WF-ACs were markedly different from those of their previous results for glycinergic currents in AII ACs (Gill et al, 2006). The sIPSCs in WF-ACs had a slow

decay time constant and slowed desensitization and deactivation kinetics (Figure 2-5). The slow kinetics along with the single-channel conductance analysis indicated that glycinergic inputs to WF-ACs are mediated by the $\alpha 2$ subunit. Later, Majumdar et al

Figure 2-5. Wide-field amacrine cells have slower kinetic profiles compared to AII amacrine cells. (A) The sIPSCs of WF-AC recorded from a rat retinal slice have much slower decay time course compared to AII ACs. The average waveforms were aligned at onset after the peak amplitudes were normalized. (B) A trace from an outside-out patch shows the slower deactivation and desensitization kinetics of WF-ACs compared to AII ACs. The average waveforms were aligned at onset after the peak amplitudes were normalized (Source: Veruki et al, 2007).

(2009) confirmed the previous results by demonstrating glycinergic currents in a group of WF-ACs are mediated by GlyR $\alpha 2$. They characterized and compared glycine evoked IPSCs (eIPSCs) and sIPSCs in a variety of WT, *Glra1^{spd-ot}*, *Glra2^{-/-}* and *Glra3^{-/-}* displaced GABAergic WF-ACs. They classified these cells based on their response to the exogenous application of glycine. Group I cells consisted of: medium-field ACs (MF-ACs) that stratified throughout the IPL, polyaxonal ACs (PA-ACs) that stratified in the innermost and outermost layers of the IPL; A17 ACs; and a WF-AC that stratified in the Off sublamina of the IPL. Group II cells consisted of: a PA-AC that stratified in the On sublamina and WF-ACs that stratified in multiple layers of the IPL. Group III cells

consisted of the ON starburst ACs. There were no differences in the glycine eIPSCs or sIPSCs between WT and the GlyR KO mice in Group I cells (Figure 2-6A).

Immunocytochemical staining showed GlyR α 2 and GlyR α 4 labeling on the dendrites of Group I cells. This suggests that glycinergic currents elicited in WT and all three GlyR KOs are mediated by GlyR α 2 and GlyR α 4. In Group II cells, no glycinergic currents could be recorded in the *Gla2*^{-/-} mouse whereas glycinergic currents could be elicited from WT, *Gla1*^{spd-ot} and *Gla3*^{-/-} (Figure 2-6B). These results suggest that glycinergic inhibition to Group II cells is only mediated by GlyR α 2. Group III cells showed a reduction in glycine eIPSCs in *Gla2*^{-/-} suggesting glycinergic currents in ON starburst ACs are mediated by GlyR α 2, but the decay time constants became faster (Figure 2-6C). Immunocytochemistry shows a high density of GlyR α 4 on the dendrites of ON starburst ACs (Heinze et al, 2007). Together, these results suggest that the kinetics mediating glycinergic currents in ON starburst ACs are slower than GlyR α 2 and are therefore mediated by GlyR α 4.

Figure 2-6. Cumulative frequency plots of decay time constants for Group I, Group II and Group III WF-ACs. Histograms of the frequency of the decay time constants (τ) were calculated and normalized for each cell. The cumulative frequency plots are derived from the integration of these histograms for WT and the three GlyR mutants. **(A)** Group I cells. There is no difference in τ between WT and the three GlyR mutant mice. The deletion of GlyR α 1 is lethal at postnatal week three. Therefore, τ for WT juveniles were used as age matched controls and compared to *Glr1^{spd-ot}* and still the curves were similar. **(B)** Group II cells. No eIPSCs or sIPSCs could be recorded from *Glr2^{-/-}* suggesting that glycinergic currents in these cells are mediated by GlyR α 2. **(C)** Group III cells. With the exception of the τ in *Glr2^{-/-}*, the curves were similar between WT and the other GlyR mutants. The τ in the *Glr2^{-/-}* are shifted left and are faster than WT and the other GlyR mutants suggesting the presence of a receptor with slow kinetics in ON starburst ACs. Immunocytochemistry staining shows dense α 4 labeling on ON starburst AC dendrites (Heinze et al, 2007) suggesting glycinergic currents in these cells are mediated by GlyR α 4 (Source: Majumdar et al, 2009).

Localization of Glycine Receptors and Bipolar Cells

BCs receive excitatory glutamatergic signaling from the photoreceptors in the outer retina and relay this signal to the RGCs in the inner retina, and also receive GABAergic and glycinergic inhibition from ACs in the inner retina. Electrophysiological studies have reported glycinergic currents in retinal BCs (Cui et al, 2003) but were unable to determine which GlyR α subunit mediated those currents. Recently, Ivanova et al (2006) recorded eIPSCs and sIPSCs from retinal slices in WT, *Glr1^{spd-ot}* and *Glr3^{-/-}*. No glycinergic currents could be recorded from *Glr1^{spd-ot}* BCs even after the application of a high dose of glycine (10 nM). In contrast, eIPSCs did not differ between WT and *Glr3^{-/-}* BCs. Moreover, only cone HBCs and some rod DBCs had demonstrable glycinergic currents mediated by GlyR α 1, whereas cone DBCs do not receive any glycinergic inhibition. In addition, Eggers et al (2007) demonstrated that the contribution of GlyR-mediated inhibitory inputs varies across BC type in the IPL. Cone HBCs

receive the most glycinergic input, whereas rod DBCs receive a small glycinergic input. They also showed that cone DBCs do not receive any demonstrable glycinergic input (Figure 2-7).

Figure 2-7. Glycine receptor-mediated inputs differ across BC class. Glycine receptor-mediated inputs dominate the light-evoked inhibitory postsynaptic currents (L-IPSCs) in cone HBCs. A small amount of glycine receptor-mediated input to rod DBCs is detectable. However, no glycine-mediated currents can be recorded in cone DBCs. Scale bar 10pA and 200ms (Source: Eggers et al, 2007).

Localization of Glycine Receptors and Retinal Ganglion Cells

Earlier studies applied strychnine to the retina to demonstrate the effects of glycine-mediated inhibition on the responses of RGCs; however these studies produced conflicting results. Similar effects of strychnine were reported for a variety of species that include an overall increase in the spontaneous and evoked activity of RGCs when glycinergic transmission is blocked (Burkhardt, 1972, Kirby and Enroth-Cugell, 1976, Miller and Dacheux, 1977). Caldwell et al (1978) noted that strychnine shortened or abolished the transient component of RGCs to a spot or annulus but that RF spatial organization remained intact. Saito (1981) reported strychnine had differential effects on the RF surround component of cat X and Y ON- and OFF-center RGCs. Müller et al (1988) reported that in addition to increasing the light responses of ON-center RGCs,

strychnine also increased the light responses of OFF-center RGCs even when the signaling through the On pathway was blocked. Stone and Pinto (1992) examined RF center/surround organization in a GlyR α 1 mutant mouse, *spastic*, but a decrease in the expression levels of the β subunit caused severely altered RGC responses. O'Brien et al (2003) recorded the responses of cat Y (alpha) type RGCs to a stimulus opposite in contrast to that preferred by the RF center and reported that glycinergic inhibition mediates inputs to the RF center either at the BC terminals or directly to alpha RGCs. In addition, they showed that glycine is not involved in RF surround inhibition but that inputs to the surround are mediated by GABA. This is consistent with previous reports that GABAergic, and not glycinergic inhibition, generate the RF surround response (Cook and McReynolds, 1998). A common effect of glycine-mediated inhibition on the responses of RGCs is difficult to interpret from the previous studies for the following reasons. First, strychnine is a non-specific blocker of all GlyRs and the contribution of GlyR subunit-specific inhibition cannot be determined. Second, glycinergic ACs have been shown to be involved in serial inhibitory circuits with GABAergic ACs (Zhang et al, 1997) and strychnine would not only effect the target RGC but would also effect the output of other BCs and ACs pre-synaptic to the RGC (O'Brien et al, 2003).

Only recently through the use of GlyR KO mutant models, has the contribution of GlyR subunit-specific inhibition to RGCs been examined. Majumdar et al (2007) identified three classes of A-type RGCs in the mouse retina that express GlyR α 1 and receive inhibitory synaptic input via glycinergic ACs. The three A-type RGCs (A₁, A₂-inner and A₂-outer) comprise <10% of the RGC population and have been previously characterized in rat and mouse (Sun et al, 2002a; Sun et al, 2002b). A₁ RGCs are located

in the inner On sublamina of the IPL and the A₂-inner and A₂-outer RGCs are located in the On and Off sublamina, respectively. Several studies have shown that the inhibitory actions of GABA and glycine receptors shape the excitatory responses of A-type RGCs (Pang et al, 2003; Rotolo and Dacheux, 2003). The A-type RGCs in mouse are considered the homologues of the alpha and M cells in cat and primate, respectively, and share similar functional characteristics (Wässle, 2004). This cell type detects transient changes in the visual environment and relays this information to higher cortical areas with high temporal resolution, a function that is well suited for the fast kinetics of the $\alpha 1$ subunit (Wässle, 2004; Levick, 1996). To date there are about 12 morphological types of RGCs (Wässle, 2004) and the different types of GlyR subunits that comprise the post-synaptic clusters to the remaining RGC classes have not yet been identified.

Specific Aims

The role of receptor subtypes in neuronal function is frequently studied using pharmacological manipulations of receptor subtype selective agonists and antagonists. However, the lack of specific GlyR subunit antagonists has limited this approach and the functional analysis of their contributions to the visual response properties of retinal neurons, in particular RGCs. Picrotoxinin, a GABA_AR and GABA_CR antagonist has also been shown to be an antagonist of homomeric GlyRs (Pribilla et al, 1992); however, with a heteromeric (α/β) configuration of mature GlyRs, picrotoxinin is no longer effective (Han and Slaughter, 2004). Therefore, the use of genetically manipulated animal models has provided the only way to understand the contribution of individual subunit-mediated glycinergic inhibition in RGCs and to the overall function of the retina.

The overall goal of this research is to characterize the contribution of glycinergic inhibition to the visual responses and RF organization of RGCs in GlyR α 2 and GlyR α 3 KO models and compare them to WT controls. Specifically, I performed experiments to characterize the responses to stimuli in ON- and OFF-center RGCs at light and dark adapted levels using *in vivo* electrophysiology techniques. The differences from WT responses will define more clearly how subunit specific glycinergic inhibition shapes visual processing in the cone and rod pathways as well as the On and Off pathways of the retina. Moreover, this assessment of GlyR α 2- and GlyR α 3-mediated inhibition in RGCs will be the first *in vivo* contribution to the vast morphological and *in vitro* literature currently established for the different isoforms of the GlyR.

CHAPTER 3

RESEARCH DESIGN AND METHODS

Animals

Wild type *C57BL/6J* (Jackson Labs) mice and two knockout mouse lines in which expression of the GlyR α 2 (*Gla2*^{-/-}) or the GlyR α 3 (*Gla3*^{-/-}) subunit is eliminated were used in these experiments. The *Gla2*^{-/-} mouse was a gift from Dr. Connie Cepko in the Department of Genetics at the Harvard Medical School in Boston, Massachusetts. The *Gla3*^{-/-} mouse was a gift from Drs. Heinrich Betz and Ulrike Müller in the Department of Neurochemistry at the Max-Planck Institute in Frankfurt, Germany and the Department of Bioinformatics and Functional Genomics at the Institute for Pharmacology and Molecular Biotechnology in Heidelberg, Germany, respectively. The mice were maintained at the University of Louisville on a 12:12 light/dark schedule. All experimental procedures were conducted in accordance with regulations described for the ethical care and treatment of animals in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and in compliance with a protocol approved by the University of Louisville Institutional Animal Care and Use Committee.

Glycine Receptor Alpha 2 Subunit Knock-out Mouse ($Gla2^{-/-}$)

Young-Pearse et al (2006) generated GlyR α 2 KO mice ($Gla2^{-/-}$) by eliminating exons 6 and 7 of the *Gla2* gene. This region encodes the protein for the first and second pore-forming transmembrane domains of the GlyR and its deletion abolishes GlyR α 2 function. Although GlyR α 2 is the primary subunit expressed during embryonic development, mice that lack the expression of *Gla2* develop normally and *in situ* hybridization of a P0 retinal slice revealed no abnormal morphological or molecular changes in $Gla2^{-/-}$ compared to WT (Young-Pearse et al, 2006). In addition, electroretinogram (ERG) analysis did not reveal any functional differences between $Gla2^{-/-}$ and WT mice.

Glycine Receptor Alpha 3 Subunit Knock-out Mouse ($Gla3^{-/-}$)

Harvey et al (2004) generated GlyR α 3 KO mice ($Gla3^{-/-}$) by eliminating exon 7 of the *Gla3* gene. Exon 7 encodes for the first transmembrane domain and the second pore-forming transmembrane domain of the GlyR. Its deletion abolishes GlyR α 3 function. The $Gla3^{-/-}$ mutant mice develop normally, show proper motor coordination, righting behavior and are able to reproduce. In addition, there are no gross morphological abnormalities in the retina (Haverkamp et al, 2003).

Surgical Preparation for Electrophysiology Recordings from the Optic Nerve Fibers

All surgical procedures were performed under light adapted conditions. Adult mice were anesthetized with an initial intraperitoneal injection of a Ringer's solution containing ketamine (127mg/kg) and xylazine (12mg/kg). For $Gla2^{-/-}$ mice a

concentration of 12.5% more of the initial dose was needed to properly anesthetize the animal. Anesthesia was maintained throughout the experiment with supplemental subcutaneous injections administered, as needed (~ every 45 min). Recording sessions for each RGC lasted around 2 hrs with a total recording time between 9-10 hrs. The head was secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with ear cups and a bite bar. Body temperature was maintained at 37°C with a feedback controlled heating pad (TC-1000; CWE, Ardmore, PA). Topical Mydfrin (Phenylephrine hydrochloride ophthalmic solution 2.5%) and Mydriacyl (Tropicamide ophthalmic solution 1%; Alcon Labs, Inc., Fort Worth, TX) were applied to dilate the pupils and paralyze accommodation. To prevent drying of the corneas, clear zero-powered lenses (Sagdullaev et al, 2004) moistened with artificial tears (Akwa Tears, Akorn, Inc., Buffalo Grove, IL.) were placed over the eyes. A craniotomy was performed anterior to the Bregma suture and the overlying cortex was removed to expose the right optic nerve.

In vivo Electrophysiology Recordings from Optic Nerve Fibers

Action potentials were recorded extracellularly from the optic nerve using sharpened tungsten microelectrodes (A-M Systems, Inc., Carlsborg, WA.) with a final impedance between 30-100M Ω . A reference electrode was inserted subcutaneously on the back of the neck. Action potentials from a single optic fiber were isolated, amplified (X3+Cell, slope/height window discriminator, amplifier, FHC, Bowdoinham, ME), digitized at 15 kHz (Power1401, CED, UK) and stored for offline analysis. The isolated spike trains were simultaneously displayed on an oscilloscope (60MHz, Tektronix Inc., Beaverton, OR) and computer monitor (Spike2, CED, UK) and played over an

audiomonitor (AM7, Grass Instruments, Quincy, MA) to obtain direct feedback of the cell's response.

Characterization and Responses of Retinal Ganglion Cells in the WT Mouse Retina

Each single unit was isolated under ambient room lighting and the spatial extent of its RF was mapped on a removable screen that covered the CRT display monitor (Eizo E120 FlexScan FXC7, Japan) using a hand held ophthalmoscope. The smallest and dimmest spot that could elicit the maximal response from the cell was used. Once the RF was located it was centered on the monitor and placed within a range of 20-25cm from the anterior nodal point of the right eye. All of the stimulus and RF dimensions are corrected for monitor distance and presented as degrees of visual angle. Before computer generated stimuli were presented the RF center sign (ON vs. OFF) was determined and the response duration was characterized as sustained or transient. The majority of ON-center cells have a sustained response (Cleland et al, 1971; Enroth-Cugell and Robson, 1966) and OFF-center RGCs have either a sustained or transient response (Ikeda and Wright, 1972) to a stimulus presented to the RF center. This dichotomy has been observed since the initial extracellular recordings of RGCs in a variety of vertebrate species (cat-Hartline, 1938; Kuffler, 1953; Cleland et al, 1971; mudpuppy-Werblin and Dowling, 1969; primate-Gouras, 1968; squirrel-Michael, 1966). A sustained cell responds with an initial high frequency component followed by a steady-state component during the entire duration of the stimulus; whereas a transient cell responds with high frequency firing rate at stimulus onset but adapts quickly and returns to the level of spontaneous activity, usually in less than one second (Cleland et al, 1971; Kuffler, 1953).

I find the same distribution among my population of wild-type (WT) RGCs (Figure3-1). In my experimental paradigm, I presented a spot of preferred contrast to the RF center for 2 seconds. Sustained ON- and OFF-center RGCs responded for entire duration of the stimulus while transient OFF-center RGCs responded <1.70 seconds. The difference

Figure 3-1. WT ON- and OFF-center RGCs respond in a sustained or transient fashion to a spot of preferred contrast presented to the RF center. (A) The majority of WT ON-center RGCs (97%) are sustained and respond for the entire duration of a stimulus compared to 77% of WT OFF-center RGCs. **(B)** The frequency distribution illustrates the response durations for the remaining 3% of WT ON- and 23% of WT OFF-center RGCs. These cells are characterized as transient RGCs because they respond with an initial increase in firing rate to a preferred stimulus but then decrease firing rate back to the level of spontaneous activity before the end of the stimulus presentation (<1.70 seconds).

in the kinetics of the RGC responses is thought to serve functionally different visual processes (Hamasaki and Winters, 1974). In primate and cat, sustained cells tend to have smaller, well-defined RF centers and are more sensitive to contrast changes, whereas

transient cells have less well-defined RFs and are more sensitive to temporal variations within visual stimuli (Cleland et al, 1973; Ikeda and Wright, 1972).

In our lab, we have used a variety of stimulus durations and found that a stimulus with longer durations (2 second) not only properly characterizes RGCs into sustained and transient, but also shows that there are varying degrees of sustained and transient responses. Therefore, I derived a measurement to quantify the degree of sustained and transient responses by computing a Sustained/Transient Index which is a ratio of $[(\text{Peak} - \text{Spontaneous Activity}) / (\text{Maintained Firing Rate} - \text{Spontaneous Activity})] / \text{Peak} - \text{Spontaneous Activity}$. In this way, I can determine the degree by which the response remains above baseline firing rate (<1.0 =more sustained and 1.0 = transient). This measurement is indicative of the amount of inhibitory inputs a particular RGC receives in order to shape the response and can provide hypotheses as to which type of inhibitory receptors are mediating those inputs. Figure 3-2 illustrates the distribution of Sustained/Transient Index scores for WT ON- and OFF-center RGCs.

Figure 3-2. The distribution of Sustained/Transient Index values for WT ON- and OFF-center RGCs. The Sustained/Transient Index is used to quantify the degree of a sustained and transient response to the presentation of a 2 second stimulus. An index value of 1.0=transient and less than 1.0= more sustained. For WT ON-center RGCs (solid circles), 97% respond for the entire duration of the stimulus with ratios that range from 0.22 to 0.94 whereas the remaining 3% range from 0.70-1.0. For WT OFF-center RGCs (open circles), 77% respond for the entire duration of the stimulus with ratios that range from 0.26 to 0.97 whereas the remaining 23% range from 0.70 to 1.0.

Quantitative Characterization of Retinal Ganglion Cell Visual Response Properties

To quantify the visual response properties of RGCs, a series of computer generated spot and annular stimuli (VisionWorks; Vision Research Graphics, ME) were presented on a CRT monitor with a luminance range between 0-100cd/m². Spots and annuli were of standing contrast and outer and inner diameter varied (4.6° to 52.7°), respectively. Responses were accumulated with a 50ms bin width and displayed as post-stimulus time histograms (PSTH). The average PSTH was smoothed by fitting with a raised cosine function with a 50ms smoothing interval (Sagdullaev et al, 2005). The spontaneous activity was measured from random “Blank” trials that lasted 7 seconds with

the monitor set to a luminance of 20cd/m^2 and the average defined the RGC's mean firing rate. The standard deviation of the spontaneous activity/ $\sqrt{n}$ was used to determine the standard error of the mean to define a threshold for excitatory and inhibitory responses. The standard error is an estimate of how close the sample mean is to the parametric mean. Since my samples are very large, I chose $\pm 3\text{SEM}$ because nearly the entire sample means (99.7%) would be within three standard errors of the parametric mean (www.udel.edu).

Adaptation Levels

To alter adaptation level, the mean luminance of the display monitor background was varied. At light adapted (LA) levels, the monitor background was set to 20cd/m^2 to elicit responses driven primarily from the cone pathway. To record dark adapted (DA) responses the room was completely darkened for 20 minutes and the monitor was set to 0cd/m^2 . During this adaptation period the RGC firing rate was monitored to ensure the same cell was isolated. After DA the RF was re-characterized and the appropriate protocols were performed.

Area Response Function

I used an area response function (ARF) to define spatial summation, surround suppression and the optimal stimulus for each RGC. To produce an ARF, computer generated spot stimuli of standing contrast (67%) (VisionWorks; Vision Research Graphics, ME) and varying diameter (4.6° to 52.7°) were presented to ON- and OFF-center RGCs. Bright spots for ON-center RGCs (100cd/m^2 ; Figure 3-3A) and dark spots

for OFF-center RGCs (3cd/m^2 ; Figure 3-4A) were presented on a mean luminance background (20cd/m^2). There were 8 different spot diameters and each spot was presented for 2 seconds with a 5 second inter-stimulus interval eight times for a total of 64 presentations. The peak responses were plotted as a function of spot size to construct ARFs (Figure 3-3B and 3-4B). The ascending limb of the ARF and its slope evaluates spatial summation: the rate of increase in the peak firing as spot size changes. The optimal spot diameter, defined as the stimulus that elicited the maximum response from the RGC, is the peak of the ARF curve. As the spot size increased beyond the RF center the peak firing rate decreased and the slope and magnitude of the decrease are used to define surround suppression.

Figure 3-3. An example of the stimuli presented to WT ON-center RGCs at LA levels to generate an ARF. (A) WT ON-center RGCs were presented bright spots (100cd/m^2) of varying diameter (4.6° to 52.7°). All spots were presented on a mean luminance background (20cd/m^2). The event correlations illustrate a RGC response during center stimulation with a small spot (i), a spot that matches the RF center (ii), and a large spot (iii). **(B)** An Area Response Function is the peak response plotted as a function of spot size. The ascending portion of the curve demonstrates spatial summation, the peak represents the maximum response at the optimal spot matched to the RF center, and the descending portion of the curve illustrates surround antagonism. The dotted line represents the average spontaneous activity level for WT ON-center RGCs (~ 29 spks/sec).

Figure 3-4. An example of the stimuli presented to WT OFF-center RGCs at LA levels to generate an ARF. (A) WT OFF-center RGCs were presented dark spots (3cd/m^2) of varying diameter (4.6° to 52.7°). All spots were presented on a mean luminance background (20cd/m^2). The event correlations illustrate a RGC response during center stimulation with a small spot (i), a spot that matches the RF center (ii), and a large spot (iii). (B) An Area Response Function is the peak response plotted as a function of spot size. The ascending portion of the curve demonstrates spatial summation, the peak represents the maximum response at the optimal spot matched to the RF center, and the descending portion of the curve illustrates surround antagonism. The dotted line represents the average spontaneous activity level for WT OFF-center RGCs (~ 9 spks/sec).

Measures of the Excitatory Component at Stimulus Onset

At LA levels, the excitatory component of the response was measured at the onset of a bright or dark spot for ON- and OFF-center RGCs, respectively (Figure 3-5A and C). At DA levels, ON-center RGC responses were measured at the onset of the stimulus, whereas OFF-center cell responses were measured at its offset (Figure 3-5B and D). The parameters for measuring the excitatory components remain the same for LA and DA ON-center RGCs and LA OFF-center RGCs.

The diagrams in Figure 3-5 show the various excitatory response components derived from the RGC's average PSTH at the optimal spot matched to the RF center. For the excitatory portion of the response the following parameters were quantified. The excitatory response was measured as the total excitatory area above spontaneous activity during the entire presentation of a stimulus (0-2 sec, colored regions). This measurement was further divided into a transient, peak component (0-0.4 sec) (dark shaded areas) and a sustained, maintained component (0.4-2.0 sec) (lightly shaded areas). I used the interval between 0-0.4 seconds as the transient component because 37% (or $1/e$) of the peak decay occurs within this time point for all RGCs. The time interval from stimulus onset to the peak response measured the cell's time to peak. For all measures, the onset of excitation was when the response crossed +3SEM and the offset of excitation was when the response fell below -3SEM. The duration of the response was measured as the time interval over which the cell's firing rate remained above +3SEM until the response crossed below spontaneous activity (End of response–Response Onset = Duration). Within our database of 50 WT transient RGCs the response duration did not exceed 1.7

seconds and out of 416 WT sustained RGCs the response duration was never terminated before 2.0 seconds.

Figure 3-5. The average PSTHs represent the parameters used to quantify the excitatory components of WT ON- and OFF-center RGC responses at LA and DA levels. (A) At LA levels, WT ON-center RGCs increase their firing rate to a bright spot (100cd/m^2) presented to their RF center on a light background (20cd/m^2). (B) At DA levels, WT ON-center RGCs also increase their firing rate to a dim spot (3cd/m^2) presented on a dark background (0cd/m^2). The excitatory response begins when the response crosses above $+3\text{SEM}$ of spontaneous activity and ends when the response crosses below spontaneous activity. The transient peak response ($0\text{--}0.4\text{s}$) and the maintained portion of the response ($0.4\text{--}2.0\text{s}$) are represented by the dark and light shaded regions, respectively. (C) At LA levels, WT OFF-center RGCs increase their firing rate to a dark spot (3cd/m^2) presented on a light background (20cd/m^2). The excitatory response begins when the response crosses above $+3\text{SEM}$ of spontaneous activity and ends when the response crosses below spontaneous activity. The transient peak response ($0\text{--}0.4\text{s}$) and the maintained portion of the response ($0.4\text{--}2.0\text{s}$) are represented by the dark and light shaded regions, respectively. (D) At DA levels, WT OFF-center RGCs are suppressed to the presentation of a dim spot (3cd/m^2) on a dark background (0cd/m^2), but have a large, transient peak response at the offset of the dark-adapted stimulus. The peak response at onset at LA levels and at offset at DA levels is used to make comparisons between WT OFF-center RGCs responses under different adaptation levels.

Annulus Response Function

To independently assess the inhibitory RF surround I used an Annulus Response Protocol, which isolates the surround response. ON-center RGCs have ON center and OFF surround responses (Figure 3-6); whereas OFF-center RGCs have OFF center and ON surround responses (Figure 3-7). To isolate and examine RF surround suppression, I used computer generated annular stimuli of standing contrast (67%) and varied the inner diameter ($4.6^\circ\text{--}37.8^\circ$). The annulus contrast was the same as the preferred contrast of the RF center. Thus, ON-center RGCs were stimulated with a bright annulus (100cd/m^2) and OFF-center RGCs were stimulated with a dark annulus (3cd/m^2) on a mean luminance background (20cd/m^2). An Annulus Response Function (AnRF) plots the minimum firing rate to the presentation of an annulus as a function of inner diameter. An AnRF is the inverse of an ARF due to the opposite yet equal effect each mechanism has when

stimulated independently from one another (compare Figures 3-3B; 3-4B to Figures 3-6B; 3-7B). The descending portion of the AnRF represents RF center excitation to annuli with small inner diameters. The inner diameter that elicits maximum suppression is termed the optimal annulus and all suppressive response components are compared at the optimal annulus. The suppressive response components during the presentation of an annulus (0-5.0 seconds) were quantified from the average PSTHs. The spontaneous activity was recorded for each cell during random “Blank” trials (10 seconds) and was estimated as the average firing rate with the monitor intensity at a mean luminance (20cd/m^2). The computed standard deviation of the spontaneous activity was used to set the threshold for suppressive responses. Each trial block consisted of eight stimulus presentations, including a blank trial, and each block was presented eight times for a total of 64 stimulus presentations. All measures are reported as mean $\pm 3\text{SEM}$ of spontaneous activity.

Light Adapted

ON-center RGCs

Figure 3-6. Examples of the stimuli presented to WT ON-center RGCs to generate an AnRF at LA levels. (A) WT ON-center RGCs were presented with a bright annulus (100cd/m^2) with a 20cd/m^2 inner diameter on a mean luminance background (20cd/m^2). Annuli with small inner diameters stimulate the RF center and cause an increase in firing rate (i). Annuli with an inner diameter that matches the RF center, stimulates the RF surround and causes a decrease or suppression of the cell's firing rate (ii). Annuli with large inner diameters still suppress the RF surround but the magnitude of suppression is decreased (iii). (B) An AnRF plots the firing rate as a function of inner diameter. The dotted line represents the average spontaneous activity for WT ON-center RGCs to the presentation of an annulus (~ 37 spks/s).

Figure 3-7. Examples of the stimuli presented to WT OFF-center RGCs to generate an AnRF at LA levels. (A) WT OFF-center RGCs were presented with a dark annulus (3cd/m^2) with a 20cd/m^2 inner diameter on a mean luminance background (20cd/m^2). Annuli with small inner diameters stimulate the RF center and cause an increase in firing rate (i). Annuli with an inner diameter that matches the RF center, stimulates the RF surround and causes a decrease or suppression of the cell's firing rate (ii). Annuli with large inner diameters still suppress the RF surround but the magnitude of suppression is decreased (iii). **(B)** An AnRF plots the firing rate as a function of inner diameter. The dotted line represents the average spontaneous activity for WT OFF-center RGCs to the presentation of an annulus (~ 10 spks/s).

Measures of the Suppressive Response at Stimulus Onset

At LA levels, the suppressive component of the response was measured at the onset of a bright or dark annulus for ON- and OFF-center RGCs, respectively. Figure 3-8 illustrates the measurements that were used to quantify the RF surround response. Total suppression of the isolated RF surround is measured as the total area (spikes/s²) below spontaneous activity. All RGCs that are suppressed by an annulus have a transient suppression that occurs in the initial phase of the response and some RGCs have sustained suppression that lasts for the entire presentation of an annulus (5sec). I derived a measurement to calculate the decrement of the response that falls below spontaneous activity and normalized the RF surround suppression across inner diameter. The Response Decrement for transient suppression is a ratio of the (Minimum firing rate-Spontaneous Activity)/Spontaneous Activity. The Response Decrement for maintained suppression is a ratio of the (Maintained firing rate-Spontaneous Activity)/Spontaneous Activity. The onset latency of suppression is defined as the time point at which the response crosses below -3SEM of spontaneous activity at annulus onset. In addition, the duration of the response is estimated as the time interval the cell's firing rate crosses -3SEM of spontaneous activity at annulus onset until the response returns to spontaneous activity and is calculated as: (End of suppression-Onset of suppression). Together total suppression, response decrement, onset latency and duration indicate the strength of the surround mechanism from which I can hypothesize the magnitude of inhibitory inputs RGCs receive to their RF surround and the type of receptors that are mediating these inputs.

Figure 3-8. Average PSTHs of a WT ON- and OFF-center RGC response to the presentation of an optimal annulus at LA levels. (A) At the onset of a bright annulus (100cd/m^2), the firing rate of WT ON-center RGCs drops below the level of spontaneous activity. At the offset of an annulus there is a post-stimulus excitatory response. **(B)** At the onset of a dark annulus (3cd/m^2), the firing rate of WT OFF-center RGCs drops below the level of spontaneous activity. WT OFF-center RGCs also have a post-stimulus response at stimulus offset. The onset, duration (arrows) and total suppression (shaded regions) are used to determine the strength of the isolated RF surround response.

Cluster Analysis

When visual responses of cat RGCs were first characterized in the 1950's and 60's, recordings were performed using extracellular recordings from the optic nerve. This approach yielded two types of RGCs were named X- and Y-cells (Enroth-Cugell and Robson, 1966). Subsequently, the morphological equivalents were defined (Boycott & Wässle, 1974) and X-cells were represented by β cells, while Y-cells were represented

by α cells. During this time, recordings were made directly from the cat retina and other functional classes were defined and correlated to different morphological types (Fukuda et al, 1984; Stanford 1987; Stein and Berson, 1995; Berson et al, 1998, 1999; Isayama et al, 2000). With these new experiments it became clear that the extracellular recordings were biased toward the RGCs with the largest axons, the X- and Y-cells (Fakuda et al, 1984).

Light evoked responses of A or α -type RGCs in the mouse are commonly targeted for analysis because they are easily identified by their large somas and wide branching dendritic trees (Sun et al., 2002). In the cat retina, the correlation between α RGC structure and visual function was extensively characterized (Cleland et al, 1975; Wassle et al, 1981, Peichl et al, 1987a, b) and subsequently found to comprise about <10% of the entire RGC population (Wassle, 2004). The morphology of α RGCs is reasonably conserved throughout a variety of mammalian species, including mouse (Peichl, 1991; Sun et al., 2002).

In the mouse retina a true structure/function correlation has not been established. We do not know the functional characteristics of murine RGCs with large somas, although three morphological types with large somas (α -like) have been identified (Sun et al, 2002). A₁ RGCs have dendrites that branched in the ON sublaminae of the IPL; A₂-inner RGCs also have dendrites that branch in the same sublamina, whereas the dendrites of A₂-outer branch in the OFF sublamina of the IPL. There has been no attempt to date to characterize the axon diameters of these or any murine RGCs. Function in these RGCs with large somas has been assessed to some extent. Majumdar et al (2007) characterized

their spontaneous and glycine-evoked currents; Pang et al (2003) and Van Wyk et al (2009) recorded their light-evoked currents and classified them as ON sustained, OFF sustained and OFF transient.

Because my experiments used extracellular recordings from the optic nerve of mice, I could not determine their morphological equivalents. Although we assume that α -like murine RGCs should have the largest axons, we do not know if the axon diameters of other RGC morphological classes overlap. Because of this gap in our understanding of the number of morphological and functional types of RGCs that exist in the mouse retina, I wanted to independently examine if there were different populations within my sample of ON- and OFF-center RGCs. To do this, I used a statistical approach and performed a cluster analysis based on their responses to a stimulus that matched their receptive field center size and response profile. I performed my analyses separately for ON- and OFF-center RGCs because it is clear that they are morphologically distinct and receive excitatory inputs from two different populations of bipolar cells that use different excitatory glutamate receptors to initiate their response. I used a K-means cluster analysis algorithm to identify homogenous groups of cases based on specific response properties for the cells. First, I generated a correlation matrix (Tables 2 and 3) for all response variables to determine if any of the variables were highly correlated with one another and should be eliminated. The variables total area of excitation and minimum firing rate were eliminated.

Table 2. Correlation Matrix: WT OFF-center RGCs.

	SpontAct	S/TIndex	Peak	MainFR	TTP	RFDiam	TotalSupp	DurSupp	OnsetSupp
SpontAct	1.00	0.05	0.19	-0.01	-0.18	-0.02	0.44	-0.01	-0.15
S/TIndex	0.05	1.00	0.35	-0.62	-0.54	0.24	0.17	0.08	-0.28
Peak	0.19	0.35	1.00	0.21	-0.50	-0.28	0.51	0.45	-0.16
MainFR	-0.01	-0.62	0.21	1.00	0.16	-0.37	0.09	0.09	0.16
TTP	-0.18	-0.54	-0.50	0.16	1.00	-0.09	-0.29	-0.19	0.16
RFDiam	-0.02	0.24	-0.28	-0.37	-0.09	1.00	-0.16	-0.23	-0.19
TotalSupp	0.44	0.17	0.51	0.09	-0.29	-0.16	1.00	0.59	-0.19
DurSupp	-0.01	0.08	0.45	0.09	-0.19	-0.23	0.59	1.00	0.06
OnsetSupp	-0.15	-0.28	0.16	0.16	0.17	0.19	-0.19	0.06	1.00

Table 3. Correlation Matrix: WT ON-center RGCs.

	SpontAct	S/TIndex	Peak	MainFR	TTP	RFDiam	TotalSupp	DurSupp	OnsetSupp
SpontAct	1.00	-0.10	-0.11	-0.05	-0.15	0.19	0.15	-0.01	-0.36
S/TIndex	-0.10	1.00	-0.11	-0.64	-0.31	-0.07	0.08	-0.03	-0.39
Peak	-0.10	-0.11	1.00	0.76	-0.22	0.08	0.45	0.32	-0.04
MainFR	-0.05	-0.64	0.76	1.00	-0.01	0.10	0.27	0.26	0.23
TTP	-0.15	-0.31	-0.22	-0.01	1.00	-0.11	-0.15	0.02	0.39
RFDiam	0.19	-0.07	0.08	0.10	0.11	1.00	-0.13	-0.15	-0.11
TotalSupp	0.15	0.08	0.45	0.27	-0.15	-0.13	1.00	0.80	-0.17
DurSupp	-0.01	-0.03	0.31	0.26	-0.02	-0.15	0.80	1.00	0.05
OnsetSupp	-0.36	-0.39	-0.04	0.23	0.39	-0.11	-0.17	0.05	1.00

Nine variables (Table 2) that describe specific aspects of the excitatory and suppressive aspects of the RGC response were used in a K-means algorithm. This clustering program calculates the Euclidean distance between data points to determine the variance around a centroid, or mean for that particular group. The analysis is performed

with a pre-determined number of clusters or k and the “goodness of fit” is evaluated. Several cluster numbers are evaluated and comparisons are made to determine the optimal number of clusters. For each cluster solution, a second discriminant analysis is performed to obtain a discriminant function coefficient which according to size, is interpreted as the variable(s) having more or less influence on defining the groups (Tables 4 and 5). Discriminant functions are independent and their contributions to defining groups do not overlap (www.statsoft.com). The number of discriminant functions depends on the number of groups-1. In a 2 cluster solution, there are two groups and the analysis yields one discriminant function coefficient. For example, when WT and GlyR KO ON or OFF RGCs were grouped using a 2 cluster solution, peak firing rate was the variable with the most “weight” in both and separated RGCs with low and high peak firing rates.

Table 4. WT OFF-center RGCs
Discriminant Function
Coefficients.

Peak	1.06
SpontAct	0.10
S/T Index	0.11
MainFR	0.11
TTP	0.16
RFDiam	0.08
TotalSupp	-0.11
DurSupp	0.05
OnsetSupp	0.09

Table 5. WT ON-center RGCs
Discriminant Function
Coefficients.

Peak	0.87
SpontAct	-0.01
S/T Index	0.05
MainFR	0.16
TTP	-0.05
RFDiam	0.11
TotalSupp	-0.02
DurSupp	0.22
OnsetSupp	0.08

A Principal Components (PCA) and Factor Analysis was performed to reduce the number of variables, identify variables that account for the most variance and detect structure in the relationships between those variables (www.statsoft.com). PCA transforms the number of correlated variables into a smaller number of uncorrelated variables known as the principal components. The first component accounts for the maximum variance and the successive components explain smaller portions of variance (www.statsoft.com). My analysis produced three principal components that account for 76% of the variability in the original nine variables. Figure 3-9 is the best view of 3D plot that shows the three principal components and the structure of the two groups of OFF RGCs defined by my cluster analysis.

Figure 3-9. A 3D plot of the principal components analysis. Three factors were derived from the PCA (#1: Peak Firing Rate; #2: Maintained Firing Rate; #3: Spontaneous Activity). These three factors account for 76% of the variability in the original nine variables used to define two clusters of OFF RGCs: OFF_{K1} (red squares) and OFF_{K2} (blue circles).

To evaluate the efficacy of the clustering method, I performed two different analyses. First, I evaluated two, three and four cluster solutions using the K-means algorithm by computing MANOVAs and examining the proportion of the variance each solution provided compared to the others, using Wilks' lambda statistic. If a cluster

solution with more groups better separates the RGCs than a solution with a lower number, we expect that the proportion of the variance accounted for should improve by increasing the number of groups. I then performed a cluster analysis with a second algorithm, Ward's hierarchical method, and compared the cluster solutions with those determined by K-means. The assumption here is that a robust solution will arrive at the same number of groups.

OFF-Center RGCs

I compared the Wilks' lambda for the two, three and four K-means cluster solutions and found that the variance accounted for by the three and four cluster solutions did not increase over the two cluster solution. This suggests that the two K-means cluster solution is the optimal solution. When I compared the K-means cluster solution with the Ward's solution I found that there was an 87% identity in the groups using the two techniques (Figure 3-10). For these reasons I chose a 2-cluster solution for my OFF-center RGCs, which I will refer to as OFF_{K1} and OFF_{K2}.

Wards vs K means Clustering for WT OFF RGCs

2 Cluster Solution

Figure 3-10. The two cluster solution using either Ward's or K-means produces similar groups of OFF RGCs. The Venn diagrams illustrate the overlaps in cluster membership when OFF-center RGCs are grouped using either a K-means or a Wards clustering algorithm. Of the 174 OFF-center RGCs that were grouped only 28 did not fall into the same clusters.

It is interesting to note that the proportion of sustained and transient OFF-center RGCs that make up OFF_{K1} and OFF_{K2} RGC populations are similar across WT, *Gla2*^{-/-} and *Gla3*^{-/-} OFF-center RGCs (Table 6 and Table 7). In addition, as will become evident in the rest of my dissertation results, GlyRα2 and GlyRα3 have differential effects on the OFF_{K1} and OFF_{K2} RGCs, respectively.

	OFF _{K1} RGCs		Total
	Sustained	Transient	
WT	66% (n=69)	34% (n=36)	105
<i>Gla2</i> ^{-/-}	44% (n=14)	56% (n=18)	32
<i>Gla3</i> ^{-/-}	67% (n=26)	33% (n=13)	39

Table 6. The proportions of sustained vs. transient OFF-center RGCs in the OFF_{K1} cluster.

	OFF _{K2} RGCs		Total
	Sustained	Transient	
WT	94% (n=65)	6% (n=4)	69
<i>Gla2</i> ^{-/-}	94% (n=14)	6% (n=18)	16
<i>Gla3</i> ^{-/-}	95% (n=26)	5% (n=13)	22

Table 7. The proportions of sustained vs. transient OFF-center RGCs in the OFF_{K2} cluster.

ON Center RGCs

In contrast to OFF-center RGCs, my analyses for ON-center RGCs did not yield a clear optimal clustering solution. To determine if a single cluster solution was preferable to any clustering, I produced a simulated ON-center RGC data set. The simulated data set was produced by generating a normal distribution for each variable, using the mean and the standard deviation for that variable. This data set was entered into the same cluster analyses and the Wilks' lambda was similar to that for the two, three and four cluster solutions using the K-means algorithm. As a consequence I chose to assume that the ON-center RGCs make up a homogenous group of RGCs. To provide further evidence that the ON-center RGCs form a homogenous group, I compared the responses of WT and GlyR KO ON-center RGCs with the K-means two cluster solution. The analyses showed that all ON-center RGCs are similarly changed by the absence of either GlyR α 2 or GlyR α 3 inhibition in a single or two cluster solutions. Thus providing support for the assumption that WT and GlyR KO ON-center RGCs are a single, homogenous group.

Statistical Analyses

Statistical procedures were used to compare the differences in the visual responses of each GlyR KO and WT ON- and OFF-center RGCs. I did not compare *GlyR α 2^{-/-}* and *GlyR α 3^{-/-}* responses because the GlyR α 2 and GlyR α 3: 1) have very different expression patterns in the IPL; 2) have been localized to different retinal cell types; and 3) are believed to participate in different circuits within the retina. I used the following parametric and non-parametric statistics. A non-paired Student's t-test was used to

determine differences between GlyR KO and WT ON- and OFF-center RGCs. A paired Student's t-test was used to determine differences within WT and GlyR KO ON- and OFF-center RGCs at different adaptation levels. A Chi-square test was used to compare the frequencies of cells that increased, decreased or did not change their response from light to dark adapted levels. A Mann-Whitney U test was used to determine differences in RF center diameter (ordinal data) and Sustained/Transient Index scores (interval data). A Mixed ANOVA was used to compare responses across spot and inner diameter. A linear regression analysis was used to determine changes in the slopes of the ARF and AnRF between genotype. All means are reported as $\pm$ SEM and p-values <0.05 are considered statistically significant. Statistical analyses were performed using GraphPad Prism5 Software v5.03 for Windows (San Diego, CA, USA) and PASW Statistics Software v18.0 for Windows (Chicago, IL, USA).

CHAPTER 4

PART I

THE ROLE OF GLYR α 2-MEDIATED INHIBITION IN THE RECEPTIVE FIELD CENTER/SURROUND INTERACTIONS IN RETINAL GANGLION CELLS

Introduction

The visual system adapts to a wide range of ambient intensities and this process begins in the retina. Changes in intensity are encoded in the retina by two parallel processing streams, the ON and OFF pathways, which carry excitatory information about the onset and offset of light, respectively (see Chapter 1, Figure 2). This vertical transmission of information from photoreceptors (PRs) to bipolar cells (BCs) to retinal ganglion cells (RGCs) is modulated by lateral inhibitory inputs from horizontal cells (HCs) in the outer retina and amacrine cells (ACs) in the inner retina. The purpose of this inhibition is to shape the excitatory output of the BCs as well as the excitatory responses of RGCs, which then transmit this information to higher visual processing centers.

The Amacrine Cells

The inner retina contains a diverse morphological group of ACs (>20 types; Masland, 2001) provide inhibitory inputs to BCs and RGCs. ACs comprise around 40% of the population of cells in the inner retina and differ in morphology and in the type of neurotransmitters they use; e.g. glycine, GABA, dopamine, acetylcholine and indoleamines (MacNeil and Masland, 1998). Based on dendritic field size ACs are classified into three broad categories: narrow-field (NF-ACs), medium-field (MF-ACs) and wide-field (WF-ACs). Based on their horizontal and vertical branching patterns, ACs also are classified into mono-, bi- or multi-stratified. Mono-stratified ACs have either an ON or OFF response whereas bi- or multi-stratified ACs can have either an ON, OFF or ON-OFF response. In the mammalian retina the majority of NF-ACs are glycinergic, whereas the majority of WF-ACs are GABAergic (Menger et al, 1998). Because my research focus in this chapter is on the glycine receptor alpha 2 subunit (GlyR α 2), the remainder of this review concentrates on cells with GlyR α 2-mediated currents.

Glycine Receptors and their Distribution in the Inner Retina

Recent studies have examined the expression and distribution patterns of glycine receptor (GlyR) alpha subunits (α 1- α 4) in the IPL and their localization to particular cell types (Heinze et al, 2007; Wässle et al, 2009). GlyR α 1 has been shown to mediate chloride currents in cone HBCs and A-type RGCs and GlyRs α 2 and α 3 have been shown to mediate chloride currents in WF- and NF-ACs (Ivanova et al, 2006; Majumdar et al, 2007; Weiss et al, 2008; Majumdar et al, 2009). The diverse subunit distribution,

differences in current kinetics and the proportion of receptor subunits expressed on specific cell types, leads to my hypothesis that specific GlyR subunits participate in different IPL circuits and play specific roles in visual processing in RGCs. This chapter focuses on GlyR α 2 and its role in shaping the RF excitatory center responses, as well as its contribution to the RF center/surround organization of RGCs.

GlyR α 2 in the Inner Retina

Of the four GlyR α subunits, α 2 is the most widely expressed in the retina and is located throughout the On and Off sublamina of the IPL and mediates chloride currents in a number of NF- and displaced WF-ACs (Figure 4-1; Haverkamp et al, 2004;

Figure 4-1. GlyR α 2 expression is widespread throughout all layers of the IPL. A photomicrograph shows the immunoreactivity of the α 2 subunit expressed throughout the layers of the IPL from Heinze et al, 2007 (Scale bar =50 μ m). A schematic of the retina shows the cell types that receive glycinergic inhibition via the α 2 subunit (). (ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; off, on: Off and On sublamina of the IPL; AC_{NF}: narrow-field amacrine cells; dAC_{WF}: displaced wide-field amacrine cells; AII_{AC}: AII amacrine cell, rod DBC and cone DBC: rod & cone depolarizing bipolar cells, cone HBC: cone hyperpolarizing bipolar cells, ON & OFF RGC: A type retinal ganglion cells; Gly= glycine; GABA= GABA; Glu= glutamate).

Weiss et al, 2008; Majumdar et al, 2009). Weiss et al (2008) assessed the contribution of glycinergic receptors in synaptic transmission by recording and comparing glycinergic spontaneous inhibitory post-synaptic currents (sIPSCs) in three types of NF-ACs: AII, Type 5/6 and Type 7, all of which are bi- or multi-stratified. The decay time constants of glycinergic currents in NF-ACs Type 5/6 and 7 in *Gla2*^{-/-} mice were significantly prolonged compared to WT, indicating that GlyR α 2 mediates synaptic input in these cells. In contrast, glycinergic currents did not change in NF-AII ACs suggesting that GlyR α 2 does not mediate their synaptic current. Majumdar et al (2009) characterized and compared glycine evoked IPSCs (eIPSCs) as well as sIPSCs in a variety of WF-ACs in WT and *Gla2*^{-/-} retina. They classified these WF-ACs into three groups based on their responses to exogenous glycine application. Group I consisted of GABAergic displaced MF-, WF-, polyaxonal and A17 ACs. Group II consisted of GABAergic displaced WF-ACs and polyaxonal ACs. Group III consisted of ON starburst ACs. Using these criteria, glycinergic eIPSCs and sIPSCs did not differ in Groups I and III in WT and *Gla2*^{-/-} retina. However, glycinergic currents were absent in *Gla2*^{-/-} Group II ACs, suggesting that synaptic GlyRs in these cells are dominated by the α 2 subunit. Using a similar

approach, they also showed that glycinergic inhibition to A-type RGCs is independent of GlyR α 2 (Majumdar et al, 2007). In summary, at this time we know that glycinergic inhibition is only mediated by GlyR α 2 in a select group of GABAergic displaced WF- and NF-ACs.

Predictions for GlyR α 2-mediated inhibition in RGC RF center/surround interactions

The published results lead to several predictions about the role of GlyR α 2-mediated inhibition in the spontaneous and visually-evoked responses of WT RGCs:

1. The expression pattern of GlyR α 2 throughout the On and Off sublamina of the IPL suggests that GlyR α 2-mediated inhibition could affect the responses of both ON- and OFF-center RGCs.
2. The absence of GlyR α 2 expression and currents in the primary components of the rod pathway: rod DBCs, A17 ACs and AII ACs suggests that GlyR α 2-mediated inhibition should not influence RGC responses at dark adapted levels. If a change in ON- or OFF-center RGCs occurs in *Gla2*^{-/-} mice, then GlyR α 2-mediated inhibition shapes RGC responses that arise within the secondary or tertiary rod circuitry.
3. The absence of glycine-mediated currents in cone DBCs suggests that GlyR α 2-mediated inhibition in the On pathway cannot occur via feedback inhibition onto their axon terminals. Therefore, any GlyR α 2-mediated inhibition must occur either through a serial inhibitory input to GABAergic ACs, or through a direct feedforward input to RGCs. If a change in the ON-

center RGCs response occurs in *Glra2*^{-/-} mice, the first form of inhibition will lead to an increase in excitation and the second to a decrease in excitation.

4. The presence of GlyRa2-mediated currents in bi-stratified NF-ACs (Type 5/6 and 7) suggests that GlyRa2 could influence the RF center response of ON- and OFF-center RGCs via cross-over inhibition from the Off or On pathway, respectively, or via serial inhibition. If a change in the RGC response occurs in *Glra2*^{-/-} mice, either form of inhibition will increase excitation.

The results in this dissertation are the first functional assessment of a role for GlyRa2-mediated inhibition in the spontaneous and light-evoked responses of RGCs *in vivo*. To this end, I recorded the light adapted (LA) responses of ON- (n=50) and OFF-center (n=48) RGCs in *Glra2*^{-/-} mice and compared them to WT RGCs (n=292, 174). In a subset of these cells (*Glra2*^{-/-} ON n=36, OFF n=33; WT ON n= 85; OFF n= 45) I characterized their responses after 20 minutes of dark adaptation (DA). I will begin this chapter with a description of my results for spontaneous activity in *Glra2*^{-/-} and WT OFF- and ON-center RGCs at LA and DA levels followed by the results for visually-evoked activity in ON-center WT and *Glra2*^{-/-} RGCs at LA and DA levels. The latter part of the chapter describes visually-evoked activity in two populations of OFF-center *Glra2*^{-/-} and WT RGCs that were defined by my cluster analysis (Chapter 3, page 21). Only significant differences are presented graphically and all means and standard errors are listed in Appendix A.

Spontaneous Activity of ON- and OFF-center RGCs

Tonic activity in some cells is due to the continuous pre-synaptic release of excitatory neurotransmitter and has been called spontaneous or maintained activity. In RGCs this spontaneous activity (SA) is influenced by extrinsic synaptic inputs as well as intrinsic membrane properties. The extrinsic influence reflects the balance between tonic excitatory and inhibitory synaptic inputs (Barlow and Levick, 1969) as well as differences in the presence and type of synaptic and extrasynaptic receptors (Sagdullaev et al, 2006). On average SA is lower in OFF-center RGCs compared to ON-center RGCs in a variety of species including the WT mouse (Kuffler et al, 1957; Barlow and Levick, 1969; Yarbrough, 2007; Freeman et al, 2008). In addition, the mechanisms that contribute to SA differ between WT mouse ON- and OFF-center RGCs. SA in WT ON-center RGCs requires synaptic input, whereas SA in WT OFF-center RGCs is intrinsically generated and modulated by synaptic input (Margolis and Detwiler, 2007). We have previously shown that GABA_C receptor-mediated inhibition reduces the spontaneous release of glutamate from rod and cone DBC axon terminals via reciprocal feedback from GABAergic ACs (Figure 4-12 inset) (Lukasiewicz et al, 2004; Sagdullaev, et al 2006; Yarbrough, 2007). Similarly, spontaneous BC glutamate release also is controlled by GABA_A and GlyRs influencing the SA of a variety of retinal cell types (Weiss et al, 2008; Majumdar et al, 2007; Tian et al, 1998).

To determine if GlyR α 2 subunit-specific inhibition contributes to SA in the cone and rod pathways, I recorded and compared SA in WT and *Gla2*^{-/-} ON- and OFF-center

RGCs at LA levels. I then re-assessed SA in a subset of these cells after 20 minutes of DA.

Results

A. OFF-center RGCs

Hypothesis I: *GlyRa2 does not mediate inhibitory currents in any BCs and therefore it cannot directly contribute to their tonic release of glutamate or modulate the SA of OFF-center RGCs.*

1. The SA of $Gla2^{-/-}$ OFF-center RGCs is lower than WT at LA levels.

In contrast to my hypothesis, I found that the average SA is significantly lower in $Gla2^{-/-}$ OFF-center RGCs compared to WT OFF-center RGCs at LA levels. Regardless of OFF RGC type, defined by my cluster analysis (Chapter 3, page 21), the SA of $Gla2^{-/-}$ OFF-center RGCs was significantly lower than WT (OFF_{K1}, $p=0.004$; OFF_{K2}, $p=0.005$) and when OFF-center RGC classes are pooled the SA of $Gla2^{-/-}$ OFF-center RGCs is nearly 50% lower than WT (Student's t-test, $p<0.0001$). Figure 4-2 shows the frequency distributions of SA for WT and $Gla2^{-/-}$ OFF-center RGCs and the inset shows the difference in their means.

Figure 4-2. Spontaneous activity of OFF-center RGCs is significantly lower in *Glra2*^{-/-} compared to WT at LA levels. Frequency distributions and their means show that SA at LA levels is lower in *Glra2*^{-/-} (hatched bars, 3.92 ± 0.41 spikes/sec) compared to WT (black bars, 7.05 ± 0.39 spikes/sec) OFF-center RGCs ($p < 0.0001$).

2. The SA of *Glra2*^{-/-} OFF-center RGCs is not altered further at DA levels.

The SA of WT OFF-center RGCs is higher at DA levels (Matched t-test, $p=0.007$) and the same is true for SA in *Glra2*^{-/-} OFF-center RGCs (Matched t-test, $p=0.0004$). To determine if SA differed further at DA levels in WT and *Glra2*^{-/-} OFF-center RGCs I first attempted to define the stability in the SA of WT OFF-center RGCs. I computed the change in the SA of WT OFF-center RGCs ($n=40$) measured at two different contrasts at LA levels (20cd/m^2) with an intervening 20 minute interval. The SA of WT OFF-center RGCs was very stable; and the mean (± 3 SEM) of the difference in their SA between the two trials was 0.08 ± 0.71 spikes/sec, which is only $\sim 7\%$ of the average SA. Using this measure, I knew that any difference outside the range of -0.64 to 0.79 spikes/sec was a significant change in SA between LA and DA levels. Figure 4-3A plots the distribution and means of the change in SA between LA and DA in WT and *Glra2*^{-/-} OFF-center RGCs and shows that they are similar (Student's t-test, $p=0.17$). As an additional measure, I computed and compared the percent of cells that fell into one of three groups, those whose SA increased, decreased or did not change between LA and DA levels

(Figure 4-3B). The majority of both WT and *Gla2*^{-/-} OFF-center RGCs increase their SA after 20 minutes of DA, and the degree of increase is similar (χ^2 , $p=0.48$).

Figure 4-3. GlyRa2-mediated inhibition does not further alter SA in OFF-center RGCs at DA levels. (A) Scatter plots show the distribution of the change in SA between adaptation levels in WT and *Glra2*^{-/-} OFF-center RGCs. The mean and standard error (shaded region) was computed from the SA recorded in WT OFF-center RGCs from two different trials at LA levels (see text for details). The mean difference in SA is similar between WT (-1.00 ± 0.35 spikes/sec) and *Glra2*^{-/-} (-1.78 ± 0.45 spikes/sec) OFF-center RGCs ($p=0.17$). (B) The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change SA between adaptation conditions. The majority of both WT and *Glra2*^{-/-} OFF-center RGCs increased their SA from LA to DA conditions however, the proportions did not differ (χ^2 , $p=0.48$). The black lines represent the mean difference in SA in WT and *Glra2*^{-/-} OFF-center RGCs between adaptation levels.

Consistent with my hypothesis, I found that SA at DA levels did not differ between *Glra2*^{-/-} and WT OFF-center RGCs. Inconsistent with my hypothesis, the SA of OFF-center RGCs at LA levels depends on GlyRa2-mediated input. The majority of OFF-center RGCs increase their SA from LA to DA levels. My results suggest that this shift in SA of OFF-center RGCs is independent of GlyRa2-mediated input.

B. ON-center RGCs

Hypothesis II: *GlyRa2 does not mediate inhibitory currents in any BCs and therefore it cannot directly contribute to their tonic release of glutamate or modulate the SA of ON-center RGCs.*

1. *The SA of $GlyRa2^{-/-}$ ON-center RGCs is not affected at either LA or DA levels.*

Using the same methods described for OFF-center RGCs, I recorded and compared the SA in WT and $GlyRa2^{-/-}$ ON-center RGCs at LA and DA levels. Consistent with my hypothesis, I found that SA did not differ between WT and $GlyRa2^{-/-}$ ON-center RGCs at either LA (Student's t-test, $p=0.10$) or DA levels (Student's t-test, $p=0.37$). Unlike the OFF-center RGCs, the SA of ON-center RGCs does not change between adaptation level in either WT or $GlyRa2^{-/-}$ (Matched t-test, $p=0.17$ and $p=0.18$, respectively). I used similar methods described for OFF-center RGCs to determine the stability of SA in ON-center RGCs ($n=43$). I found that the difference in SA between the two adaptation levels was -2.33 ± 1.68 spikes/sec. A negative change indicates that on average the SA between the two conditions increases by 2.33spks/sec, which accounts for only ~6% of the average SA and indicates that the SA of WT ON-center RGCs is also stable Overall, my results suggest that SA of ON-center RGCs at LA and DA levels is independent of GlyRa2-mediated input. The means and standard errors for ON-center RGC SA at LA and DA levels are listed in Table 8.

Table 8. Spontaneous Activity: WT vs. *Glra2*^{-/-} ON RGCs.

	WT ON (N=292)	<i>Glra2</i> ^{-/-} ON (N=95)	P-value	Statistical Test
SpontAct @ LA	28.42 ± 0.78	25.14 ± 1.36	0.10	unpaired t-test, 2-tailed
	WT ON (N=83)	<i>Glra2</i> ^{-/-} ON (N=36)		
Change in SpontAct @ DA	-0.52 ± 0.76	-1.80 ± 1.30	0.37	unpaired t-test, 2-tailed

Summary

A role for GlyRa2-mediated inhibition in the maintained response of RGCs

Consistent with my hypothesis and the absence of GlyRa2-mediated currents in DBCs as well as in the known components of the rod pathway, SA in ON-center RGCs is independent of GlyRa2 at LA and DA levels. In contrast, my results show an unanticipated role for GlyRa2-mediated inhibition in modulating SA of OFF-center RGCs at LA levels. Because this is SA, it must be a tonic synaptic input that is local and mediated only by synaptic and not extrasynaptic inputs. A decrease in tonic excitatory activity that I observe in *Glra2*^{-/-} OFF-center RGCs suggests that GlyRa2 modulates the release of neurotransmitter from a secondary inhibitory mechanism. If GlyRa2 mediates a direct inhibitory input to OFF-center RGCs in the WT retina, its absence should increase SA. Eliminating a direct inhibition to BCs increases glutamate release and increases the SA of RGCs (Lukasiewicz et al, 2004; Sagdullaev et al, 2006); therefore

this mechanism cannot account for the change I observe in the SA of *Gla2*^{-/-} OFF-center RGCs. Since GlyR α 1 mediates chloride currents in cone HBCs (Ivanova et al, 2006) feedback inhibition to BCs cannot account for a decrease in the SA of *Gla2*^{-/-} OFF-center RGCs. Therefore, a decrease in *Gla2*^{-/-} OFF-center RGCs SA is most likely created by disinhibition via one of two serial inhibitory circuits (Figure 4-4). Circuit A (WT retina) illustrates a NF-AC that expresses GlyR α 2 and directly inhibits an OFF-center RGC. Elimination of GlyR α 2 expression in the NF-AC will create disinhibition and increase its direct tonic inhibition to the RGC, reducing its SA. Circuit B (WT retina) illustrates a NF-AC that expresses GlyR α 2 that directly inhibits a cone HBC. Elimination of GlyR α 2 expression in the NF-AC will again create disinhibition and increase tonic inhibition to the cone HBC. This will result in a decrease in cone HBC glutamate release and reduce OFF-center RGC SA.

The identity of the “disinhibited” NF-AC in either circuit is unknown. In circuit A, if the NF-AC is glycinergic the direct inhibition onto the OFF RGC can be mediated by GlyR α 1 currents (Majumdar et al, 2007). If the NF-AC is GABAergic then GABA_ARs, which are the only GABARs expressed on RGCs (Lukasiewicz and Shields, 1998), would mediate direct inhibition onto the OFF RGCs. In circuit B, glycinergic inhibition of the cone HBCs will be mediated by GlyR α 1 currents (Ivanova et al, 2006). If the NF-AC is GABAergic the most likely direct inhibition onto the cone HBC is via GABA_CRs because GABA_CRs are more sensitive to GABA, have slower current kinetics and do not desensitize quickly compared to GABA_ARs (Eggers et al, 2007). In addition, GABA_CRs have been shown to modulate SA of OFF-center RGCs *in vivo* (Yarbrough, 2007). The identity of the NF-AC can be determined by applying GABAR and GlyR

antagonists, such as TPMPA (GABA_CR), bicuculline (GABA_AR), or strychnine (GlyRs), using a whole-cell patch clamp approach.

Figure 4-4. A circuit diagram of the WT mouse retina illustrating GlyR α 2-mediated serial inhibition that modulates SA of OFF-center RGCs. **Circuit A: Serial inhibition at the level of the OFF-center RGCs. GlyR α 2-mediated serial inhibition reduces direct tonic inhibition of a NF-AC that synapses with an OFF-center RGC. When GlyR α 2 expression is eliminated, this direct tonic inhibition from the NF-AC to the OFF-center RGC increases and SA is reduced. **Circuit B: Serial inhibition at the level of the cone HBC terminals.** GlyR α 2-mediated serial inhibition reduces the pre-synaptic release of glutamate from cone HBCs via direct inhibitory inputs from a glycinergic or GABAergic NF-AC that expresses GlyR α 2. When GlyR α 2 expression is eliminated, the NF-AC that directly inhibits the cone HBC becomes more depolarized and increases inhibition to the cone HBC terminals, decreasing glutamate release and SA in OFF RGCs. HBC= cone hyperpolarizing bipolar cells; AC= narrow-field amacrine cells; OFF GC= OFF-center RGC; Gly=glycine; GABA= GABA; Glu=glutamate; Off=off sublayer of IPL; On=on sublayer of IPL.**

Visually Evoked Responses of ON and OFF RGCs

Receptive field center/surround organization is a common characteristic across most RGCs and is present at LA and DA levels, although surround contribution declines at DA levels (Enroth-Cugell and Lennie, 1975). The RF center is derived from direct excitatory inputs from BCs to RGCs (Werblin, 1991) whereas the surround is generated by lateral inhibitory inputs from HCs in the outer retina (Dowling, 1970; Mangel, 1991) and ACs in the inner retina (Werblin and Dowling, 1969; Cook and McReynolds, 1998). For simplicity of discussion, all types of lateral inhibition that may modulate excitation include feedback, feedforward and serial. The spatial organization of RFs has been modeled as a Difference of Gaussians (Figure 4-5; Rodieck and Stone, 1965) that represents separate, co-extensive Gaussian profiles for the RF center and surround mechanisms. Because these RF components are spatially contiguous inhibition also shapes the excitatory RF center response. Thus, the excitatory phase of the RGC

Figure 4-5. A Difference of Gaussians (DOG) model for RF center/surround spatial organization. The spatial organization of a RGC's RF is represented by two co-extensive mechanisms with different sensitivity profiles that sum together to create a response profile (purple) for each individual RGC. The size of the RF center (+) roughly matches the dendritic span of RGCs and has a steep Gaussian profile (blue). The area of the RF surround (-) extends over large regions of the retina and has a shallow Gaussian profile (red) (Source: Modified from Rodieck and Stone, 1965).

response reflects excitatory inputs to its RF center along with any local inhibitory modulation evoked by inputs that are spatially co-extensive. For example, reciprocal feedback inhibition at the axon terminal of the BC modulates the excitatory input to the RF center. Here the pre-synaptic glutamatergic BC excites an inhibitory AC that provides reciprocal inhibition and changes the excitatory output of the same BC (see Figure 4-12 inset). Because the RF center and surround have opposite signs (ON-center RGCs have an OFF surround and vice versa), mechanisms have been hypothesized to include interactions between the IPL On and Off pathways in RF organization. In one, referred as a “push-pull” model, McGuire et al (1986) proposed that when the RF center of ON RGCs is illuminated they received excitation from cone DBCs and inhibition from cone HBCs. Under the same stimulus conditions, OFF-center RGCs would receive inhibition from cone DBCs and a decreased excitation from cone HBCs. Although the “push-pull” model was consistent with interactions between RF ON and OFF components it requires inhibitory BCs, for which there is no evidence. This model has been modified to incorporate glycinergic NF-ACs as the components mediating the interactions between the ON and OFF RF components and the mechanism is referred to as “cross-over” inhibition (Roska and Werblin, 2001; Roska et al, 2006; Molnar and Werblin, 2007; Hsueh et al, 2008; Van Wyk et al, 2009). Figure 4-6 illustrates cross-over inhibition to an OFF-center RGC from the On pathway. A cone DBC depolarizes a bi-stratified NF-AC that releases glycine to cone HBCs or to OFF-center RGCs. The glycinergic NF-AC also receives inhibitory inputs from a neighboring AC. Excitation from the On pathway and inhibition from the Off pathway produce a synergistic effect such that when excitation increases, inhibition decreases (Roska et al, 2006). Therefore,

cross-over inhibition is hypothesized to enhance rather than oppose excitation in RGCs (Roska et al, 2006; Molnar et al, 2009; Werblin, 2010).

Figure 4-6. Cross-over inhibition in the retina. Cross-over inhibition in the retina is mediated by glycinergic narrow-field ACs (NF-AC). ON ACs receive excitation from cone DBCs and provide OFF inhibition to cone HBCs or OFF RGCs. Similarly, OFF ACs receive excitation from cone HBCs and provide ON inhibition to cone DBCs and ON RGCs (circuit not shown).

Even though I show that the absence of GlyR α 2 has no effect on the SA of ON-center RGCs, its expression pattern throughout the On and Off sublamina of the IPL still predicts that it could modulate visually-evoked activity in the ON- and/or OFF-center RGCs. The lack of GlyR α 2 currents in BCs (Ivanova et al, 2006; Eggers et al, 2007) and A-Type RGCs (Majumdar et al 2007), suggests it does not have a direct effect on the visually-evoked responses of RGCs and most likely participates in serial inhibition. Both the disynaptic nature of serial inhibition coupled with the slower kinetics of GlyR α 2-mediated currents should restrict its effects to the later stages of the excitatory response. Finally, the role of GlyR α 2-mediated inhibition at DA levels is unknown. I characterized and quantified the excitatory responses of WT and *Gla2*^{-/-} ON- and OFF-center RGCs. I used a spot whose size was matched to the cell's RF center at LA and DA levels. This

stimulus configuration maximizes the input from mechanisms that contribute to the RF center response.

Results

A. ON-center RGCs

Hypothesis III: *GlyRa2 mediated changes in visually-evoked responses of ON-center RGCs are via serial inhibition.*

1. The maintained, but not transient, component of visually-evoked responses of $Glr2^{-/-}$ ON-center RGCs is lower than WT at LA levels.

The excitatory response profile of all WT ON-center RGCs recorded from the optic nerve *in vivo* (n=292) increase their firing rate above SA at the onset of bright spot and continue to respond for the entire duration of the stimulus (Figure 4-7A). I compared the total excitatory response of WT and $Glr2^{-/-}$ ON-center RGCs. I found that the average excitatory response to an optimal spot diameter is lower in $Glr2^{-/-}$ compared to WT (Figure 4-7B; Student's t-test, $p=0.0007$) and is also lower at large spot diameters (Figure 4-7C; Mixed ANOVA, no interaction $p=0.14$, but a significant effect of genotype ($p<0.0001$) and spot size ($p<0.0001$)). The initial transient peak of the response (0.0-0.4 sec after stimulus onset), and the maintained component of the response that persists throughout the presentation of our 2 second stimulus (0.4-2.0 sec) are mediated by different inhibitory receptors with fast and slow kinetics, respectively. Therefore, I

examined these two components of the excitatory response separately. Consistent with my hypothesis, peak firing rate is similar (Figure 4-8A, Student's t-test, $p=0.06$) but the

Figure 4-7. In the absence of GlyR α 2-mediated inhibition the total excitatory response is lower in ON-center RGCs at LA levels. (A) Representative post-stimulus time histogram (PSTH) of WT ON-center RGC response to a bright spot whose size is matched to the cell's RF center. The stimulus profile shows a bright spot (100cd/m²) presented on a LA background (20cd/m²) for duration of 2 sec. The peak amplitude of the response occurs within the first 0.4 sec (dark red shaded region). A maintained response occurs from 0.4-2.0 sec (light red shaded region). (B) The average total excitatory response at the optimal spot is lower in *Gla2*^{-/-} ON-center RGCs (white bars, 77.37 ± 3.72 spikes/sec²) compared to WT (black bars, 95.70 ± 2.12 spikes/sec², $p=0.0007$). (C) ARF plots the total excitatory response as a function of the percent of optimal spot diameter. Across spot diameter, the total excitatory response is lower in *Gla2*^{-/-} (open circles) ON-center RGCs compared to WT (closed circles). Although a mixed ANOVA did not show a significant interaction ($p=0.14$) there was an effect of genotype ($p<0.0001$) and spot diameter ($p<0.0001$).

maintained portion of the response is significantly lower in *Gla2*^{-/-} ON-center RGCs compared to WT (Figure 4-8B; Student's t-test, $p=0.004$). This indicates either a decrease in visually-evoked excitation or an increase in the direct inhibition to these ON-center RGCs. Direct inhibition cannot explain this result because glycinergic currents in ON-center RGCs are not mediated by GlyR α 2. These results again predict that GlyR α 2 participates in a serial inhibitory circuit within the On pathway.

Figure 4-8. In the absence of GlyR α 2-mediated inhibition the maintained, but not the peak firing rate is lower in ON-center RGCs at LA levels. (A) Frequency distributions and means (inset) compare and show that peak firing rate in *Glra2*^{-/-} (white bars, 42.64 ± 2.4 spikes/sec) and WT (black bars, 47.31 ± 1.18 spikes/sec) ON-center RGCs do not differ ($p=0.06$). **(B)** Frequency distributions and means (inset) compare and show that maintained firing rate in *Glra2*^{-/-} ON-center RGCs (12.54 ± 1.5 spikes/sec) is significantly lower compared to WT (17.57 ± 0.66 spikes/sec, $p=0.004$).

2. The visually-evoked responses of *Glra2*^{-/-} ON-center RGCs are not altered further at DA levels.

Consistent with my hypothesis based on the absence of GlyR α 2 currents in rod DBCs and AII ACs, I found no changes in the visually-evoked responses at DA levels between *Glra2*^{-/-} and WT ON-center RGCs. A matched pairs analysis shows that the peak and maintained firing rates are significantly lower within WT and *Glra2*^{-/-} ON-center RGCs from LA to DA levels ($p<0.0001$ for all groups). To determine if there were changes in peak and maintained firing rates between WT and *Glra2*^{-/-} ON-center RGCs from LA to DA levels, I subtracted WT ON-center RGC's LA from DA peak firing rates

($LA_{PK}-DA_{PK}$) and maintained firing rates ($LA_{MFR}-DA_{MFR}$) and computed the means and ± 3 standard errors of the difference. Using this measure, I defined significant changes in peak and maintained firing rates as any difference outside the range of 20.85 ± 6.03 and 6.61 ± 3.68 spikes/sec, respectively. Figure 4-9 plots the distributions of the differences for WT and *Gla2*^{-/-} ON-center RGCs and shows that the means for both peak (Student's t-test, $p = 0.98$) and maintained firing rates (Student's t-test, $p = 0.38$) are similar between adaptation levels. My results suggest that GlyR $\alpha 2$ -mediated inhibition does not contribute to either the SA or the visually-evoked activity of WT ON-center RGCs at DA levels.

Figure 4-9. GlyR α 2-mediated inhibition does not further alter visually-evoked responses of ON-center RGCs at DA levels. (A) A scatter plots shows the distribution of the change in peak between adaptation levels in WT and *Gla2*^{-/-} ON-center RGCs. The values within the shaded region represent no change in peak firing rate between adaptation levels (see text for details) and the mean difference is similar between WT (20.85 ± 2.02 spikes/sec) and *Gla2*^{-/-} (20.96 ± 2.93 spikes/sec) ON-center RGCs ($p=0.98$). (B) The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change their peak firing rate between adaptation conditions. The proportions of cells within the three groups were similar (χ^2 , $p=0.86$). The solid lines represent the mean difference for WT and *Gla2*^{-/-} ON-center RGCs. (C) A scatter plots shows the distribution of the change in maintained firing rate between adaptation levels in WT and *Gla2*^{-/-} ON-center RGCs. The values within the shaded region represent no change in maintained firing rate between adaptation levels (see text for details) and the mean difference is similar between WT (6.61 ± 1.22 spikes/sec) and *Gla2*^{-/-} (8.47 ± 1.64 spikes/sec) ON-center RGCs ($p=0.38$). (D) The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change their maintained firing rate between adaptation conditions. The proportions of cells within the three groups are similar (χ^2 , $p=0.64$). The solid lines represent the mean difference for WT and *Gla2*^{-/-} ON-center RGCs.

3. *The temporal kinetics of visually-evoked responses in Gla2^{-/-} ON-center RGCs is more transient than WT ON-center RGCs at LA levels.*

WT ON-center RGCs that respond throughout the entire 2 second stimulus presentation are defined as sustained cells and comprise 97% of our total WT ON-center RGC population ($n=282$). Within the sustained population of WT ON-center RGCs, the nature of the response varies and can be quantified by computing a ratio of the peak and maintained firing rates. To examine changes within single RGCs I computed their Sustained/Transient Index (S/T Index). The S/T Index of WT ON-center RGCs ranged from 0.22 (very sustained) to 0.94 (more transient; Figure 4-10), whereas the S/T Index of *Gla2*^{-/-} ON-center RGCs ranged from 0.34 to 0.94.

Figure 4-10. Sustained/Transient Index. The Sustained/Transient Index is a ratio of the RF center peak and maintained firing rates corrected for SA. **(A)** Example waveforms of a WT ON-center RGC illustrating the response of a RGC that has a much sustained response (gray trace), less sustained response (dotted trace) or a very transient response (black trace). **(B)** The frequency distribution represents the range of sustained/transient ratios in WT (0.22-0.94) and *Glra2*^{-/-} (0.34-0.94) ON-center RGCs.

The average S/T Index is significantly higher in *Glra2*^{-/-} ON-center RGCs (Figure 4-11A; Mann-Whitney U, $p = 0.001$). This result is due to a population of sustained WT ON-center RGCs with high peak and maintained firing rates (~8%) that are absent from the sustained *Glra2*^{-/-} mice (Figure 4-11B). This suggests that in the WT retina, GlyR α 2-mediated inhibition reduces inhibitory inputs to the maintained component of the RF center response. A small percentage of WT ON-center RGCs (~3%) are transient and their maintained firing rate decreases to SA levels in ≤ 1.70 sec. The similar proportion (4%) of these transient cells is present in *Glra2*^{-/-} mice and they have similar peak and maintained firing rates (17.20 ± 2.73 vs. 19.80 ± 8.18 spikes/sec). This indicates that

GlyRa2-mediated inhibition does not play a role in the pathway that generates transient ON-center RGCs.

Figure 4-11. In the absence of GlyRa2-mediated inhibition, ON-center RGC responses become more transient at LA levels. (A) A histogram compares the means of S/T Index and shows *Glra2*^{-/-} ON-center RGCs have a higher index (0.71 ± 0.02) compared to WT (0.63 ± 0.01 , $p=0.001$). (B) Peak and maintained firing rates are plotted separately for sustained WT (open circles) and *Glra2*^{-/-} (closed circles) ON-center RGCs. The slopes between sustained WT and *Glra2*^{-/-} ON-center RGCs are similar ($p=0.08$).

None of the other excitatory aspects of the visually-evoked response properties differed between *Glra2*^{-/-} and WT ON-center RGCs. The means and standard errors for all visually-evoked response properties for ON-center RGCs at LA and DA levels are listed below in Tables 9 and 10.

Table 9. Optimal RF Center Response: WT vs. *Gla2*^{-/-} ON RGCs at LA levels.

	WT _{ON} (N=292)	<i>Gla2</i> ^{-/-} _{ON} (N=50)	P-value	Statistical Test
Peak	47.31 ± 1.18	42.47 ± 2.48	0.11	unpaired t-test, 2-tailed
MainFR	17.57 ± 0.66	12.54 ± 1.51	0.004	unpaired t-test, 2-tailed
TTP	0.13 ± 0.00	0.13 ± 0.00	0.09	unpaired t-test, 2-tailed
S/T Index	0.64 ± 0.01	0.72 ± 0.02	0.002	Mann-Whitney U
RF Diameter	20.81 ± 0.69	16.82 ± 1.03	0.08	Mann-Whitney U

Table 10. Optimal RF Center Response: WT vs. *Gla2*^{-/-} ON RGCs at DA levels.

	WT _{ON} (N=78)	P-value	<i>Gla2</i> ^{-/-} _{ON} (N=35)	P-value	Statistical Test
Peak	50.58 ± 2.13 vs. 29.73 ± 1.70	<0.0001	45.50 ± 2.75 vs. 24.54 ± 2.05	<0.0001	paired t-test, 2-tailed
MainFR	17.95 ± 1.32 vs. 11.34 ± 0.90	<0.0001	14.98 ± 1.97 vs. 6.51 ± 0.95	<0.0001	paired t-test, 2-tailed
TTP	0.13 ± 0.00 vs. 0.16 ± 0.01	0.0001	0.13 ± 0.01 vs. 0.16 ± 0.01	0.003	paired t-test, 2-tailed
S/T Index	0.65 ± 0.02 vs. 0.63 ± 0.02	0.44	0.69 ± 0.03 vs. 0.74 ± 0.03	0.15	Wilcoxon matched- pairs test
RF Diameter	19.89 ± 1.31 vs. 19.16 ± 0.94	0.60	15.49 ± 0.83 vs. 19.78 ± 1.67	0.01	Wilcoxon matched- pairs test

Summary

A selective role for GlyR α 2-mediated inhibition in the maintained component of visually-evoked responses of ON-center RGCs

Although GlyR α 2-mediated inhibition had no effect on the SA of ON-center RGCs, my results demonstrate that it modifies their visually-evoked responses. Specifically, my results show that in the WT retina the GlyR α 2 modulates direct inhibition via a serial inhibitory mechanism that serves to increase maintained firing rate and produce a more sustained response to light. Similar to SA, there are two serial inhibitory circuits that are most likely to produce this result in ON-center RGCs (Figure 4-12). Circuit A (WT retina) illustrates GlyR α 2-mediated serial inhibition onto a NF-AC that directly inhibits an ON-center RGC. If GlyR α 2 expression is eliminated, the NF-AC is disinhibited and its direct inhibitory output to the RGC increases reducing maintained firing rate. Circuit B (WT retina) illustrates GlyR α 2-mediated serial inhibition of a reciprocal feedback synapse that reduces pre-synaptic release of glutamate onto an inhibitory AC that directly inhibits a cone DBC. If GlyR α 2 is eliminated, the AC that directly inhibits the cone DBC becomes disinhibited and increases its inhibition to the cone DBC terminal, thus glutamate release and maintained firing rate are decreased. Although the identity of the NF-AC and the type of inhibitory input are not known, a general rule in the mammalian retina is that NF-ACs are primarily glycinergic. While GlyR α 1 has recently been found to mediate currents in murine A-type ON RGCs (Majumdar et al, 2007), there is no evidence that glycine $\rightarrow$ glycine serial inhibition is present in the retina (Zhang et al, 1997, Hsueh et al, 2008; Eggers and Lukasiewicz,

2010). However, my data predicts that there is either a previously unknown glycine serial inhibitory circuit or GlyR α 2 participates in a serial glycine $\rightarrow$ GABA inhibitory circuit modulating ON-center RGCs.

I think that Circuit B is the most likely scenario to explain an indirect increase in inhibition in the absence of GlyR α 2 for the following reasons. First, a decrease in maintained firing rate occurs for all *Gla*2^{-/-} ON-center RGCs. Second, a common alteration suggests a change to an input shared by the ON-center RGCs, the cone DBCs. Third, an alteration in only the maintained component of the response supports GlyR α 2 in a disynaptic circuit because only the slow inhibitory component is eliminated. This prediction can be tested by directly assessing the magnitude of inhibitory and excitatory currents at the level of cone DBC terminals or ON-center RGCs using a whole-cell patch clamp approach.

Figure 4-12. A circuit diagram of the WT mouse retina illustrating GlyRa2-mediated serial inhibition that modulates visually-evoked responses of ON-center RGCs. Circuit A: Serial inhibition at the level of the ON-center RGCs. GlyRa2-mediated serial inhibition reduces direct tonic inhibition of a NF-AC that synapses with an ON-center RGC. When GlyRa2 expression is eliminated, this direct tonic inhibition from the NF-AC to the ON-center RGC increases and maintained firing rate is reduced. **Circuit B: Serial inhibition at the level of the cone DBC terminals.** GlyRa2-mediated serial inhibition reduces the pre-synaptic release of glutamate via a direct inhibitory GABAergic NF-AC. This circuit includes a reciprocal feedback circuit (inset; modified from Sagdullaev et al, 2006) between cone DBCs and the GABAergic NF-AC that expresses GlyRa2. When GlyRa2 expression is eliminated, the GABAergic NF-AC that directly inhibits the cone DBC becomes more depolarized and increases feedback inhibition onto the cone DBC terminals, decreasing glutamate release and maintained firing rates in ON-center RGCs. DBC= cone depolarizing bipolar cells; AC= narrow-field amacrine cells; ON GC= ON-center RGC; Gly=glycine; GABA= GABA; Glu=glutamate; Off=off sublayer of IPL; On=on sublayer of IPL.

B. OFF-center RGCs

Hypothesis IV: *GlyRa2 mediated changes in visually-evoked responses of OFF-center RGCs are via serial inhibition.*

1. The maintained, but not transient, component of visually-evoked responses in a subpopulation of $GlyRa2^{-/-}$ OFF-center RGCs is lower compared to WT at LA levels.

All WT OFF-center RGCs recorded from the optic nerve *in vivo* (n=174) increase their firing rate above SA at the onset of a dark spot, and the majority (~77%) continue to respond for the entire duration of the stimulus (Figure 4-13A). I compared the total excitatory response separately for two subpopulations of OFF RGCs defined by my cluster analysis in WT and $GlyRa2^{-/-}$. I found that the total excitatory response in $GlyRa2^{-/-}$ OFF_{K1} but not OFF_{K2} is lower than WT at the optimal (Figure 4-13B; Student's t-test, p=0.0003 and p=0.09, respectively) and at the larger spot sizes (Figure 4-13C; Mixed ANOVA, no interaction p=0.16; effect of spot diameter (p<0.0001) and genotype (p<0.0001)).

Figure 4-13. In the absence of GlyRa2-mediated inhibition the total excitatory response is lower in a subpopulation of OFF-center RGCs at LA levels. (A) Representative post-stimulus time histogram (PSTH) of OFF-center response to a dim spot whose size is matched to the cell's RF center. The stimulus profile shows a dim spot (3cd/m²) presented on a LA background (20cd/m²) for duration of 2 seconds. The peak amplitude of the response occurs within the first 0.4 sec (dark blue shaded region). A maintained response occurs from 0.4-2.0 sec (light blue shaded region). **(B)** The total excitatory response at an optimal spot matched to the RF center is lower in *Glra2*^{-/-} (white bars, 13.46 ± 1.79 spikes/sec²) compared to WT OFF_{K1} RGCs (black bars, 23.25 ± 1.34 spikes/sec², $p=0.0003$). However, the total excitatory response to an optimal spot is similar in *Glra2*^{-/-} (gray checkered bars, 32.09 ± 3.58 spikes/sec²) and WT (gray bars, 39.71 ± 1.93 spikes/sec², $p=0.09$) OFF_{K2} RGCs. **(C)** ARF plots the total excitatory response as a function of the percent of optimal spot diameter. Across spot diameter, the total excitatory response is lower in *Glra2*^{-/-} OFF_{K1} RGCs (open circles) compared to WT (closed circles). Although a mixed ANOVA did not show a significant interaction ($p=0.16$) there was an effect of genotype ($p<0.0001$) and spot diameter ($p<0.0001$).

I also examined the peak and the maintained components of the excitatory response separately for *Gla2*^{-/-} and WT OFF_{K1} and OFF_{K2} RGCs. The peak firing rate distributions of all *Gla2*^{-/-} and WT OFF-center RGCs were similar regardless of OFF cell class, K1 and K2, defined by the cluster analysis (Figure 4-14A & C, Student's t-test, OFF_{K1} p=0.97; and OFF_{K2}, p=0.62). However, the maintained firing rate of *Gla2*^{-/-} OFF_{K1} RGCs was significantly lower than WT OFF_{K1} RGCs (Figure 4-14B, Student's t-test, OFF_{K1} p=0.004). In contrast, the maintained firing rate of *Gla2*^{-/-} and WT OFF_{K2} RGCs were similar (Figure 4-14D, Student's t-test, p=0.99).

There is strong morphological and physiological evidence for two types of OFF RGCs with large A-type morphology (Sun et al, 2002; Pang et al, 2003; Majumdar et al, 2007; Van Wyk et al, 2009). Although the morphology of RGCs from which I am recording is unknown, I am most likely recording from RGCs with A-type morphology given their large axon diameters. My results also suggest that there are two functional types of OFF-center RGCs among those that I record in the optic nerve. In one group, GlyRα2 mediates inhibition, whereas in the other it does not. The similarity in the effects of the absence of GlyRα2 between ON and OFF_{K1} RGCs, the lack of GlyRα2-mediated currents in BCs and A-type RGCs, and lower maintained firing rates all suggest that GlyRα2 is involved in a serial inhibitory circuit within both the On and Off pathways. Further, the NF-ACs that express GlyRα2 (Types 5/6 and 7) are bi-stratified which supports the possibility that the same NF-AC could mediate inputs to ON- and OFF-center RGCs. This, however, has not been demonstrated in the retina thus far.

Figure 4-14. In the absence of GlyRa2-mediated inhibition the maintained, but not the peak firing rate is lower in OFF_{K1} RGCs at LA levels. (A) Frequency distributions and means (inset) compare and show that peak firing rates between *Glra2*^{-/-} (checkered bars, 29.68 ± 2.05 spikes/sec) and WT (black bars, 29.60 ± 1.21 spikes/sec) OFF_{K1} RGCs are similar ($p=0.97$). (B) Frequency distributions and means (inset) compare and show that maintained firing rate of *Glra2*^{-/-} OFF_{K1} RGCs (2.29 ± 0.48 spikes/sec) is significantly lower than WT OFF_{K1} RGCs (5.08 ± 0.50 spikes/sec) ($p=0.004$). (C) Frequency distributions and means (inset) compare and show that peak firing rates between *Glra2*^{-/-} (grey checkered bars, 71.06 ± 4.26 spikes/sec) and WT (grey bars, 73.36 ± 1.96 spikes/sec) OFF_{K2} RGCs are similar ($p=0.62$). (D) Frequency distributions and means (inset) compare and show that maintained firing rate of *Glra2*^{-/-} OFF_{K2} RGCs (7.65 ± 1.51 spikes/sec) is significantly lower than WT OFF_{K2} RGCs (7.63 ± 0.57 spikes/sec) ($p=0.99$).

2. The visually-evoked responses of $Gla2^{-/-}$ OFF-center RGCs are not altered further at DA levels.

At DA levels the stimulus used for ON- and OFF-center RGCs must be the same, a dim light increment on a black background. As a consequence, the excitatory response profile for OFF-center RGCs occurs at the offset of this stimulus (Figure 4-15B). This response is initiated in the rod photoreceptors and conveyed to the RGCs via rod DBCs and the AII ACs. In this circuit the depolarization of AII ACs by rod DBCs results in the release of glycine onto cone HBCs, reducing their release of glutamate and ultimately the firing rate of OFF-center RGCs. At stimulus offset AII AC inhibition of cone HBCs is released, glutamate release increases and OFF-center RGCs increase their firing rate. This excitation is therefore inherently different from the excitation generated by a dark spot on a LA background. In addition, the nature of the stimulus differs; the dark spot is a stationary, sustained stimulus whereas the removal of a dim spot represents a transient change. Because this is not a maintained response I could only compare OFF responses at LA to DA levels using their transient component.

Figure 4-15. The response profiles of ON- and OFF-center RGCs to RF center stimulation at DA levels. (A) At stimulus onset, the presentation of a dim spot (3cd/m^2) on a black background (0cd/m^2) elicits an increase in glutamate release from cone DBCs and an excitatory response in ON-center RGCs. (B) At stimulus onset, the presentation of a dim spot (3cd/m^2) on a black background (0cd/m^2) elicits an increase in glutamate release from rod DBCs, thus depolarizing the AII AC. A depolarized AII AC increases the release of glycine onto cone HBCs terminals, thereby decreasing glutamate release and the firing rate of OFF-center RGCs. At stimulus offset, inhibition is released; glutamate release increases and OFF-center RGCs increase their firing rate.

Consistent with my hypothesis and the absence of GlyR α 2 currents in rod DBCs and AII ACs, I found no further changes in the visually-evoked responses of *Glra2*^{-/-} OFF-center RGCs compared to WT at DA levels. Regardless of OFF cell class, a matched pairs analysis showed that peak firing rate did not change within *Glra2*^{-/-} or WT OFF-center RGCs from LA to DA levels. Therefore, I pooled all WT OFF-center RGCs and calculated the mean and $\pm 3\text{SEM}$ of the difference in peak firing rate from LA to DA levels ($\text{LA}_{\text{PK}} - \text{DA}_{\text{PK}}$). Using this measure, I defined a significant change in peak as any difference outside the range of -3.90 ± 9.88 spikes/sec. A negative change indicates that

on average peak firing rates increase at DA levels. Figure 4-16 plots the distributions of the difference in peak firing rate in *Gla2*^{-/-} and WT OFF-center RGCs and shows that their distributions and means are similar (Student's t-test, $p = 0.84$).

Figure 4-16. GlyRa2-mediated inhibition does not further alter peak firing rate in OFF-center RGCs at DA levels. (A) A scatter plots shows the distribution of the change in peak between adaptation levels in WT and *Gla2*^{-/-} OFF-center RGCs. The values within the shaded region represent no change in peak firing rate between adaptation levels (see text for details) and the means of the difference (solid lines) is similar between *Gla2*^{-/-} (-5.18 ± 5.8 spikes/sec) and WT (-3.90 ± 3.39 spikes/sec) OFF-center RGCs ($p=0.84$). (B) The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change their peak firing rate between adaptation conditions. The proportions of cells within the three groups were similar (χ^2 , $p=0.48$).

3. The temporal kinetics of visually-evoked responses in *Gla2*^{-/-} OFF_{KL} RGCs is altered at LA levels.

The majority of WT OFF-center RGC responses are sustained (77%) and they respond throughout the entire 2 second stimulus presentation. The remaining 23% have

responses that are transient and whose maintained firing rate decreases to SA levels in ≤ 1.70 sec. The maintained firing rate in *Gla2*^{-/-} OFF_{K1} RGCs is lower than WT but their peak firing rate is not different. To examine changes within single OFF_{K1} and OFF_{K2} RGCs, I computed their S/T Index. I found that *Gla2*^{-/-} OFF_{K1} RGCs are more transient compared to WT OFF_{K1} RGCs (Figure 4-17A; Mann-Whitney U, $p=0.002$) and that the S/T Index for *Gla2*^{-/-} and WT OFF_{K2} RGCs does not differ (Mann-Whitney U, $p=0.54$). The reason for the difference seen in *Gla2*^{-/-} OFF_{K1} RGCs is shown in Figure 4-17B. Similar to ON-center RGCs, there is a group of WT OFF_{K1} RGCs that have high maintained firing rates that are absent in the *Gla2*^{-/-} mice. Overall, my results suggest that GlyRa2-mediated inhibition contributes to the temporal kinetics OFF_{K1} RGCs by reducing inhibitory inputs to these cells in the WT retina.

Figure 4-17. In the absence of GlyRa2-mediated inhibition, OFF_{K1} RGC are more transient at LA levels. (A) The average S/T Index in *Gla2*^{-/-} OFF_{K1} RGCs (checked bars; 0.90 ± 0.02) is higher compared to WT (black bars; 0.81 ± 0.01 , $p=0.002$). (B) A scatter plot of the peak and maintained firing rates in WT ($R^2=0.04$) and *Gla2*^{-/-} ($R^2=0.0003$) shows that their slopes are similar ($p=0.57$).

The S/T Index for OFF-center RGCs at DA levels were similar in WT and *Gla2*^{-/-}. There were no other differences in the remaining DA visually-evoked responses of WT or *Gla2*^{-/-} OFF-center RGCs. In addition, none of the other excitatory aspects of the visually-evoked response properties differed between *Gla2*^{-/-} and WT OFF_{K1} or OFF_{K2} RGCs. All means and standard errors for all visually-evoked response properties at LA and DA levels are listed in Tables 11-13.

Table 11. Optimal RF Center Response: WT vs. *Gla2*^{-/-} OFF_{K1} RGCs at LA levels.

	WT _{OFFK1} (N=105)	<i>Gla2</i> ^{-/-} _{OFFK1} (N=32)	P-value	Statistical Test
Peak	29.60 ± 1.21	29.68 ± 2.05	0.97	unpaired t-test, 2-tailed
MainFR	5.08 ± 0.50	2.30 ± 0.95	0.004	unpaired t-test, 2-tailed
TTP	0.16 ± 0.00	0.15 ± 0.00	0.26	unpaired t-test, 2-tailed
S/T Index	0.81 ± 0.02	0.90 ± 0.02	0.002	Mann-Whitney U
RF Diameter	22.48 ± 1.08	25.14 ± 1.94	0.13	Mann-Whitney U

Table 12. Optimal RF Center Response: WT vs. *Gla2*^{-/-} OFF_{K2} RGCs at LA levels.

	WT _{OFFK2} (N=69)	<i>Gla2</i> ^{-/-} _{OFFK2} (N=16)	P-value	Statistical Test
Peak	73.36 ± 1.97	71.06 ± 4.26	0.62	unpaired t-test, 2-tailed
MainFR	7.63 ± 0.57	7.65 ± 1.51	0.99	unpaired t-test, 2-tailed
TTP	0.13 ± 0.00	0.14 ± 0.00	0.09	unpaired t-test, 2-tailed
S/T Index	0.89 ± 0.01	0.88 ± 0.03	0.54	Mann-Whitney U
RF Diameter	17.53 ± 0.73	16.55 ± 1.73	0.39	Mann-Whitney U

Table 13. Optimal RF Center Response: WT vs. *Gla2*^{-/-} OFF RGCs at DA levels.

	WT _{OFF} (N=44)	P-value	<i>Gla2</i> ^{-/-} _{OFF} (N=33)	P-value	Statistical Test
Peak	45.99 ± 4.44 vs. 49.89 ± 5.00	0.24	43.95 ± 4.15 vs. 49.13 ± 6.02	0.38	paired t-test, 2-tailed
TTP	0.15 ± 0.01 vs. 0.15 ± 0.01	0.13	0.15 ± 0.01 vs. 0.15 ± 0.01	0.91	paired t-test, 2-tailed
RF Diameter	21.44 ± 1.48 vs. 20.59 ± 1.26	p=0.80	23.41 ± 2.05 vs. 22.57 ± 2.47	0.53	Wilcoxon matched-pairs

Summary

A role for GlyRa2-mediated inhibition in visually-evoked responses of OFF-center RGCs

My results show that GlyRa2-mediated inhibition controls inhibitory inputs to OFF-center RGCs that govern only the maintained portion of the RF center response, but only in one subpopulation (OFF_{K1}) of these cells. I also show that the changes in visually-evoked responses of OFF_{K1} RGCs are dependent on adaptation level, and that GlyRa2 participates in the cone and not the rod pathway. This result is consistent with the known absence of GlyRa2 expression in the rod circuit. Also consistent with my predictions is that the absence of GlyRa2 expression alters portions of the RF center response to a sustained stimulus which has been shown to be mediated by receptors with slow kinetics. In addition, lower SA in the absence of GlyRa2 indicates GlyRa2 affects a

pre-synaptic circuit that controls tonic release to all OFF-center RGCs. Lower maintained firing rates in *Gla2*^{-/-} OFF_{K1} RGCs indicates GlyR α 2 affects a different post-synaptic circuit that controls evoked activity in a subpopulation of OFF-center RGCs.

My results suggest the following role for GlyR α 2-mediated inhibition in Off pathway in the WT retina. First, the effect I observed in the OFF_{K1} and not the OFF_{K2} RGCs, suggests that separate OFF circuits exist within the IPL and can be defined by their inputs via GlyR α 2-mediated inhibition. Second, there are at least two types of cone HBCs, one whose output is indirectly modulated by GlyR α 2 and one whose output is independent of GlyR α 2-mediated inhibition. Third, GlyR α 2 can modulate the release of neurotransmitter from an inhibitory NF-AC that directly inhibits an OFF_{K1} but not an OFF_{K2} RGC. Based on the changes in *Gla2*^{-/-} OFF_{K1} RGC responses, I interpret my results to suggest that in the WT retina this receptor increases maintained firing rate by modulating direct inhibition via a serial inhibitory mechanism within at least one Off cone pathway.

Circuit A (WT retina, Figure 4-18) illustrates GlyR α 2-mediated serial inhibition onto a NF-AC that directly inhibits an OFF_{K1} RGC. If GlyR α 2 expression is eliminated, the NF-AC is disinhibited and its direct inhibition to the RGC increases, reducing its maintained firing rate. Similarly, Circuit B (WT retina) illustrates GlyR α 2-mediated serial inhibition that will reduce pre-synaptic release of glutamate onto an inhibitory NF-AC through feedback inhibition to a cone HBC. If GlyR α 2 expression is eliminated, the NF-AC the directly inhibits the cone HBC becomes disinhibited and increases its inhibitory input to cone HBC terminals, reducing the maintained firing rate of an OFF_{K1}

RGC. Since GlyR α 1 mediates chloride currents to cone HBCs and A-type OFF RGCs, my results support a novel role for GlyR α 2 in a glycine (GlyR α 2) $\rightarrow$ glycine (GlyR α 1) serial inhibitory circuit that has not been shown previously in the literature.

It is important to note that my results cannot be explained by cross-over inhibition. While cross-over inhibition is mediated by glycinergic NF-ACs and the Off pathway is more often the recipient of inhibition generated by the On pathway (Roska and Werblin, 2006; Molnar and Werblin, 2007; Hsueh et al, 2008; Molnar et al, 2009; Van Wyk et al, 2009), it cannot account for lower maintained firing rates in OFF-center RGCs. If cross-over inhibition was the explanation then a dark spot would hyperpolarize a cone DBC that makes synaptic contact with a glycinergic NF-AC, decreasing glycine release to the Off pathway. In this scenario the maintained firing rate of the OFF_{KI} RGC would increase, not decrease as I observed.

Figure 4-18. A circuit diagram of the WT mouse retina to illustrate how GlyR α 2-mediated serial inhibition modulates visually evoked RF center responses in OFF-center RGCs at LA levels. GlyR α 2-mediated serial inhibition reduces direct inhibition of a NF-AC that synapses with an OFF RGC (circuit A) or to a cone HBC (circuit B). When α 2 expression is eliminated, this direct inhibition from the NF-AC to the OFF RGC or cone HBC terminal increases and the maintained firing rate is reduced. OFF GC=OFF-center RGC, HBC=cone hyperpolarizing bipolar cells, AC= OFF amacrine cells; Glu=glutamate, Gly=glycine; GABA=GABA, Off=Off sublamina, On=On sublamina.

The results described previously suggest a role for GlyR α 2-mediated inhibition in the RF center/surround interactions of ON- and OFF-center RGCs at LA but not DA levels. The following section will describe a role for GlyR α 2-mediated inhibition to the contribution of the isolated RF surround response in ON- and OFF-center RGCs at LA levels.

PART II

THE ROLE OF GLYR α 2-MEDIATED INHIBITION IN THE ISOLATED RECEPTIVE FIELD SURROUND OF RETINAL GANGLION CELLS

Introduction

A fundamental feature of RGC visually-evoked responses is their spatial tuning. In most species, some RGCs are tuned to high spatial frequencies while others are tuned to low spatial frequencies (Enroth-Cugell and Robson, 1966). It is the organization, size and strength of the receptive field (RF) components that tune RGCs to specific spatial patterns (Frishman et al, 1987). The RFs of RGCs consist of two general mechanisms, their excitatory center and their antagonistic surround. ON-center RGCs have OFF surrounds whereas OFF-center RGCs have ON surrounds (Kuffler, 1953; Rodieck and Stone, 1965). It is important to note that while I discuss a RF center and surround each component represents multiple mechanisms. This is illustrated by the dynamic nature of the RF whose size and sensitivity change depending on ambient light levels (Enroth-Cugell and Lennie, 1975; Peichl and Wässle, 1983). Although the components of RGC RFs are well established, the individual mechanisms that contribute to their interactions are still not completely understood. The RF center of RGCs is generated by the recruitment of the excitatory inputs from their pre-synaptic BCs and its spatial extent

roughly matches its dendritic arbor (Figure 4-19A) (Lukasiewicz and Werblin, 1990; Werblin, 1991). At this time, it is unknown how many BC types comprise a RGC's RF center. This excitatory RF center is modulated by inhibition at the level of the OPL as well as pre-synaptic inhibition at the BC terminals in the IPL. Lateral inhibitory inputs over a much larger region of the retina generate the RF surround via feedforward, feedback, serial and cross-over inhibitory circuits. In the OPL, inhibitory input from HCs forms the antagonistic surround in BCs and plays a role in encoding slow, sustained differences in ambient intensity (Werblin and Dowling, 1969; Dowling, 1970). In the IPL, the AC network amplifies and refines the BC output to RGCs (Figure 4-19B) (Cook and McReynolds, 1998; Roska et al, 2000). The spatial organization of the RF center/surround, their interactions and overall sensitivity has been mathematically modeled as a difference of Gaussians (Rodieck and Stone, 1965; see Figure 4-5).

Figure 4-19. The RF center/surround mechanisms are generated from different inputs. (A) The RF center mechanism of RGCs is derived from the recruitment of its pre-synaptic BCs. The RF center is limited to the spatial extent of the RGC's dendritic arbor. (B) HCs in the OPL contribute to the RF surround mechanism in BCs whose output to RGCs is then refined in the IPL by ACs. The RF surround collects information over a much larger region compared to the RF center.

Spatial opponency in the inner retina

It is important to note that although there have been reports of GlyR α 1 expression and GlyR α 1-mediated currents in the outer retina of amphibians (Maple and Wu, 1998; Ge et al, 2007; Jiang and Shen, 2010), there is no evidence that GlyR α 1-mediated inhibition in the outer retina contributes to the RF surround of RGCs in the mammalian

retina. The inhibitory processes of ACs in the IPL mediate the dynamic aspects of visual processing such as contrast enhancement, spatial tuning and motion detection (Dowling, 1970; Kamermans and Spekreijse, 1999; Werblin, 1991; Cook and McReynolds, 1998a). ACs carry out these processes through feedback, feedforward, serial and cross-over inhibition onto BCs or RGCs using either GABA or glycine (Lukasiewicz and Shields, 1998; Euler and Wässle, 1998; Wässle et al, 1998; Zhang et al, 1997; Roska et al, 2006; Molnar and Werblin, 2007; Hsueh et al, 2008). The majority of NF-ACs in the mammalian retina are glycinergic and are proposed to mediate inputs to the RF center (MacNeil and Masland, 1998; O'Brien et al, 2003). In comparison, WF-ACs are primarily GABAergic and are thought to mediate inputs to the RF surround (Cook and McReynolds, 1998; Menger et al, 1998; Roska et al, 2000; O'Brien et al, 2003). Recently, GlyR α 2 expression and currents have been observed in displaced GABAergic WF-ACs (Majumdar et al, 2009), suggesting GlyR α 2 could modulate GABAergic inputs to the RF surround.

Glycine and the RF surround

The distribution of GlyRs in the retina is widespread and strychnine is a non-specific antagonist to all GlyRs. Bath application of strychnine only allows the most proximal effect to be observed. Thus, it is no surprise that the literature is filled with diverse effects of strychnine in the vertebrate retina and some are conflicting. First, strychnine has been reported to increase ON- and OFF-center RGC's spontaneous and evoked activity at LA levels (Kirby and Enroth-Cugell, 1976; Miller et al, 1977; Müller et al, 1988) and block light responses of OFF- but not ON-center RGCs at DA levels

(Müller et al, 1988). Second, strychnine produces differential effects on RF spatial organization dependent on RGC class. Strychnine attenuates the RF surround response of ON and OFF X-type RGCs and shifts it to a more center-like response, but does not affect the RF surround of ON or OFF Y-type RGCs (Kirby, 1977; Saito, 1981). Third, strychnine abolishes the transient (Caldwell and Daw, 1978; Belgum et al, 1983, 1984; O'Brien et al, 2003) and sustained (Stone and Pinto, 1992) components of RGC responses.

Only one study has been published investigating the visually-evoked responses of RGCs in a GlyR mutant mouse. Stone and Pinto (1992) characterized RF organization of RGCs in the *spastic* mouse mutant, which has a reduced number of GlyR α 1 receptors; while the function and structure of the GlyR protein remains unchanged (Becker, 1990). Extracellular recordings from all types of RGC responses (ON, OFF, and ON-OFF) to spots and annuli centered in their RF were compared between WT and *spastic* mice. The onset of a spot of preferred contrast elicited excitatory RF center responses in all WT and *spastic* RGCs. However, an annular stimulus failed to elicit a surround-type response in all *spastic* RGCs. The presentation of an annulus and light stimulation of the periphery only produced center-like responses. In contrast, spots with larger diameters attenuated the RF center response in WT and *spastic* RGCs suggesting a local RF surround response is mediated by an alternative glycinergic synapse other than GlyR α 1 whereas lateral RF surround suppression requires a glycinergic pathway that involves GlyR α 1. Further, the application of strychnine attenuated the maintained component of *spastic* ON and OFF RGCs but it did not affect the transient peak response. No effect on the transient component of the RF response is in direct contrast to findings previously reported for

salamander (Belgum et al, 1983, 1984) and cat Y-type RGCs (O'Brien et al, 2003). In the *spastic* mutant there is an up-regulation of GABA_AR expression in portions of the CNS (Biscoe and Fry, 1982; Becker, 1990). If a similar effect occurred in the retina, clear interpretations of any changes in the spastic mouse would be difficult to interpret.

Predictions for GlyR α 2-mediated inhibition in RGC RF surround response

The published data lead to the following predictions about a role for GlyR α 2-mediated inhibition in RF surround responses in WT RGCs:

1. The expression pattern of GlyR α 2 throughout the On and Off sublamina of the IPL suggests that GlyR α 2-mediated inhibition could affect the RF surround responses of both ON- and OFF-center RGCs.
2. The expression of GlyR α 2 and GlyR α 2-mediated currents in bi- or multi-stratified GABAergic WF-ACs (Majumdar et al, 2009) suggests that GlyR α 2 could mediate long range inhibitory connections that influence the RF surround response of both ON- and OFF-center RGCs. If an effect occurs in the form of direct feedforward inhibition, then GlyR α 2 will increase inhibition to the RF surround. If an effect occurs via serial inhibition, then GlyR α 2 will decrease inhibition to the RF surround.

In this section I present the results of my experiments that investigate the role of GlyR α 2-mediated inhibition in the formation of the isolated RF surround. I characterized, quantified and compared the RF surround duration and total suppression, the minimum firing rate and the onset of suppression in ON- and OFF-center RGCs in

Gla2^{-/-} and compared them to WT at LA levels. I found that the absence of GlyRa2-mediated inhibition produces similar effects on RF surround suppression regardless of OFF RGC class, previously defined by the cluster analysis (WT OFF_{K1} n= 46; OFF_{K2} n= 35), and data from all cells are therefore pooled and presented together. My comparisons of these responses for WT and *Gla2*^{-/-} ON-center RGCs (WT n=120; *Gla2*^{-/-} n=44) follows the discussion for OFF-center RGCs.

Quantifying local RF surround suppression

Because the RF center and surround components are spatially contiguous, I used an annular stimulus with a fixed outer diameter (52°) and varied its inner diameter to determine the RF center extent and quantify the isolated RF surround suppressive response. The annulus contrast was the same as the preferred contrast of the RF center. Thus, ON-center RGCs were stimulated with a bright annulus (100cd/m²) presented on a 20cd/m² background, and OFF-center RGCs were stimulated with a dark annulus (3cd/m²) presented on a 20cd/m² background (Figure 4-20). In this stimulus configuration input from the RF center will be minimized. I defined the optimal annulus as the inner diameter that elicited the maximum suppressive response. I used the response characteristics produced by the optimal annulus and compared WT and *Gla2*^{-/-} ON- and OFF-center RGCs.

As the inner diameter of an annulus increases the surface area stimulated decreases. I was able to determine an optimal annulus response in nearly all WT and *Gla2*^{-/-} ON (95%, 98%) and OFF-center RGCs (93%, 89%) because responses become less suppressed at the larger inner diameters. This indicates that 1) an adequate surface

area was stimulated for surround suppression; 2) increasing the inner diameter characterizes the spatial extent of the RGC's RF surrounds; and 3) reduced suppression at larger inner diameters denotes inhibition from an activated network outside the RF surround. The small percentages of cells with no surround suppression were excluded from the analysis.

Figure 4-20. Stimulus examples used to quantify RF center and surround responses at the optimal spot and annulus, respectively. ON-center RGCs are presented with a bright spot (100cd/m^2) on a LA background (20cd/m^2) to assess the RF center response and a bright annulus (100cd/m^2) with an inner diameter the same luminance as the LA background (20cd/m^2) is used to assess the isolated RF surround response. Similarly, OFF-center RGCs are presented with a dark spot (3cd/m^2) on a LA background (20cd/m^2) to assess the RF center response and a dark annulus (3cd/m^2) with an inner diameter the same luminance as the LA background (20cd/m^2) is used to assess the isolated RF surround response.

Quantifying lateral RF surround suppression

As an additional measure of RF surround suppression I generated an ARF to characterize the responses of RGCs to spots with diameters that exceeded the RF center and antagonized or decreased the excitatory response below the maximum response. A

measure of maximum antagonism was computed by subtracting the maximum response at the optimal spot from the response at the largest spot. I compared Maximum Antagonism to Total Suppression at the optimal annulus. Figure 4-21B plots Maximum Antagonism as a function of Total Suppression for WT ON- and two subpopulations of OFF-center RGCs and shows that they are not correlated. This suggests that stimulating the RF center and surround simultaneously measures a different aspect of antagonism than when an annulus stimulus isolates the RF surround.

Figure 4-21. Maximum antagonism and total suppression are used to quantify RF surround suppression. An ARF plots the peak firing rate of a WT OFF- (A) and ON- (C) RGC as a function of spot size (refer to Chapter 3 for details). The descending portion of the curve illustrates the decrease in RF center response due to surround antagonism. Changes in the slope are indicative of the magnitude of suppression. (B & D) Two ways to quantify RF surround suppression. Maximum antagonism is the difference between the peak response at an optimal spot matched to RF center and the response at the largest spot. Total suppression is the magnitude of suppression measured as area under the curve. Maximum antagonism and total suppression are not correlated between OFF_{K1} ($R^2 = 0.02$), OFF_{K2} ($R^2 = 0.12$) or ON-center RGCs ($R^2 = 0.11$).

Results

A. OFF-center RGCs

Hypothesis I: *GlyRa2 has been shown to mediate currents in bi-stratified GABAergic WF-ACs and therefore can directly contribute to the RF surround responses of OFF-center RGCs.*

1. The duration of RF surround suppression is shorter in $Gla2^{-/-}$ OFF-center RGCs compared to WT.

I observed a significantly shorter suppression duration in $Gla2^{-/-}$ OFF-center RGCs compared to WT (Student's t-test, $p = 0.0002$). Figure 4-22 compares the frequency distributions of duration of suppression for WT and $Gla2^{-/-}$ OFF-center RGCs and the inset shows the difference in their means. In addition, 20% of WT OFF-center RGCs were suppressed for the full 5 second presentation of an annulus while none of the $Gla2^{-/-}$ OFF-center RGCs were suppressed for the entire duration. The longest duration

among *Gla2*^{-/-} OFF-center RGCs was 2.31 seconds. The absence of cells with suppression over 5 seconds could indicate either that all cells shift their duration of suppression or that a subpopulation of OFF RGCs is absent in *Gla2*^{-/-} retina. The cluster analysis argues that the latter explanation is unlikely because the cluster solution of WT OFF RGCs alone or when *Gla2*^{-/-} OFF RGCs are included is similar. However, to examine this possibility further I excluded all WT RGCs with suppression duration of 5 seconds. When I recalculated and compared the means of suppression duration they remained significantly different ($p=0.01$). This decrease in the duration of the suppressive response is consistent with the absence of a current mediated by a receptor with slow, sustained kinetics, like the GlyR α 2. The only way that suppression duration can decrease in the absence of GlyR α 2 is through the removal of a direct inhibitory input to the RF surround.

Figure 4-22. In the absence of GlyRa2-mediated inhibition, the duration of RF surround suppression is shorter in OFF-center RGCs. (A) Frequency distribution and means (inset) show that the duration of suppression in *Glra2*^{-/-} OFF_{K1} RGCs (white bars; 0.87 ± 0.15 sec) is significantly shorter compared to WT (black bars; 1.66 ± 0.22 sec) OFF-center RGCs ($p=0.03$). **(B)** The distribution and means show that the

duration of suppression in *Glra2*^{-/-} OFF_{K2} RGCs (checked bars; 0.89 ± 0.14 sec) is significantly shorter compared to WT (black bars; 2.73 ± 0.31 sec) OFF-center RGCs ($p=0.002$). **(C)** Example waveforms of a RGC's RF surround response to the presentation of an annulus where in the absence of GlyRa2-mediated inhibition the duration of suppression is shorter in *Glra2*^{-/-} OFF-center RGCs (dotted waveform) compared to WT (solid waveform).

2. Total suppression is lower in *Glra2*^{-/-} OFF-center RGCs compared to WT.

Consistent with my hypothesis, I found that total suppression was significantly lower in *Glra2*^{-/-} OFF-center RGCs compared to WT (Student's t-test, $p=0.003$). Figure 4-23 compares the frequency distributions of total suppression for WT and *Glra2*^{-/-} OFF-center RGCs and the inset shows the difference in their means. I also excluded WT OFF-

center RGCs with total suppression ≥ 5 seconds, recalculated the means and they remained significantly different ($p=0.02$). Less total suppression suggests a reduction in either the amount of inhibitory input to the RF surround or an increase in excitation, but this is inconsistent with a decrease in SA in OFF-center RGCs. Again, the only way that total suppression can be lower in the absence of GlyR $\alpha 2$ is through the removal of direct inhibition to the RF surround of OFF-center RGCs.

Figure 4-23. In the absence of GlyR $\alpha 2$ -mediated inhibition, total suppression of RF surround response is lower in OFF-center RGCs. (A) Frequency distribution and means (inset) show that total suppression in *Glra2*^{-/-} OFF_{K1} RGCs (white bars; 1.24 ± 0.30 spikes/sec²) is significantly lower compared to WT (black bars; 2.84 ± 0.45 spikes/sec²) OFF-center (B) The distribution and means show that total suppression in *Glra2*^{-/-} OFF_{K2} RGCs (checkered bars; 1.76 ± 0.34 spikes/sec²) is significantly lower compared to WT (black bars; 7.55 ± 1.36 spikes/sec²) OFF-center RGCs ($p=0.03$). (C) Example waveforms of a RGC's RF surround response to the presentation of an annulus where in the absence of GlyR $\alpha 2$ -mediated inhibition total suppression is lower in *Glra2*^{-/-} OFF-center RGCs (dotted waveform) compared to WT (solid waveform).

3. *The minimum firing rate is lower in $Gla2^{-/-}$ OFF-center RGCs compared to WT.*

Similar to peak firing rate at the onset of a spot in the ARF, I examined the minimum firing rate at the onset of an annulus in the AnRF. Figure 4-24 compares the frequency distributions of minimum firing rates for WT and $Gla2^{-/-}$ OFF-center RGCs and the inset shows the differences in their means. A caveat to analyzing minimum firing rate is that a RGC cannot be suppressed past 0 spikes but the underlying inhibitory currents could still increase. The minimum firing rates of over half of the WT (54%) and 41% of the $Gla2^{-/-}$ OFF-center RGCs were 0 spikes/sec. For these cells the magnitude of suppression by the annulus is underestimated. Therefore, I compared WT and $Gla2^{-/-}$ OFF-center RGCs with minimum firing rates >0 spikes/sec. I found the minimum firing rate is lower in $Gla2^{-/-}$ OFF-center RGCs compared to WT (Student's t-test, $p=0.004$). A reduction in minimum firing rate (less suppression) is consistent with the removal of a direct inhibitory input to the RF surround.

Figure 4-24. In the absence of GlyR α 2-mediated inhibition, the minimum firing rate of OFF-center RGCs is lower. (A) Frequency distribution and means **(B)** show that the minimum firing rate in *Glra2*^{-/-} OFF RGCs (hatched bars; 4.83 ± 0.58 sec) is significantly shorter compared to WT (black bars; 10.11 ± 1.26 sec) OFF-center RGCs ($p=0.004$). **(C)** Example waveforms of a RGC's RF surround response to the presentation of an annulus where in the absence of GlyR α 2-mediated inhibition the minimum firing rate is lower in *Glra2*^{-/-} OFF-center RGCs (dotted waveform) compared to WT (solid waveform).

4. The onset of RF suppression is slower in *Glra2*^{-/-} OFF-center RGCs than WT.

In RGCs the RF surround mechanism is delayed in relation to the RF center mechanism by ~6-8ms (Frishman et al, 1987; Benardete and Kaplan, 1997). These differences in the temporal properties of RF center and surround mechanisms are hypothesized to reduce the redundancy of low spatiotemporal frequencies inherent in visual images and increase the RGC's efficiency of information coding (Tokutake and

Freed, 2008). The mechanisms governing the delay in RF surround signaling are not well known. To determine if GlyRa2-mediated inhibition is involved, I examined the onset latency of suppression in WT and *Gltra2*^{-/-} OFF-center RGCs. Figure 4-25 compares the frequency distributions of onset of suppression for WT and *Gltra2*^{-/-} OFF-center RGCs and the inset shows the difference in their means. I found that the onset of suppression was significantly slower in *Gltra2*^{-/-} OFF-center RGCs compared to WT (Student's t-test, $p = 0.006$). Even when WT OFF-center RGCs with suppression duration >5 seconds were excluded from the analysis the means remained significantly different ($p = 0.04$).

Figure 4-25. In the absence of GlyRa2-mediated inhibition, onset of RF surround suppression is slower in OFF-center RGCs. (A) Frequency distribution and means (inset) show that suppression onset in *Gltra2*^{-/-} OFF_{K1} RGCs (white bars; 0.12 ± 0.02 sec) is significantly slower compared to WT (black bars; 0.08 ± 0.01 sec) OFF-center RGCs ($p=0.05$). **(B)** The distribution and means show that suppression onset in *Gltra2*^{-/-}

OFF_{K2} RGCs (checkered bars; 0.14 ± 0.02 sec) is significantly slower compared to WT (black bars; 0.09 ± 0.00 sec) OFF-center RGCs ($p=0.03$). (C) Example waveforms of a RGC's RF surround response to the presentation of an annulus where in the absence of GlyR α 2-mediated inhibition suppression onset is slower in *Gla2*^{-/-} OFF-center RGCs (dotted waveform) compared to WT (solid waveform, arrow).

These results are consistent with my predictions that GlyR α 2 expression and currents in GABAergic WF-ACs could influence the RF surround response in RGCs. Specifically, my results show that GlyR α 2-mediated inhibition contributes to the duration, magnitude and onset of the RF surround response to an annulus. In addition, the effects I observe are consistent with the removal of a direct feedforward inhibitory input to the RF surround. I interpret my results in the *Gla2*^{-/-} retina to suggest that in the WT retina, the overall role of GlyR α 2-mediated inhibition is to increase inhibitory inputs to the local RF surround, presumably to enhance the spatial tuning of the OFF-center RGCs.

5. Lateral RF surround suppression is greater in Gla2^{-/-} OFF-center RGCs compared to WT.

The previous sections characterized and compared local RF surround antagonism at the optimal annulus in WT and *Gla2*^{-/-} OFF-center RGCs. I also analyzed lateral surround antagonism during RF center stimulation to large spots (ARF) and during RF surround stimulation to annuli with large inner diameters (AnRF). Figure 4-26A plots the

RF center peak response (transient component) as a function of the percent of optimal spot diameter. A decrease in peak firing rate at large spots indicates inputs from the antagonistic surround. The slopes in the descending portion of the ARF for WT and *Gla2*^{-/-} OFF-center RGCs are similar ($p=0.14$), which suggests that surround antagonism of the peak response during RF center stimulation is not significantly altered in the absence of GlyRa2. However, this is not the case when I plot the total excitatory RF center response (transient and maintained components) as a function of the percent of optimal spot diameter (Figure 4-26B). Although there is no significant interaction ($p=0.30$), an effect of genotype ($p<0.0001$) and spot size ($p<0.0001$) shows that the total excitatory response in *Gla2*^{-/-} OFF-center RGCs is significantly lower compared to WT and that the slopes of the descending portion of the curve are significantly different ($p=0.04$). The slope for the WT curve is steeper than *Gla2*^{-/-} which suggests increased surround antagonism to the RF center which is consistent with GlyRa2-mediated inhibition in GABAergic WF-ACs.

Figure 4-26. Area response functions for WT and *Gla2*^{-/-} OFF-center RGCs. (A) ARFs plot the peak firing rate as a function of percent of optimal spot diameter. A decrease in the peak firing rate at the larger spots is due to increased inhibitory inputs from the antagonistic RF surround. The slopes (red) in WT (-0.09) and *Gla2*^{-/-} (-0.06) OFF-center RGCs are similar ($p=0.14$). (B) ARFs plot that total excitatory response as a function of spot diameter. The descending slopes (red) of WT (-0.04) and *Gla2*^{-/-} (-0.03) are significantly different ($p=0.04$). A mixed ANOVA (*) shows an effect of genotype ($p<0.0001$) and spot size ($p<0.0001$) on the total excitatory response between WT and *Gla2*^{-/-} OFF-center RGCs at the larger spot diameters.

To investigate further the change in response at the larger spots, I compared the responses of WT and *Gla2*^{-/-} OFF-center RGCs to annuli as the inner diameter increases. Figures 4-27 illustrates a change in the balance of center excitation and surround suppression as a function of inner diameter. The outer diameter of the annulus is the same contrast preferred by an OFF-center RGC so a small inner diameter will elicit excitation because the center mechanism dominates the RGC response (Figure 4-27A). A null point is reached when bright inner diameters match the RF center due to equal center and surround inputs (Figure 4-27C). As the bright inner diameter increases, the recruitment of inhibitory inputs increases and the RGC is suppressed (Figure 4-27D). As the largest inner diameter exceeds the spatial extent of the RGC surround, the recruitment of additional lateral inputs suppresses the RF surround resulting in decreased suppression or the absence of a response (Figure 4-27F). The entire response profile is plotted in the insets in Figure 4-28.

Figure 4-27. OFF-center RGC's RF surround response to annuli with increasing inner diameters. (A-B) A fixed outer diameter is the same contrast preferred by the OFF-center RGC and a small inner diameter elicits an excitatory response because the center dominates. (C) A null point occurs when an inner diameter matches the RF center because the center and surround are equal. (D-E) Once the bright annulus extends out from the center, the RGC is suppressed because the surround dominates the response. (F) The largest inner diameter does not produce a response due to the recruitment of additional lateral inputs that suppress the surround.

Similar to the excitatory response profile, RGCs that respond to an annulus have a fast, transient suppression that occurs in the first 0.4 seconds and a slower, sustained suppression that can last for the entire annulus presentation (0.4-5.0 seconds) (Figure 4-28A). I characterized and compared the transient and sustained suppressive components

separately for *Gla2*^{-/-} and WT OFF-center RGCs at inner diameters larger than the RF center. I found that RF surround suppression at the large inner diameters does not change in *Gla2*^{-/-} OFF-center RGCs whereas in the WT RGCs suppression is reduced. A repeated measures ANOVA shows that there is a significant interaction for the transient (Figure 4-28B, p=0.001) and sustained (Figure 4-28C, p=0.0005) suppressive components for *Gla2*^{-/-} OFF-center RGCs compared to WT. This means that a mechanism that reduces WT OFF-center RGC suppression at the margins of the RF is absent in *Gla2*^{-/-} OFF-center RGCs. I interpret my results in the *Gla2*^{-/-} retina to suggest that in the WT retina, GlyRa2 reduces inhibition to the lateral RF surround in WT OFF-center RGCs and does so through a serial or cross-over inhibitory circuit.

Figure 4-28. In the absence of GlyR α 2-mediated inhibition, lateral RF surround suppression is greater in *Glra2*^{-/-} OFF-center RGCs compared to WT. (A) Representative average PSTH of a WT OFF-center RGC to the presentation of an annulus optimized to elicit the maximum suppression. The response profile consists of a transient suppressive component that occurs within the first 0.4 seconds (cursor 1- cursor 2), followed by sustained suppression (0.4-5sec; cursor 2- cursor 3). **(B)** Transient suppression is greater at the largest inner diameter in *Glra2*^{-/-} OFF-center RGCs compared to WT (Mixed ANOVA, p=0.001). The slopes also differ significantly between WT (0.01) and *Glra2*^{-/-} (0.0002) OFF-center RGCs (p=0.02). **(C)** *Glra2*^{-/-} OFF-center RGCs RF surround remains suppressed at larger inner diameters compared to WT (Mixed ANOVA, p=0.0005). The slopes also differ significantly between WT (0.003) and *Glra2*^{-/-} (0.0007) OFF-center RGCs (p=0.04).

Summary

A role for GlyR α 2-mediated inhibition in the RF surround of OFF-center RGCs

My results show that GlyR α 2-mediated inhibition has differential effects on the RF center responses of two populations of OFF-center RGCs. In contrast, GlyR α 2-mediated effects to the RF surround are similar for all OFF-center RGCs. I interpret my results in *Glra2*^{-/-} retina to suggest that in the WT retina GlyR α 2 participates in two different inhibitory circuits within the IPL: one that increases inhibitory inputs to the local RF surround and one that decreases inhibitory inputs to the lateral RF surround.

The only published report on GlyR-subunit specific inhibition and its affect on RF spatial organization in mouse RGCs were by Stone and Pinto (1992). They showed that reduced GlyR α 1 expression in the *spastic* mutant has different effects on the RF center and surround response for all types of RGCs (ON, OFF and ON-OFF) compared to WT.

First, *spastic* RGCs responded to a preferred contrast spot presented to the RF center with a transient excitatory peak but lacked a maintained component. Second, larger spots of preferred contrast presented to the RF center attenuated the peak response but no suppression could be elicited during the presentation of an annulus, only center-like responses. Third, strychnine abolished the RF center response suggesting another type of GlyR present in the *spastic* retina.

The absence of a maintained component is similar to my results in the RF visually-evoked responses of *Gla2*^{-/-} ON- and one subpopulation of OFF-center RGCs. My results show that participation of GlyR α 2 in a disynaptic circuit with a GABAergic/glycinergic mechanism, and not the receptor kinetics, produced more transient responses compared to WT. In the *spastic* mutant, GABA_ARs are up-regulated and GlyR α 1 are down-regulated. It is possible that GlyR α 1 also participates in a disynaptic circuit with GABA_ARs and in their absence, only the fast transmission of inhibitory input to the RF center is observed in the *spastic* retina. The attenuation of the RF center response to large spots in *spastic* RGCs suggests there is some RF surround antagonism. In addition, the fact that this response can be abolished by the application of strychnine also suggests the presence of a GlyR other than GlyR α 1. Lastly, a center-like response to the presentation of an annulus in *spastic* RGCs is puzzling given that it suggests there is no inhibitory input to the stimulated RGC. The absence of a surround response in both ON and OFF RGCs along with the up-regulation GABA in these mutants makes this particular result difficult to interpret.

In *Gla2*^{-/-} OFF-center RGCs, duration and total suppression of the RF surround sustained component is attenuated in the absence of GlyR α 2-mediated inhibition. In addition, onset of suppression was slower. RF surround signals are delayed compared to RF center responses (Frishman et al, 1987) and the mechanism for this delay is not well known. My results suggest a possible role for GlyR α 2-mediated inhibition in modulating the temporal properties of suppression onset in the RF surround. Although GlyR α 2-mediated currents have not yet been observed in OFF-center RGCs, an attenuated surround response in the absence of an inhibitory receptor can only occur via a direct feedforward inhibition to the RGCs. Because the effect I observe is at the optimal annulus, this direct feedforward inhibition can arise through the network connections of BCs and ACs within the Off sublamina. In Figure 4-29, Circuit A (WT retina) illustrates GlyR α 2-mediated direct feedforward inhibition to the local RF surround in WT retina. The outer diameter is the same contrast preferred by OFF-center RGCs and depolarizes neighboring cone HBCs, resulting in increased glutamate release to ACs and direct inhibition to the local RF surround of OFF-center RGCs. In the absence of GlyR α 2 expression, direct inhibition to an OFF-center RGC is reduced, resulting in shorter duration and an overall lower RF surround suppression.

In contrast to the effects at the optimal annulus, surround suppression is greater at the larger inner diameters. In WT OFF-center RGCs, the largest inner diameter does not elicit suppression but in *Gla2*^{-/-} RGCs they remained suppressed. The most parsimonious explanation for an increase in suppression in the absence of an inhibitory receptor is via cross-over inhibition from the On pathway. In Figure 4-30, Circuit B (WT retina) illustrates a large, bright inner diameter depolarizes a cone DBC that contacts a bi-

stratified WF-AC that expresses GlyR α 2. The WF-AC also receives inhibitory input from a neighboring AC. This WF-AC then releases inhibitory neurotransmitter in the Off sublamina directly to the RF surround of an OFF-center RGC. If GlyR α 2 expression is eliminated, the bi-stratified WF-AC is further depolarized by a cone DBC and releases more inhibitory neurotransmitter to the OFF RGC surround, increasing suppression. The effects I observe cannot occur via serial inhibition within the Off pathway due to the stimulus. A large bright inner diameter would hyperpolarize any cone HBCs which would result in a decrease of inhibitory neurotransmitter release from a WF-AC, not an increase.

My results are interesting because they suggest differential roles for GlyR α 2 in local versus lateral RF surround in OFF-center RGCs. My results also are consistent with GlyR α 2 expression and currents in GABAergic WF-ACs which have been shown to contribute to the RF surround in RGCs (Cook and McReynolds, 1998). In conclusion, I interpret my results in the *Gla2*^{-/-} retina to suggest two novel roles for GlyR α 2-mediated inhibition in the RF surround of OFF-center RGCs in the WT retina. One that increases inhibitory inputs to the local RF surround via feedforward inhibition and one that decreases inhibitory inputs to the lateral RF surround via cross-over inhibition.

Figure 4-29. A circuit diagram of the WT mouse retina to illustrate how GlyRa2-mediated serial inhibition modulates visually evoked RF surround responses in OFF-center RGCs at LA levels. The GlyRa2 receptor is localized to the dendrites of OFF-center RGCs. GlyRa2-mediated feedforward inhibition increases direct inhibition to OFF-center RGC RF surround (Circuit A). When $\alpha 2$ expression is eliminated, inhibitory input to OFF-center RGC's decreases, reducing RF surround suppression. OFF GC=OFF-center RGC, HBC=cone hyperpolarizing bipolar cells, AC= OFF amacrine cells; Glu=glutamate, Gly=glycine; GABA=GABA, Off=Off sublamina, On=On sublamina.

Figure 4-30. A circuit diagram of the WT mouse retina to illustrate how GlyRa2-mediated cross-over inhibition modulates visually evoked RF surround responses in OFF-center RGCs at LA levels. The GlyRa2 receptor is localized to the dendrites of bi-stratified, displaced GABAergic WF-ACs (Majumdar et al, 2009). A large, bright inner diameter depolarizes cone DBCs in the extended RF surround in OFF-center RGCs. The GABAergic WF-AC expressing GlyRa2 releases GABA to the RF surround of OFF-center RGCs. The elimination of GlyRa2 expression disinhibits the GABAergic WF-AC, increasing GABA release and RF surround suppression. OFF GC=OFF-center RGC, HBC=cone hyperpolarizing bipolar cells, DBC=cone depolarizing bipolar cells; AC_{WF}= displaced GABAergic wide-field ACs; AC= OFF amacrine cells; Glu=glutamate, Gly=glycine; GABA=GABA, Off= Off sublamina, On=On sublamina.

B. ON-center RGCs

Hypothesis II: *GlyRa2 has been shown to mediate currents in bi-stratified GABAergic WF-ACs and therefore can directly contribute to the RF surround responses of ON-center RGCs.*

1. GlyRa2-mediated inhibition does not contribute to RF surround in ON-center RGCs.

In contrast to my hypothesis, GlyRa2 does not contribute to the RF surround mechanisms of ON-center RGCs. I used the same methods described for OFF-center RGCs to characterize and compare duration and total suppression, minimum firing rate and onset of suppression at the optimal annulus. All of these parameters were similar in WT and *GlyRa2*^{-/-} ON-center RGCs. The means and standard errors are listed in Table 14.

I examined suppression in the lateral RF surround by comparing the responses at inner diameters greater than the optimal annulus. GlyRa2-mediated inhibition does not contribute to ON-center RGC suppression in the lateral RF surround. I characterized and compared the transient and sustained suppression at the larger inner diameters separately for ON-center RGCs and found that they do not differ. My results show that GlyRa2-mediated inhibition differentially affects the RF surround component in OFF- but not ON-center RGCs and predicts different roles for GlyRa2 in two inhibitory circuits within the Off pathway of the IPL.

Table 14. Optimal RF Surround Response: WT vs. *Gla2*^{-/-} ON RGCs at LA Levels.

	WT _{ON} (N=120)	<i>Gla2</i> ^{-/-} _{ON} (N=44)	P-value	Statistical Test
Duration	2.58 ± 0.19	2.72 ± 0.30	0.70	unpaired t-test, 2-tailed
Total Area	23.44 ± 2.66	19.79 ± 3.61	0.46	unpaired t-test, 2-tailed
Minimum Firing Rate	18.01 ± 2.09	19.17 ± 1.58	0.66	unpaired t-test, 2-tailed
Onset Latency	0.03 ± 0.00	0.04 ± 0.00	0.07	unpaired t-test, 2-tailed
Transient Resp. Dec. @ Optimal	-0.71 ± 0.05	-0.65 ± 0.04	0.43	unpaired t-test, 2-tailed
Sustained Resp. Dec. @ Optimal	-0.21 ± 0.04	-0.22 ± 0.04	0.85	unpaired t-test, 2-tailed

CHAPTER 5

PART I

THE ROLE OF GLYR α 3-MEDIATED INHIBITION IN THE RECEPTIVE FIELD CENTER/SURROUND INTERACTIONS IN RETINAL GANGLION CELLS

To determine a role for GlyR α 3-mediated inhibition in the retina, I used the same methods described in Chapters 3 and 4 to compare WT RGCs responses to those in mice that lack functional GlyR α 3 (*Gla3*^{-/-}). In the following section I describe what is currently known about the expression pattern of GlyR α 3 in the IPL and its localization to specific retinal cell types. Based on the published literature, I formulated hypothesis and tested them. My results describe the changes in spontaneous activity and visually-evoked responses in *Gla3*^{-/-} ON- and OFF-center RGCs at LA and DA levels and the role of GlyR α 3 in mediating those responses.

Introduction

GlyR α 3 in the Inner Retina

Immunocytochemical studies show GlyR α 3 labeling in four discrete bands in the IPL, with the densest labeling in the Off sublamina and reduced labeling in the On sublamina (Haverkamp et al, 2003; Figure 5-1). In addition, the analysis of glycine

evoked currents (eIPSCs) and spontaneous inhibitory post-synaptic currents (sIPSCs) in identified retinal neurons shows that glycinergic inhibition to the AII AC is mediated by GlyR α 3 (Gill et al, 2006; Weiss et al, 2008). Given these results and expression pattern of GlyR α 3 in the Off sublamina suggests that GlyR α 3 is localized on the lobular appendages of the AII ACs (Heinze et al, 2007).

Figure 5-1. The localization of GlyR α 3 in the inner retina. A photomicrograph shows the immunoreactivity of the α 3 subunit is expressed in four discrete bands of IPL with the densest label in the Off sublamina. (Source: Heinze et al, 2007, Scale bar = 50 μ m). A schematic of the retina shows that, to date, the AII AC is the only cell type that receives glycinergic inhibition via the α 3 subunit (∇). (ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; AII_{AC}: AII amacrine cell; rod DBC: rod depolarizing bipolar cell; cone HBC: cone hyperpolarizing bipolar cell; cone DBC: cone depolarizing bipolar cell; OFF, ON RGC: ON and OFF RGC: A-type retinal ganglion cells; Gly=glycine signal; Glu=glutamate signal.

The AII Amacrine Cell

The most widely studied glycinergic AC is the NF AII AC. The AII AC comprises about 13% of the total AC population (MacNeil et al, 1999) and along with the rod DBCs, are the crucial interneurons that transmit information from rod photoreceptors to the RGCs (Famiglietti and Kolb, 1975) under scotopic conditions. AII ACs receive glutamatergic input from rod DBCs and relay this excitatory signal to the On pathway via gap junctions with cone DBCs. In contrast, the AII AC uses a sign-inverting glycinergic synapse to relay an inhibitory signal to the Off pathway through cone HBCs (Famiglietti and Kolb, 1975). This inhibitory signal from AII ACs to cone HBCs is mediated by GlyR α 1 (Sassoè-Pognetto et al, 1994) whereas inhibitory inputs to AII ACs is mediated by GlyR α 3 (Weiss et al, 2008).

Although the AII AC is best known for its role at scotopic levels, recent studies show that AII ACs also function at photopic levels (Xin and Bloomfield, 1999; Pang et al, 2007; Manookin et al, 2008). This suggests OFF RGC responses should receive a mixture of excitation from cone HBCs and the removal of AII AC inhibition (disinhibition) from DBCs. For example, at LA levels light onset depolarizes cone DBCs which will share their depolarization with AII ACs through bi-directional gap junctions (Trexler et al, 2005; Veruki et al, 2002). Depolarization of the AII AC will increase its glycine release onto cone HBCs and decrease excitation of OFF RGCs. Light offset will depolarize cone HBCs and hyperpolarize cone DBCs. Cone DBC hyperpolarization will hyperpolarize the AII AC again via gap junctions, reduce glycine release onto cone HBCs

and alter their glutamate release via disinhibition (Manookin et al, 2008). This should augment the OFF RGC's excitatory response and could expand their dynamic range.

Predictions for GlyR α 3-mediated inhibition in RGC RF center/surround interactions

These published results lead to several predictions about the role of GlyR α 3-mediated inhibition in the visual responses of WT RGCs:

1. The expression pattern of GlyR α 3 in both the On and Off sublamina of the IPL suggests that GlyR α 3-mediated inhibition could affect the responses of both ON- and OFF-center RGCs.
2. The presence of GlyR α 3-mediated currents in AII ACs suggests that GlyR α 3 should influence the RF center responses of ON- and OFF-center RGCs in the following ways:
 - a. The absence of GlyR α 3 expression and currents in AII ACs should result in a more depolarized resting membrane potential than normal. This should increase tonic glycine release, tonic hyperpolarization in cone HBCs and decrease glutamate signaling to OFF-center RGCs and decrease their SA and visually-evoked responses.
 - b. Conversely, a more depolarized AII AC should result in a more depolarized cone DBC resulting in increased glutamate signaling to ON-center RGCs and potentially increase their SA and visually-evoked responses.

- c. Given the integral role of AII ACs in the rod circuit, GlyR α 3-mediated inhibition should influence the responses of both ON- and OFF-center RGCs at DA levels.
3. Glycinergic currents in cone HBCs and OFF A-type RGCs are mediated by GlyR α 1 and not GlyR α 3. Therefore, GlyR α 3-mediated inhibition in the Off pathway cannot occur via direct feedforward inhibition. Any effects that I observe should result from its role in a serial inhibitory input to either GABAergic or glycinergic ACs. In the WT retina, this form of inhibition should lead to a decrease in inhibition and increase in excitatory responses and /or a decrease in suppression.
4. The absence of any glycine-mediated currents in cone DBCs suggests that GlyR α 3-mediated inhibition in the On pathway cannot occur via feedback inhibition onto their axon terminals. Therefore, if GlyR α 3 mediates inhibition in the On pathway most likely participates in either a serial glycine $\rightarrow$ GABA circuit to cone DBCs, a serial glycine $\rightarrow$ glycine circuit to ON RGCs, or a direct feedforward input to ON RGCs. In the WT retina, these two forms of inhibition will be differentiated by increases or decreases in the excitatory response, respectively. Similarly, they may be evident as decreases or increases in suppression.

Numerous studies have been published on the diversity of GlyR subunit kinetics, expression patterns in the IPL and the localization of the receptors to specific cell types. However, more insight into the functional role of the inhibitory processes mediated by the specific subunits can only come from measuring light responses and RF properties of

retinal neurons. My results are the first functional assessment of a role for GlyR α 3-mediated inhibition in the spontaneous and light-evoked responses of RGCs. To this end, I recorded the LA responses of *Gla3*^{-/-} ON- (n=95) and OFF-center (n=61) RGCs and compared them to the same WT RGCs (n= 292, 174) used in Chapter 4 to examine changes in *Gla2*^{-/-} retina. In a subset of cells I re-characterized their responses after 20 minutes of DA (*Gla3*^{-/-} ON n= 48, OFF n= 23; WT ON n= 85; OFF n= 45). I will begin this chapter with a description of my results for spontaneous activity in *Gla3*^{-/-} and WT ON- and OFF-center RGCs at LA and DA levels followed by the results for visually-evoked activity in ON-center *Gla3*^{-/-} and WT RGCs at LA and DA levels. The latter part of the chapter describes visually-evoked activity in two populations of OFF-center *Gla3*^{-/-} and WT RGCs that were defined by the cluster analysis (Chapter 3, page 21).

Results

A. ON-center RGCs

Hypothesis I: *GlyRa3 does not mediate inhibitory currents in any BCs and therefore it cannot directly contribute to their tonic release of glutamate or modulate the SA of ON-center RGCs at LA levels.*

1. *The SA of *Gla3*^{-/-} ON-center RGCs is lower than WT at LA levels.*

Figure 5-2 compares the frequency distributions of SA for WT and *Gla3*^{-/-} ON-center RGCs and the inset shows the differences in their means. I found that the average SA in *Gla3*^{-/-} ON-center RGCs was significantly lower compared to WT ON-

center RGCs at LA levels (Student's t-test, $p=0.001$). This result indicates that the absence of an inhibitory input within the On pathway lowers tonic excitation which is not consistent with a direct effect at the AII AC.

Figure 5-2. Spontaneous activity is significantly lower in *Glra3*^{-/-} ON-center RGCs compared to WT at LA levels. Frequency distributions and their means show SA at LA levels is lower in *Glra3*^{-/-} (white bars, 24.50 ± 1.16 spikes/sec) compared to WT (black bars, 28.50 ± 0.78 spikes/sec) ON-center RGCs ($p=0.001$).

2. The SA of *Glra3*^{-/-} ON-center RGCs is not altered further at DA levels.

The SA within WT and *Glra3*^{-/-} ON-center RGCs did not change at DA levels (Matched t-test $p=0.17$ and $p=0.06$). To determine if SA differed between WT and *Glra3*^{-/-} ON-center RGCs at DA levels, I computed the change in the SA of WT ON-center RGCs ($n=43$) as described in Chapter 4 (page 73). The mean (± 3 SEM) of the difference in the SA of WT ON-center RGCs between two contrast conditions was -2.33 ± 1.68 spikes/sec. A negative change indicates that on average the SA between the two conditions increased by 2.33spks/sec, which is only ~6% of the mean SA indicating that SA does not change much. Using this measure, I knew that any difference outside the range of -4.01 to -0.65 spks/sec was a significant change in SA from LA to DA levels. Figure 5-3A plots the distribution of the change in SA between LA and DA in WT and *Glra3*^{-/-} ON-center RGCs and shows that their means are

similar (Student's t-test, $p=0.11$). As an additional measure, I computed and compared the percent of cells that fell into one of three groups, those whose SA increased, decreased or did not change between LA and DA levels (Figure 5-3B). Overall, the majority of both WT and *Gla3*^{-/-} ON-center RGCs decrease their SA after 20 minutes of DA and this decrease is independent of GlyRa3. However, the proportions of cells that do not change their SA at DA levels are significantly different (χ^2 , $p=0.009$). A higher percentage of *Gla3*^{-/-} ON-center RGCs (31%) do not change their SA at DA levels compared to 14% that do not change in WT. This implies that in a subpopulation

Figure 5-3. GlyRa3-mediated inhibition does not further alter SA in ON-center RGCs at DA levels. (A) A scatter plots shows the distribution of the change in SA between adaptation levels in WT and *Glra3*^{-/-} ON-center RGCs. The mean and standard error (shaded region) was computed from the SA recorded in WT ON-center RGCs at two different trials at LA levels (see Chapter 4, page 10 for details). The mean difference in SA is similar between WT (-0.52 ± 0.76 spikes/sec) and *Glra2*^{-/-} (-1.38 ± 0.87 spikes/sec) ON-center RGCs ($p=0.11$). The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change SA between adaptation conditions. The majority of both WT and *Glra3*^{-/-} ON-center RGCs decreased their SA form LA to DA conditions and the proportions were significantly different (χ^2 , $p=0.009$). The black lines represent the mean difference in WT and *Glra3*^{-/-}.

of ON-center RGCs GlyR α 3 is important. However, the sampling size is relatively small and given there is no known direct input to ON RGCs via GlyR α 3 further studies are needed.

I found two results. First, the SA levels did not differ between *Gla3*^{-/-} and WT ON-center RGCs as a function of adaptation level. Second, the SA of ON-center RGCs at LA levels is lower in the absence of GlyR α 3. Between LA and DA there is a shift in SA and the majority of WT (54%) and *Gla3*^{-/-} (57%) ON-center RGCs decrease their SA. My results suggest that this shift in SA within WT and *Gla3*^{-/-} ON-center RGCs is independent of GlyR α 3-mediated inhibition.

B. OFF-center RGCs

Hypothesis II: *GlyR α 3 does not mediate inhibitory currents in any BCs and therefore it cannot directly contribute to their tonic release of glutamate or modulate the SA of OFF-center RGCs at LA levels.*

1. *The SA of *Gla3*^{-/-} OFF-center RGCs is not affected at either LA or DA levels.*

I recorded and compared the SA in WT and *Gla3*^{-/-} OFF-center RGCs. Regardless of OFF RGC type, I found that SA did not differ between WT and *Gla3*^{-/-} OFF-center RGCs at LA levels (OFF_{K1}, p=0.24; OFF_{K2} p=0.87). In general, the SA within WT and *Gla3*^{-/-} OFF-center RGCs increases at DA levels (Matched t-test, p=0.006 and p=0.003, respectively). To determine if the increase in SA differed between WT and *Gla3*^{-/-} OFF-center RGCs between adaptation levels, I computed a

change in the SA described in Chapter 4 (page 73). I defined a significant change as any difference outside the range of -0.63 to 0.79 spikes/sec. The change in SA between LA and DA in WT and *Glra3*^{-/-} OFF-center RGCs is similar (Student's t-test, p=0.09). I also classified and computed the percent of cells whose SA increased, decreased or did not change between adaptation levels and the proportions of WT and *Glra3*^{-/-} OFF-center RGCs also are similar (χ^2 , p=0.10). All means and standard errors for OFF-center RGC SA at LA and DA levels are listed in Table 15.

Table 15. Spontaneous Activity: WT vs. *Glra3*^{-/-} OFF RGCs at LA Levels.

	WT OFF _{K1} (N=103)	<i>Glra3</i> ^{-/-} OFF _{K1} (N=39)	P-value	Statistical Test
SpontAct @ LA	6.42 ± 0.41	5.42 ± 0.88	0.24	unpaired t-test, 2-tailed
	WT OFF _{K1} (N=28)	<i>Glra3</i> ^{-/-} OFF _{K1} (N=11)		
Change in SpontAct @ DA	-0.76 ± 0.44	-1.50 ± 0.48	0.34	unpaired t-test, 2-tailed
	WT OFF _{K2} (N=68)	<i>Glra3</i> ^{-/-} OFF _{K2} (N=22)	P-value	Statistical Test
SpontAct @ LA	7.53 ± 0.60	7.33 ± 0.88	0.87	unpaired t-test, 2-tailed
	WT OFF _{K2} (N=17)	<i>Glra3</i> ^{-/-} OFF _{K2} (N=11)		
Change in SpontAct @ DA	-1.41 ± 0.60	-2.82 ± 1.22	0.26	unpaired t-test, 2-tailed

GABAergic AC that directly inhibits the cone DBC is disinhibited and its direct tonic inhibition to the cone DBC increases, decreasing glutamate release and SA in ON-center RGCs. Although currently there is no evidence of GlyRa3 currents in other ACs besides the AII AC (Weiss et al, 2008), immunolabeling has shown positive GlyRa3 clustering between AC → AC synapses (Haverkamp et al, 2003). Given that the absence of GlyRa3 from AII AC would predict an increase and not a decrease in SA, my results suggest an AC other than the AII AC that expresses GlyRa3 modulates GABAergic feedback inhibition to cone DBCs as shown in Circuit B.

Figure 5-4. A circuit diagram of the WT mouse retina illustrating GlyR α 3-mediated serial inhibition that modulates SA of ON-center RGCs. Circuit A: Serial inhibition at the level of ON-center RGCs. GlyR α 3-mediated serial inhibition reduces direct tonic inhibition of a GABAergic AC that synapses with an ON-center RGC. When GlyR α 3 expression is eliminated, this direct tonic inhibition from the GABAergic AC to the ON-center RGC increases and SA is reduced. **Circuit B: Serial inhibition at the level of cone DBC terminals.** GlyR α 3-mediated serial inhibition reduces the pre-synaptic release of glutamate via a direct inhibitory GABAergic AC. This circuit includes a reciprocal feedback circuit (inset; modified from Sagdullaev et al, 2006) between cone DBCs and a GABAergic AC that expresses GlyR α 3. When GlyR α 3 expression is eliminated, the GABAergic AC that directly inhibits the cone DBC becomes more depolarized and increases feedback inhibition onto the cone DBC terminals, decreasing glutamate release and SA in ON-center RGCs. DBC= cone depolarizing bipolar cells; AC= GABAergic AC; ON GC= ON-center RGC; Gly= glycine-mediated inhibition; GABA= GABA-mediated inhibition; Glu= glutamate; Off= Off sublamina of the IPL; On= On sublamina of the IPL.

Visually- Evoked Responses of ON and OFF RGCs

Even though the absence of GlyR α 3 has no effect on the SA of OFF-center RGCs, its expression pattern in the Off sublamina of the IPL still predicts that it could modulate visually-evoked activity of OFF-center RGCs. GlyR α 3 expression and currents in AII ACs, but not in BCs (Ivanova et al, 2006) or RGCs (Majumdar et al, 2007) predicts that if GlyR α 3 plays a role in visually-evoked responses of OFF-center RGCs, this mechanism is most likely a modulation of a direct input to cone HBCs or OFF-center RGCs. In addition, the medium-fast kinetics of GlyR α 3-mediated currents should restrict its effects to the earlier stages of the excitatory responses in any of these cells. To test these predictions, I used the same methods described previously (refer to Chapter 3 for details) to characterize and quantify the excitatory responses of WT and *Gla3*^{-/-} ON- and OFF-center RGCs. I used a spot whose size and contrast were matched to the cell's RF center sign and size at LA and DA levels. The stimulus

configuration maximizes the input from mechanisms that contribute to the RF center response.

Results

A. ON-center RGCs

Hypothesis III: *GlyRa3 does not mediate inhibitory currents in any type of BCs or any RGCs characterized to date and therefore any changes in visually-evoked responses of ON-center RGCs must be consistent with serial inhibition at LA levels. GlyRa3 mediates inhibitory currents in the AII ACs and therefore any changes in visually-evoked responses must be consistent with a direct effect onto ON-center RGC at DA levels.*

1. The maintained, but not transient, component of visually-evoked responses of $Gla3^{-/-}$ ON-center RGC is lower than WT at LA levels.

The excitatory response profile of all $Gla3^{-/-}$ ON-center RGCs is similar to that described for WT (Figure 5-5A). I compared the total excitatory response of WT and $Gla3^{-/-}$ ON-center RGCs. Figure 5-5B shows that the mean excitatory response at the optimal spot diameter is lower in $Gla3^{-/-}$ ON-center RGCs compared to WT (Student's t-test, $p=0.0004$) and at the smaller spot diameters (Mixed ANOVA, no interaction ($p=0.33$) but a significant effect of genotype ($p<0.0001$) and spot size ($p<0.0001$)). In addition, the WT slope is steeper compared to $Gla3^{-/-}$ ($p=0.002$) suggesting GlyRa3 increases surround antagonism in WT ON-center RGCs. I then examined the transient

peak and maintained components separately for *Gla3*^{-/-} and WT ON-center RGCs to determine if GlyRα3-mediated inhibition governs one or both components of the excitatory RF center response. The peak firing rate is similar between *Gla3*^{-/-} and WT

Figure 5-5. In the absence of GlyRα3-mediated inhibition, the total excitatory response is lower in ON-center RGCs at LA levels. (A) Representative post-stimulus time histogram (PSTH) of WT ON-center RGC response to a bright spot whose size is matched to the cell's RF center. The stimulus profile shows a bright spot (100cd/m²) presented on a LA background (20cd/m²) for duration of 2 sec. The peak amplitude of the response occurs within the first 0.4 sec (dark red shaded region). A maintained response occurs from 0.4-2.0 sec (light red shaded region). (B) The average total excitatory response at the optimal spot is lower in *Gla3*^{-/-} ON-center RGCs (white bars, 80.42 ± 3.82 spikes/sec²) compared to WT (black bars, 95.70 ± 2.12 spike/sec², p=0.0004). (C) ARF plots the total excitatory response as a function of the percent of optimal spot diameter. Across the smaller spot diameters, the total excitatory response is lower in *Gla3*^{-/-} (open circles) ON-center RGCs compared to WT (solid circles). Although a mixed ANOVA did not show a significant interaction (p=0.33) there was an effect of genotype (p<0.0001) and spot diameter (p<0.0001). The slopes (red lines) are significantly different (p=0.002) and the WT slope is steeper (-0.06) compared to *Gla3*^{-/-} (-0.04; p=0.002) suggesting increased surround antagonism.

ON-center RGCs (Figure 5-6A, Student's t-test, p=0.06) but the maintained portion is significantly lower in *Gla3*^{-/-} compared to WT (Figure 5-6B, Student's t-test, p=0.004).

Lower maintained firing rates in the absence of GlyRα3 must result from either a decrease in excitation or an increase in inhibition in the ON-center RGCs. The

elimination of a direct inhibitory input to either the BC or RGC cannot explain this result because there are no glycinergic currents in cone DBCs (Ivanova et al, 2006; Eggers et al, 2007) and no GlyR α 3-mediated currents in RGCs (Majumdar et al, 2007). Therefore, the most likely explanation is a role for GlyR α 3 in a serial inhibitory circuit that reduces inhibition within the On pathway, suggesting that a disynaptic circuit most likely governs the timing of inhibition rather than the kinetics of the receptor.

Figure 5-6. In the absence of GlyR α 3-mediated inhibition the maintained, but not the peak firing rate is lower in ON-center RGCs at LA levels. (A) Frequency distributions and means (inset) show that peak firing rate in *Glra3*^{-/-} (white bars, 42.95 ± 1.70 spikes/sec) and WT (black bars, 47.31 ± 1.18 spikes/sec) ON-center RGCs are similar ($p=0.06$). (C) Frequency distributions and means (inset) compare and show that maintained firing rate in *Glra3*^{-/-} ON-center RGCs (13.38 ± 1.05 spikes/sec) is significantly lower compared to WT (17.57 ± 0.66 spikes/sec, $p=0.004$).

2. The visually-evoked responses of *Glra3*^{-/-} ON-center RGCs are not altered further at DA levels.

A matched pairs analysis shows that the peak and maintained firing rates are significantly lower within WT and *Glra3*^{-/-} ON-center RGCs from LA to DA levels ($p<0.0001$ for all groups). To determine if there were changes in peak and maintained

firing rates between WT and *Gla3*^{-/-} ON-center RGCs from LA to DA levels, I used the same method to quantify change as described in Chapter 4 (page 85). Using this measure, I defined significant changes in peak and maintained firing rates as any difference outside the ranges of 20.85 ± 6.03 and 6.61 ± 3.68 spikes/sec, respectively. Figure 5-7 plots the distributions of the differences for WT and *Gla3*^{-/-} ON-center RGCs and shows that the means for both peak (Student's t-test, $p=0.69$) and maintained firing rates (Student's t-test, $p=0.77$) are similar. My results suggest that GlyR $\alpha 3$ -mediated inhibition does not contribute to either the SA or the visually-evoked activity of WT ON-center RGCs at DA levels.

Figure 5-7. GlyR α 3-mediated inhibition does not further alter visually-evoked responses of ON-center RGCs at DA levels. (A) A scatter plots shows the distribution of the change in peak between adaptation levels in WT and *Gltra3*^{-/-} ON-center RGCs. The values within the shaded region represent no change in peak firing rate between adaptation levels (see Chapter 4, page 19 for details) and the mean difference is similar between WT (20.85 ± 2.02 spikes/sec) and *Gltra3*^{-/-} (19.66 ± 1.87 spikes/sec) ON-center RGCs ($p=0.98$). (B) The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change their peak firing rate between adaptation conditions. The proportions of cells within the three groups were similar (χ^2 , $p=0.45$). The solid lines represent the mean difference for WT and *Gltra3*^{-/-} ON-center RGCs. (C) A scatter plots shows the distribution of the change in maintained firing rate between adaptation levels in WT and *Gltra3*^{-/-} ON-center RGCs. The values within the shaded region represent no change in maintained firing rate between adaptation levels (see Chapter 4, page 19 for details) and the mean difference is similar between WT (6.61 ± 1.22 spikes/sec) and *Gltra3*^{-/-} (6.07 ± 1.18 spikes/sec) ON-center RGCs ($p=0.38$). (D) The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change their maintained firing rate between adaptation conditions. The proportions of cells within the three groups are similar (χ^2 , $p=0.43$). The solid lines represent the mean difference for WT and *Gltra3*^{-/-} ON-center RGCs.

3. *The temporal kinetics of visually-evoked responses is more transient in Glra3^{-/-} ON-center RGCs compared to WT at LA levels.*

The majority of WT ON-center RGCs (97%) respond throughout the entire 2 second stimulus presentation. We call these sustained RGCs. In contrast, only a small percentage of WT ON-center RGCs (3%) are transient and their maintained rates decrease to SA levels soon after stimulus onset (≤ 1.70 seconds). To quantitatively evaluate the sustained/transient nature of the sustained WT ON-center RGCs I used a ratio (S/T Index) of the peak and the maintained firing rates. The maintained firing rate of *Gltra3*^{-/-} ON-center RGCs as a group is lower, whereas their peak firing rate is unchanged compared to WT. To examine changes within single RGCs, I computed their S/T Index and compared *Gltra3*^{-/-} to WT ON-center RGCs. The S/T Index of WT ON-center RGCs ranged from 0.22 (very sustained) to 0.94 (more transient; refer to Figure 4-

10). The S/T Index of *Glra3*^{-/-} ON-center RGCs ranged from 0.27 to 1.0 and were significantly more transient than WT (Figure 5-8A; Mann-Whitney U, $p=0.01$). Figure 5-8B plots the peak and maintained firing rates for sustained WT and *Glra3*^{-/-} ON-center RGCs. As described previously in Chapter 4 (page 88), a small percentage of WT ON-center RGCs (8%) have high peak and maintained firing rates. Cells with these characteristics do not appear among sustained *Glra3*^{-/-} ON-center RGCs. These data suggest that in the WT retina GlyR α 3-mediated inhibition reduces inhibitory inputs to the maintained component of the RF center response. The proportion of transient cells within WT (3%) and *Glra3*^{-/-} (9%) ON-center RGCs are similar and they have similar peak and maintained firing rates.

Figure 5-8. In the absence of GlyR α 3-mediated inhibition, ON-center RGC responses become more transient at LA levels. (A) A histogram compares the means of S/T Index and shows *Glra3*^{-/-} ON-center RGCs have a higher index (0.67 ± 0.01) compared to WT (0.63 ± 0.01 , $p=0.01$). (B) Peak and maintained firing rates are plotted separately for sustained WT (open circles) and *Glra3*^{-/-} (closed circles) ON-center RGCs. In WT, about 8% of sustained cells have high peak and maintained firing rates (dotted ring) that are not present in *Glra3*^{-/-} sustained cells. The absence of this population in *Glra3*^{-/-} supports a higher S/T Index in sustained *Glra3*^{-/-} ON-center RGCs. The slopes between sustained WT and *Glra3*^{-/-} ON-center RGCs are similar ($p=0.33$).

None of the other excitatory aspects of the visually-evoked response properties differed between *Glra3*^{-/-} and WT ON-center RGCs. The means and standard errors for all visually-evoked response properties for ON-center RGCs at LA and DA levels are listed below in Tables 16 and 17.

Table 16. Optimal RF Center Response: WT vs. *Glra3*^{-/-} ON RGCs at LA levels.

	WT _{ON} (N=292)	<i>Glra3</i> ^{-/-} _{ON} (N=95)	P-value	Statistical Test
Peak	47.31 ± 1.18	42.97 ± 1.70	0.06	unpaired t-test, 2-tailed
MainFR	17.57 ± 0.66	13.82 ± 1.05	0.004	unpaired t-test, 2-tailed
TTP	0.13 ± 0.00	0.13 ± 0.00	0.76	unpaired t-test, 2-tailed
S/T Index	0.64 ± 0.01	0.70 ± 0.02	0.001	Mann-Whitney U
RF Diameter	20.81 ± 0.69	20.28 ± 1.22	0.50	Mann-Whitney U

Table 17. Optimal RF Center Response: WT vs. *Glra3*^{-/-} ON RGCs at DA levels.

	WT _{ON} (N=78)	P-value	<i>Glra3</i> ^{-/-} _{ON} (N=47)	P-value	Statistical Test
Peak	50.58 ± 2.13 vs. 29.73 ± 1.70	<0.0001	43.14 ± 2.55 vs. 23.48 ± 2.27	<0.0001	paired t-test, 2-tailed
MainFR	17.95 ± 1.32 vs. 11.34 ± 0.90	<0.0001	14.63 ± 1.66 vs. 8.56 ± 1.38	<0.0001	paired t-test, 2-tailed
TTP	0.13 ± 0.00 vs. 0.16 ± 0.01	0.0001	0.13 ± 0.01 vs. 0.17 ± 0.01	0.0002	paired t-test, 2-tailed
S/T Index	0.65 ± 0.02 vs. 0.63 ± 0.02	0.44	0.69 ± 0.02 vs. 0.70 ± 0.03	0.35	Wilcoxon matched- pairs test
RF Diameter	19.89 ± 1.31 vs. 19.16 ± 0.94	0.60	17.28 ± 1.45 vs. 18.03 ± 1.38	0.43	Wilcoxon matched- pairs test

Summary

A selective role for GlyR α 3-mediated inhibition in the maintained component of visually-evoked responses of ON-center RGCs

My results demonstrate a role for GlyR α 3 in the visually-evoked responses of ON-center RGCs. In the absence of GlyR α 3-mediated inhibition, the maintained firing rate is lower and the temporal kinetics (S/T Index) produce more transient responses. I interpret my results in the *Gla3*^{-/-} retina to suggest that in the WT retina GlyR α 3 modulates the visual responses of RGCs via a serial inhibitory mechanism that serves to increase the maintained firing and produce more sustained responses to light. The position of GlyR α 3 in this disynaptic circuit is responsible for slowing the timing of the inhibitory input and the selective effect on the sustained and not the transient portion of the excitatory response. The same two serial inhibitory circuits discussed for SA may govern these changes in light-evoked activity (Figure 5-9). Circuit A (WT retina) illustrates GlyR α 3-mediated serial inhibition onto a GABAergic AC that directly inhibits an ON-center RGC. If GlyR α 3 expression is eliminated, the GABAergic AC is disinhibited and its direct inhibitory output to the RGC increases reducing maintained firing rate. Circuit B (WT retina) illustrates GlyR α 3-mediated serial inhibition that modulates the release of inhibitory neurotransmitter from a GABAergic AC that directly inhibits a cone DBC via a reciprocal feedback synapse. If GlyR α 3 expression is eliminated, the GABAergic AC that directly inhibits the cone DBC is disinhibited and its direct tonic inhibition to the cone DBC increases, decreasing glutamate release and maintained firing in ON-center RGCs.

The differences I observe in visually-evoked responses of ON-center RGCs do not support a role for indirect modulation of AII AC inputs to the On pathway by GlyR α 3. At LA levels, *Gla3*^{-/-} ON-center RGCs have lower maintained firing rates. When the GlyR α 3 inputs to the AII ACs are eliminated, the AII AC is most likely more depolarized and this is shared via gap junctions with the cone DBCs, which should increase glutamate release and increase, not decrease, the ON-center RGC's response. The expression pattern of GlyR α 3 in the On sublamina of the IPL suggests post-synaptic clustering of GlyR α 3 between other AC $\rightarrow$ AC synapses (Haverkamp et al, 2003) that do not involve the AII AC. Taken together, my results support a role for GlyR α 3-mediated serial inhibition of a NF-AC other than the AII AC.

Figure 5-9. A circuit diagram of the WT mouse retina illustrating GlyR α 3-mediated serial inhibition that modulates visually evoked responses of ON-center RGCs. **Circuit A: Serial inhibition at the level of ON-center RGCs. GlyR α 3-mediated serial inhibition reduces direct tonic inhibition of a GABAergic AC that synapses with an ON-center RGC. When GlyR α 3 expression is eliminated, this direct tonic inhibition from the GABAergic AC to the ON-center RGC increases and maintained firing is reduced. **Circuit B: Serial inhibition at the level of cone DBC terminals.** GlyR α 3-mediated serial inhibition reduces the pre-synaptic release of glutamate via a direct inhibitory GABAergic AC. This circuit includes a reciprocal feedback circuit (inset; modified from Sagdullaev et al, 2006) between cone DBCs and GABAergic AC that expresses GlyR α 3. When GlyR α 3 expression is eliminated, the GABAergic AC that directly inhibits the cone DBC becomes more depolarized and increases feedback inhibition onto the cone DBC terminals, decreasing glutamate release and maintained firing in ON-center RGCs. DBC= cone depolarizing bipolar cells; AC= GABAergic AC; ON GC= ON-center RGC; Gly= glycine-mediated inhibition; GABA= GABA-mediated inhibition; Glu= glutamate; Off= Off sublamina of the IPL; On= On sublamina of the IPL.**

B. OFF-center RGCs

Hypothesis IV: *GlyR α 3 does not mediate inhibitory currents in any type of BC or any RGCs characterized to date and therefore any changes in visually-evoked responses of OFF-center RGCs must be consistent with serial inhibition. At DA levels, GlyR α 3 mediates inhibitory currents in All ACs and therefore any changes in visually-evoked responses must be consistent with a direct effect onto OFF-center RGCs.*

1. The transient, but not maintained, component of visually-evoked responses in a subpopulation of Glra3^{-/-} OFF-center RGCs is higher compared to WT at LA levels.

OFF-center RGCs have a transient peak in their firing rate within the first 0.4 seconds of stimulus onset. Twenty-three percent of WT OFF-center RGCs are transient

and their maintained response returns to baseline (≤ 1.70 seconds) before stimulus offset (Figure 5-10A). I compared the total excitatory response separately for WT and *Glra3*^{-/-} OFF_{K1} and OFF_{K2} cells defined by the cluster analysis. Figure 5-10B shows that the total excitatory response is similar between *Glra3*^{-/-} and WT OFF_{K1} (Student's t-test, $p=0.56$) and OFF_{K2} RGCs (Student's t-test, $p=0.13$) at the optimal spot and across spot sizes (Mixed ANOVA, $p=0.99$ and $p=0.61$, respectively).

Figure 5-10. In the absence of GlyRa3-mediated inhibition does not alter the total excitatory response in OFF-center RGCs at LA levels. (A) Representative post-stimulus time histogram (PSTH) of OFF-center response to a dim spot whose size is matched to the cell's RF center. The stimulus profile shows a dim spot (3cd/m^2) presented on a LA background (20cd/m^2) for duration of 2 seconds. The peak amplitude of the response occurs within the first 0.4 sec (dark blue shaded region). A maintained response occurs from 0.4-2.0 sec (light blue shaded region). (B) The total excitatory response also is similar at the optimal spot matched to the RF center in *Gla3*^{-/-} (white bars, 21.75 ± 2.48 spikes/sec²) and WT OFF_{K1} RGCs (black bars, 23.25 ± 1.34 spikes/sec², $p=0.56$) and *Gla3*^{-/-} (gray checkered bars, 46.37 ± 4.89 spikes/sec²) and WT OFF_{K2} RGCs (gray bars, 39.71 ± 1.93 spikes/sec², $p=0.13$). (C) ARF plots the total excitatory response as a function of the percent of optimal spot diameter. A Mixed ANOVA shows the total excitatory response is similar across spots in *Gla3*^{-/-} (open circles) and WT OFF_{K1} RGCs (closed circles, $p=0.99$) and *Gla3*^{-/-} (open squares) and WT OFF_{K2} RGCs (closed squares; $p=0.61$).

To determine if GlyRa3-mediated inhibition contributes to one or both portions of the excitatory response in OFF-center RGCs, I compared the peak and maintained components of the excitatory response separately for *Gla3*^{-/-} and WT OFF_{K1} and OFF_{K2} RGCs. The peak firing rates in the *Gla3*^{-/-} OFF_{K2} RGCs were significantly higher compared to WT OFF_{K2} RGCs (Figure 5-11A; Student's t-test, $p<0.0001$). In contrast, the peak firing rates of *Gla3*^{-/-} and WT OFF_{K1} RGCs were similar (Figure 5-11C; Student's t-test, $p=0.67$). Regardless of OFF cell class, the maintained firing rates of *Gla3*^{-/-} OFF-center RGCs were similar to WT (Figure 5-11B and D; Student's t-test, OFF_{K1}: $p=0.79$ and OFF_{K2}: $p=0.41$).

Figure 5-11. In the absence of GlyR α 3-mediated inhibition the peak, but not the maintained firing rate is higher in a subpopulation of OFF-center RGCs at LA levels. (A) Frequency distributions and means (inset) show that the peak firing rate of *Glra3*^{-/-} OFF_{K1} RGCs (white checkered bars, 30.62 ± 2.17 spikes/sec) is similar to WT (black bars, 29.60 ± 1.21, p=0.67). (B) Frequency distribution and means (inset) compare and show that the maintained firing rate of *Glra3*^{-/-} OFF_{K1} RGCs (white checkered bars, 4.94 ± 0.88 spikes/sec) and WT (black bars, 5.63 ± 0.49 spikes/sec) are similar (p=0.47). (C) Frequency distributions and means (inset) show that the peak firing rate of *Glra3*^{-/-} OFF_{K2} RGCs (gray checkered bars, 91.78 ± 4.27 spikes/sec) is significantly higher than WT (gray bars, 73.36 ± 1.97 spikes/sec) (p<0.0001). (D) Frequency distribution and means (inset) compare and show that maintained firing rate of *Glra3*^{-/-} (gray checkered bars, 8.62 ± 1.08 spikes/sec) and WT OFF_{K2} RGCs (gray bars, 7.63 ± 0.57 spikes/sec) are similar (p=0.41).

These results are consistent with my predictions that an increase in excitation will result from the absence of a direct inhibitory input and the initial, transient portion of the response would be affected because of the medium-fast kinetics of the GlyR α 3. Because there is no evidence of GlyR α 3 expression or currents in any OFF RGCs, this effect must occur via the AII AC and disinhibition from the On pathway. A culmination of the absence of GlyR α 3 from AII ACs and hyperpolarized cone DBCs to a dark spot results in no net current to the AII AC. Therefore, OFF_{K2} RGCs do not receive any inhibitory modulation of its glutamatergic signal from cone HBCs, increasing the peak firing rate in these cells. My results argue that if this effect occurs via the AII AC circuit then the effect is pre-synaptic and demonstrates a novel glycine (GlyR α 3) $\rightarrow$ glycine (GlyR α 1) serial inhibitory circuit in the retina.

2. The visually-evoked responses of Glra3^{-/-} OFF-center RGCs are altered further at DA levels

To evaluate the response of OFF-center RGCs at DA levels I used a dim spot on a black background. As a consequence, the excitatory response profile for WT OFF-center RGCs occurs at the offset of this stimulus (refer to Figure 4-15). This response is initiated in rod photoreceptors and conveyed to RGCs via the rod DBCs and AII ACs. Depolarization of AII ACs results in the release of glycine onto cone HBCs, reducing their release of glutamate and the firing rate of OFF-center RGCs. At stimulus offset, excitation from rod DBCs to AII ACs is released, as is the inhibition to cone HBCs. Glutamate release should increase along with the firing rate of OFF-center

RGCs. This excitation is therefore inherently different from the excitation generated by a dark spot on a LA background. In addition, the nature of the stimulus differs, the dark spot is a stationary, sustained stimulus whereas the removal of a dim spot on is a transient stimulus. Therefore, to directly compare OFF responses at LA and DA levels I only characterized and compared the transient components of *Gla3^{-/-}* and WT OFF-center RGC's response.

Consistent with my hypothesis that GlyR α 3-mediated inhibition via the AII AC would have an effect on the DA RF center responses of OFF-center RGCs, I found that peak firing rate from LA to DA levels differed between *Gla3^{-/-}* and WT. Regardless of OFF cell class, a matched pairs analysis (from LA to DA levels) showed that the peak firing rate did not change within the two classes of WT or *Gla3^{-/-}* OFF-center RGCs and therefore, all OFF-center RGC data were pooled. Using the criteria described in Chapter 4 (page 98) I defined a significant change in peak from LA to DA as any difference outside the range of -3.90 ± 9.88 spikes/sec. A negative change indicates that on average peak firing rates increase at DA levels. Figure 5-12 plots the distributions of the difference in peak firing rates in *Gla3^{-/-}* and WT OFF-center RGCs and shows that their means are significantly different (Student's t-test, $p=0.05$). I interpret my results in the *Gla3^{-/-}* retina to indicate that in the WT retina GlyR α 3 reduces inhibitory inputs to the Off pathway at DA levels and is consistent with my predictions that GlyR α 3 participates in a serial inhibitory circuit. The effect is also consistent with GlyR α 3 expression on AII ACs and their role in the DA circuit. This result suggests that GlyR α 3 participates in a novel glycine (glycine (GlyR α 3) $\rightarrow$

glycine (GlyR α 1) serial inhibitory circuit.

Figure 5-12. GlyR α 3-mediated inhibition alters peak firing rate in OFF-center RGCs at DA levels. (A) A scatter plots shows the distribution of the change in peak between adaptation levels in WT and *Glra3*^{-/-} OFF-center RGCs. The values within the shaded region represent no change in peak firing rate between adaptation levels (see Chapter 4, page 32 for details). The means of the difference (solid lines) is shows that the peak response in *Glra3*^{-/-} (6.96 ± 4.4 spikes/sec) is lower compared to WT (-3.90 ± 3.39 spikes/sec) OFF-center RGCs ($p=0.05$). (B) The inset histogram plots the percent of cells that fell into one of three groups: those that increased, decreased or did not change their peak firing rate between adaptation conditions. Although the peak response in *Glra3*^{-/-} OFF-center RGCs is lower than WT, the proportions of cells that change their peak firing rate are similar (χ^2 , $p=0.54$).

3. The temporal kinetics of visually-evoked responses is not altered in *Glra3*^{-/-} OFF_{K1} or OFF_{K2} RGCs at LA levels.

Since there was an effect in the visually-evoked response of one subpopulation of OFF RGCs and not the other, I examined if there were any changes within individual OFF_{K1} and OFF_{K2} RGCs by computing their S/T Index and comparing *Glra3*^{-/-} and WT. The S/T Index was similar between *Glra3*^{-/-} and WT OFF_{K1} (Mann-Whitney U, $p=0.97$)

and OFF_{K2} RGCs (Mann-Whitney U, p=0.23). No change in the S/T Index is consistent with no difference in the maintained component of *Gla3*^{-/-} or WT OFF_{K1} or OFF_{K2} RGCs.

At DA levels, the offset of the DA stimulus results in S/T Index ratios close to 1.0 for nearly all WT and *Gla3*^{-/-} OFF-center RGCs and no differences were noted. There were no other differences in the remaining visually-evoked responses of WT or *Gla3*^{-/-} OFF-center RGCs at LA or DA levels. The means and standard errors for all visually-evoked response properties are listed in Tables 18-20.

Table 18. Optimal RF Center Response: WT vs. *Gla3*^{-/-} OFF_{K1} RGCs at LA levels.

	WT _{OFFK1} (N=105)	<i>Gla3</i> ^{-/-} _{OFFK1} (N=39)	P-value	Statistical Test
Peak	29.60 ± 1.21	30.62 ± 2.17	0.67	unpaired t-test, 2-tailed
MainFR	5.08 ± 0.50	4.81 ± 0.87	0.79	unpaired t-test, 2-tailed
TTP	0.16 ± 0.00	0.17 ± 0.01	0.29	unpaired t-test, 2-tailed
S/T Index	0.81 ± 0.02	0.82 ± 0.03	0.97	Mann-Whitney U
RF Diameter	22.48 ± 1.08	17.98 ± 1.18	0.06	Mann-Whitney U

Table 19. Optimal RF Center Response: WT vs. *Gla3*^{-/-} OFF_{K2} RGCs at LA levels.

	WT _{OFFK2} (N=69)	<i>Gla3</i> ^{-/-} _{OFFK2} (N=22)	P-value	Statistical Test
Peak	73.36 ± 1.97	91.78 ± 4.27	<0.0001	unpaired t-test, 2-tailed
MainFR	7.63 ± 0.57	8.62 ± 1.08	0.41	unpaired t-test, 2-tailed
TTP	0.13 ± 0.00	0.14 ± 0.00	0.13	unpaired t-test, 2-tailed
S/T Index	0.84 ± 0.01	0.09 ± 0.01	0.23	Mann-Whitney U
RF Diameter	17.53 ± 0.73	17.90 ± 2.54	0.38	Mann-Whitney U

Table 20. Optimal RF Center Response: WT vs. *Gla3*^{-/-} OFF RGCs at DA levels.

	WT _{OFF} (N=44)	P-value	<i>Gla3</i> ^{-/-} _{OFF} (N=23)	P-value	Statistical Test
Peak	45.99 ± 4.44 vs. 49.89 ± 5.00	0.24	59.46 ± 7.08 vs. 52.50 ± 7.38	0.13	paired t-test, 2-tailed
MainFR	5.63 ± 0.64 vs. 0.84 ± 0.25	<0.0001	7.71 ± 1.15 vs. 1.37 ± 0.52	<0.0001	paired t-test, 2-tailed
TTP	0.15 ± 0.01 vs. 0.15 ± 0.01	0.13	0.15 ± 0.01 vs. 0.15 ± 0.01	1.0	paired t-test, 2-tailed
S/T Index	0.84 ± 0.02 vs. 0.96 ± 0.01	<0.0001	0.84 ± 0.03 vs. 0.95 ± 0.02	0.001	Wilcoxon matched- pairs
RF Diameter	21.44 ± 1.48 vs. 20.59 ± 1.26	0.80	15.07 ± 1.31 vs. 15.75 ± 1.93	0.48	Wilcoxon matched- pairs

Summary

A role for GlyR α 3-mediated inhibition in visually-evoked responses of OFF-center RGCs

Consistent with my predictions and the expression of GlyR α 3 in the Off pathway, my results demonstrate a role for GlyR α 3 in visually-evoked responses of OFF-center RGCs. Generally, my results show that GlyR α 3 controls inhibitory inputs to OFF_{K2} RGCs and not OFF_{K1} RGCs at LA levels, but that all OFF-center RGCs share a common input pathway at DA levels that is modulated by GlyR α 3. Specifically, my results show that GlyR α 3-mediated inhibition controls the inhibitory inputs that govern the transient portion of the RF center response in one subpopulation of OFF-center RGCs at LA and all OFF-center RGCs at DA levels via a novel glycine → glycine serial inhibitory circuit that involves the AII AC.

At LA levels the peak firing rates *Gla3*^{-/-} OFF_{K2} RGCs are higher than WT and suggests that in the WT retina this receptor attenuates the peak response through direct feedforward inhibition to cone HBCs or OFF-center RGCs. However, there is no evidence in the literature of GlyR α 3 expression or currents in any of these cell types, only AII ACs. Therefore, my results cannot be explained by feedforward inhibition. Alternatively, my results can be explained by the newly found role of signal processing through AII ACs at LA levels, GlyR α 3's modulation of its glycine release to cone HBCs and disinhibition from the On pathway (Xin and Bloomfield, 1999; Pang et al, 2007; Manookin et al, 2008). Circuit A (WT retina, Figure 5-13) illustrates that an excitatory OFF-center RGC response is driven by two pathways; conventional glutamatergic signaling within the Off pathway (cone PRs → cone HBCs → OFF

RGCs), and the removal of inhibition, or disinhibition, from the On pathway (Manookin et al, 2008). Within the On pathway, a dark spot hyperpolarizes cone DBCs and this hyperpolarization is shared with the AII ACs via bi-directional gap junction coupling. A hyperpolarized AII AC decreases glycine release to cone HBCs and/or OFF-center RGCs enhancing their excitatory response. The AII AC also receives inhibitory inputs mediated by GlyR α 3. If GlyR α 3 expression is eliminated, the AII AC does not receive inhibitory input via GlyR α 3 nor does it receive input from the hyperpolarized cone DBCs through gap junctions. A combination of the absence GlyR α 3 and disinhibition from the On pathway essentially renders the AII AC isopotential resulting in no net inhibitory current to the Off pathway, increasing peak firing rate in OFF_{K2} RGCs. AII ACs are coupled to other AII ACs and provide inhibitory inputs to one another via gap junctions. In this scenario, the AII AC network would broadly distribute a small inhibitory signal so much so that it would result a zero net input to the Off pathway, increasing peak firing rate in OFF_{K2} RGCs. I interpret my results in the *Gla3*^{-/-} retina to suggest that in the WT retina GlyR α 3 reduces glycine release from the intermediary AII AC in a novel glycine (GlyR α 3) → glycine (GlyR α 1) serial inhibitory circuit.

At DA levels, the peak firing rate in all *Gla3*^{-/-} OFF-center RGC is lower than WT. Consistent with my predictions these results suggest that in the WT retina GlyR α 3 participates in a serial inhibitory circuit with the AII AC to reduce direct inhibition to cone HBCs and/or OFF-center RGCs. Circuit B (WT retina; Figure 5-13) illustrates GlyR α 3-mediated serial inhibition with the AII AC to reduce glycinergic inhibition to cone HBCs in the DA circuit. If GlyR α 3 is eliminated, the AII AC is more depolarized

by inputs from rod DBCs, increasing glycine release to cone HBCs thereby decreasing the peak firing rates of OFF-center RGCs.

Overall, the effects that I observed in OFF-center RGCs suggest that in the WT retina GlyR α 3 differentially governs inhibitory inputs to the Off pathway depending on adaptation level. At LA levels, GlyR α 3 increases inhibition to the RF center when signaling in the retina is high, and reduces inhibition to the RF center at DA levels when signaling in the retina is low.

Figure 5-13. A circuit diagram of the WT mouse retina illustrating GlyR α 3-mediated serial inhibition in the cone and rod pathways and its effect on the visually-evoked response of OFF-center RGCs. Circuit A: GlyR α 3-mediated indirect inhibition combined with disinhibition from the On pathway reduces excitatory drive in OFF-center RGCs. The onset of a dark spot elicits excitatory drive through the Off pathway and also hyperpolarizes the On pathway, which hyperpolarizes the electrically coupled AII AC. This results in decreased glycine release to cone HBC and an excitatory OFF RGC response. GlyR α 3 also modulates inhibitory inputs to AII AC. When GlyR α 3 is eliminated, the AII AC does not receive inhibitory or excitatory inputs resulting in no net inhibitory current to the Off pathway and an increase in the peak response of OFF RGCs. **Circuit B:** At DA levels, the excitatory response is initiated in the rod photoreceptors and is relayed to RGCs via rod DBCs and AII ACs. In this circuit the depolarization of AII ACs by the rod DBC results in glutamate signaling to the On pathway through sign-conserving gap junctions and glycinergic signaling to the Off pathway via sign-inverting glycinergic synapses. If GlyR α 3 is eliminated, the AII AC does not receive inhibitory inputs and becomes more depolarized, increasing glycine release and decreasing OFF-center RGC peak firing rate. HBC= cone hyperpolarizing bipolar cell; DBC= cone depolarizing bipolar cells; AII AC= AII amacrine cell; AC= glycinergic amacrine cell; OFF GC= OFF-center RGC; Gly= glycine-mediated inhibition; Glu= glutamate; Off= Off sublamina of the IPL; On= On sublamina of the IPL.

The results described previously predict a role for GlyR α 3-mediated inhibition in the RF center/surround interactions of ON- and OFF-center RGCs at LA and DA levels.

The following section will describe a role for GlyR α 3-mediated inhibition to the contribution of the isolated RF surround response in ON- and OFF-center RGCs at LA levels.

PART II

THE ROLE OF GLYR α 3-MEDIATED INHIBITION IN THE ISOLATED RECEPTIVE FIELD SURROUND OF RETINAL GANGLION CELLS

Introduction

GABAergic inhibition is important in the generation of the RF surround mechanism (Cook and McReynolds, 1998; Menger et al, 1998; Roska et al, 2000; O'Brien et al, 2003). In contrast, the effects of GlyR subunit specific inhibition in the RF surround are not known because the only available antagonist, strychnine, blocks all GlyR subunit combinations. This has lead to conflicting reports and difficulty in interpreting the effects of strychnine in the vertebrate retina (see Chapter 4, page 109-110). Therefore, my research is the first to assess the role of the GlyR α 3 subunit-specific inhibition in the RF surround responses of RGCs.

Predictions for GlyR α 3-mediated inhibition in RGCs RF surround response

Previous data lead to the following predictions about a role for GlyR α 3-mediated inhibition in the RF surround responses in WT RGCs that I have tested by comparing visually-evoked responses in WT and *Gla3*^{-/-} RGCs.

1. The expression pattern of GlyR α 3 in the On and Off sublamina of the IPL suggests that GlyR α 3-mediated inhibition could affect the RF surround responses of both ON- and OFF-center RGCs.
2. WF-ACs modulate inputs to the RF surround and NF-ACs modulate inputs to the RF center. Given GlyR α 3 expression and currents in NF-ACs only, GlyR α 3-mediated inhibition most likely will not contribute to the RF surround response in ON- or OFF-center RGCs.

I used the same methods described in Chapter 4 to determine how GlyR α 3-mediated inhibition contributes to visually-evoked suppression from the RF surround in *Gla3*^{-/-} ON- and OFF-center RGCs. I characterized, quantified and compared the RF surround duration and total suppression, minimum firing rate and the onset of suppression in ON-center RGCs (WT n=120; *Gla3*^{-/-} n=61) and in two subpopulations of OFF-center RGCs defined by the cluster analysis (WT OFF_{K1} n= 46; OFF_{K2} n= 35) (*Gla3*^{-/-} OFF_{K1} n= 26; OFF_{K2} n= 17).

In the mammalian retina WF-ACs are hypothesized to generate RF surround responses in RGCs due to their large dendritic arbors by which they can mediate long range inhibitory interactions within a single layer of the IPL. In contrast, NF-ACs have small dendritic arbors and are narrowly stratified in multiple layers of the IPL. NF-ACs are hypothesized to mediate inhibitory inputs to the RF center and have not been implicated in the generation of the RF surround response. Given the expression and currents of GlyR α 3 in NF AII ACs and my previous results which show governing of inhibitory inputs the RF center of OFF-center RGCs by AII ACs, I hypothesize that

GlyR α 3 will not play role in the RF surround responses of ON- or OFF-center RGCs.

In conclusion, my results show that GlyR α 3-mediated inhibition does not mediate any inhibitory input to any aspect of the local or lateral RF surround response in ON- or OFF-center RGCs. The means and standard errors for all suppression variables are listed in Table 21-22.

Table 21. Optimal RF Surround Response: WT vs. *Gla3*^{-/-} ON RGCs at LA Levels.

	WT _{ON} (N=120)	<i>Gla3</i> ^{-/-} _{ON} (N=61)	P-value	Statistical Test
Duration	2.58 ± 0.28	2.88 ± 0.29	0.39	unpaired t-test, 2-tailed
Total Area	23.44 ± 2.66	26.75 ± 4.01	0.48	unpaired t-test, 2-tailed
Minimum Firing Rate	18.01 ± 2.09	22.04 ± 1.44	0.10	unpaired t-test, 2-tailed
Onset Latency	0.03 ± 0.00	0.04 ± 0.00	0.15	unpaired t-test, 2-tailed
Transient Resp. Dec. @ Optimal	-0.71 ± 0.04	-0.78 ± 0.03	0.18	unpaired t-test, 2-tailed
Sustained Resp. Dec. @ Optimal	-0.39 ± 0.05	-0.37 ± 0.04	0.73	unpaired t-test, 2-tailed

Table 22. Optimal RF Surround Response: WT vs. *Gla3*^{-/-} OFF RGCs at LA Levels.

	WT _{OFF} (N=81)	<i>Gla3</i> ^{-/-} _{OFF} (N=43)	P-value	Statistical Test
Duration	2.07 ± 0.19	2.17 ± 0.26	0.75	unpaired t-test, 2-tailed
Total Area	4.87 ± 0.68	5.98 ± 1.19	0.38	unpaired t-test, 2-tailed
Minimum Firing Rate	7.89 ± 0.96	8.30 ± 0.96	0.78	unpaired t-test, 2-tailed
Onset Latency	0.09 ± 0.00	0.10 ± 0.01	0.43	unpaired t-test, 2-tailed
Transient Resp. Dec. @ Optimal	-0.94 ± 0.02	-0.93 ± 0.02	0.66	unpaired t-test, 2-tailed
Sustained Resp. Dec. @ Optimal	-0.37 ± 0.03	-0.38 ± 0.02	0.86	unpaired t-test, 2-tailed

CHAPTER 6

GENERAL DISCUSSION

The balance of excitatory and inhibitory inputs to neurons is crucial for proper functioning in the CNS. An alteration in the balance of synaptic input provokes adverse events in the CNS such as disruption in the maturation of synapses, epileptic seizures and abnormal brain development (Sun, 2007). In cortical neurons, inhibitory and excitatory inputs are highly correlated with inhibition lagging behind excitation by a few milliseconds (Okun and Lampl, 2008). The coupling of these inputs suggests that changes in excitation and inhibition reflect changes within a network of neurons rather than individual cells (Okun and Lampl, 2008). Networks of excitatory and inhibitory inputs form unique, individual neural circuits that are refined by the inhibitory neurotransmitters, GABA and glycine. The exact mechanisms that match excitatory and inhibitory inputs to balance individual neural circuits is not well established (Sun, 2007). The receptive fields (RFs) of RGCs as well as other sensory neurons are a product of balanced excitatory inputs to their RF center and inhibitory inputs to their RF surround. In this sense, they also represent a physical topography that is a representation of the retinal circuit as well as visual space. Therefore, assessing RGC responses in the absence

of an inhibitory input provides a way to examine the contribution of these inputs to the overall response and RF spatial organization in RGCs.

The role of GABAergic inhibition in the retina is well established. Antagonists against the ionotropic GABA_A and GABA_CRs have helped to elucidate their localization to specific retinal neurons and their contribution to the RF surround in RGCs (Cook and McReynolds, 1998; Eggers and Lukasiewicz, 2010). These experiments have verified the hypothesis that the kinetic differences in these two GABARs translates into different functions in shaping the visual response (Eggers et al, 2006; Eggers et al, 2007). The different GlyRs also differ in their receptor kinetics (Chapter 2, Table 1) and while the overall role of glycinergic inhibition in the retina has also been investigated, the contribution of individual GlyRs in visual processing is not well known due to the absence of specific GlyR α subunit antagonists. Strychnine has been used to characterize glycinergic inhibition in the retina but it blocks all subunit combinations. Anatomical and morphological studies show individual GlyR α subunit expression and currents in specific retinal cell types (for example in WF- and NF-ACs), which suggest their involvement in different circuits to fulfill specific roles in visual processing. My research is the first functional assessment of GlyR subunit-specific inhibition in the responses of RGCs, their RF center/surround organization, and overall contribution to the balance of inputs to individual retinal circuits. Based on these data several common themes arise. First, GlyR α 2- and GlyR α 3-mediated inhibition differentially affects the maintained and visually-evoked responses of ON- and OFF-center RGCs at LA and DA levels. Therefore, they participate in separate retinal circuits. Second, within the On pathway GlyR α 2 and GlyR α 3 participate in glycine $\rightarrow$ GABA serial inhibition whereas in the Off

pathway GlyR α 2 and GlyR α 3 participate in glycine $\rightarrow$ glycine serial inhibition. Third, when a receptor is expressed on NF-ACs it affects the RF center, and when a receptor is expressed on WF-ACs it affects the RF surround. Finally, when a receptor participates in direct inhibition it affects the early phase of the response, and when a receptor participates in serial inhibition it affects the later phase of the response. In the following sections I will summarize how my results fit into these common themes.

At LA levels, GlyR α 2 and GlyR α 3 reduce inhibitory input to the RF center via serial inhibition to regulate the spontaneous release of glutamate in OFF- and ON-center RGCs, respectively.

Gla2^{-/-} OFF-center RGCs and *Gla3*^{-/-} ON-center RGCs have lower SA than WT. This suggests an increase in inhibitory input in their absence. Previously, we have shown that GABA_CRs mediate a selective and direct inhibition onto rod and cone DBC axon terminals (Sagdullaev et al, 2006). When GABA_CR inhibition is eliminated spontaneous glutamate release from the pre-synaptic terminals increases, as does RGC SA (Lukasiewicz et al, 2004; Sagdullaev et al, 2006; Yarbrough, 2007). Therefore, a decrease in SA is inconsistent with a direct pre-synaptic inhibition of GlyRs. Similarly, eliminating direct feedforward inhibition to RGCs also would increase SA. The absence of GlyR α 2 and GlyR α 3 expression and currents in any BCs or A-Type RGCs suggests that they must participate in a serial inhibitory circuit that modulates a direct tonic inhibitory input from a NF-AC to cone HBC and DBC terminals, respectively. I interpret my results to suggest that in the WT retina, GlyR α 2- and GlyR α 3-mediated serial inhibition reduces inhibitory inputs to ON- and OFF-center RGCs.

Differences in SA of WT ON and OFF RGCs are due to differences in retinal circuitry (Barlow and Levick, 1969), the presence of synaptic and extrasynaptic receptors (Sagdullaev et al, 2006), and intrinsic vs. extrinsic mechanisms (Margolis and Detwiler, 2007). These asymmetries between ON and OFF RGCs can now be extended to include distinct GlyR α 2 and GlyR α 3-mediated inhibitory circuits that limit spontaneous glutamate release from cone HBCs and DBCs, respectively.

At LA levels, both GlyR α 2 and GlyR α 3 reduce inhibitory input to the RF center of ON RGCs via serial inhibition.

The maintained firing rates generated by a stimulus in the RF center are lower in both *Gla2*^{-/-} and *Gla3*^{-/-} ON-center RGCs compared to WT. In addition, the temporal kinetics of their RF center responses is more transient. This change when GlyR-mediated inputs are removed also suggests an increase in inhibition. The excitatory response of nearly all WT ON-center RGCs consists of a transient peak and a slower, sustained component. Inhibitory receptors with fast and slow kinetics differentially modulate the time course of the peak and maintained component, respectively (Han et al, 1997). Similar to GABA_A and GABA_C receptors, glycinergic currents also are composed of fast and slow components (Pan and Lipton, 1995; Han et al, 1997). The fast component of a glycinergic current peaks around 200ms (Han et al, 1997) and is presumably mediated by GlyR α 1 (Singer and Berger, 1992; Takahashi et al, 1992). A reduced maintained response matches the kinetics of GlyR α 2 (slow), but not GlyR α 3 (medium-fast). However, both share underlying disynaptic mechanisms. This similar effect argues that a

lower maintained firing rate of ON-center RGCs is more likely due to the synaptic mechanisms rather than the kinetics of the receptors.

Alternatively, the difference could be related to the kinetics of their inputs from cone DBCs. Four different morphological types of cone DBCs exist in the mouse retina (Ghosh et al, 2004) and produce either a more transient or more sustained response to light (Awatramani and Slaughter, 2000). It is plausible that GlyR α 2 and GlyR α 3 indirectly modulate inhibitory inputs to the RF center that could alter the time course of inhibition in two morphologically distinct types of cone DBCs. In this case, GlyR α 3-mediated inhibition would shape the maintained component of an ON-center RGC with faster peak and decay kinetics whereas GlyR α 2-mediated inhibition would shape the maintained component of an ON-center RGC with fast peak and slow decay kinetics. I interpret my results to suggest that in the WT retina, GlyR α 2- and GlyR α 3-mediated serial inhibition modulates the temporal response properties of ON-center RGCs by reducing inhibitory input to the RF center and producing more sustained responses to light. Since there are no glycinergic currents in cone DBCs and neither GlyR α 2 nor GlyR α 3 currents have been recorded in A-type ON RGCs, a serial inhibitory circuit with a GABAergic NF-AC must occur pre-synaptically at the cone DBCs terminals, or post-synaptically at the dendrites of ON-center RGCs. Future experiments measuring the magnitude of inhibitory and excitatory currents at the cone DBC or RGC level are required to explore this hypothesis further.

At DA levels, visually-evoked RF center responses of ON RGCs are independent of GlyRa2 and GlyRa3-mediated inhibition.

Neither the absence of GlyRa2- or GlyRa3-mediated inhibition affects the overall RF center responses of ON-center RGCs at DA levels. This is expected for *Gla2^{-/-}* ON-center RGCs given the absence of GlyRa2 expression or GlyRa2-mediated currents in the components of the rod pathway. In contrast, this was not expected for *Gla3^{-/-}* ON-center RGCs given the expression of GlyRa3 and GlyRa3-mediated currents in AII ACs, the major interneuron in the rod pathway. Without GlyRa3, AII ACs within the RF center should rest at a more depolarized state and this polarization will be shared with all cone DBCs via gap junctions. Thus, a small local depolarization may be dissipated within the AII AC network. No change in visually-evoked responses in *Gla3^{-/-}* ON-center RGCs could occur if our adaptation level is such that it cannot detect signaling through the rod pathways. However, my results from OFF-center RGCs in *Gla3^{-/-}* mice show that this is unlikely to be the case, since all OFF-center RGCs are affected (Chapter 5, page 163).

At LA levels, GlyRa2- and GlyRa3-mediated inhibition defines two circuits that differentially modulate visually-evoked RF center responses in separate OFF RGCs populations.

Two subpopulations of OFF-center RGCs (OFF_{K1} and OFF_{K2}) were defined by the cluster analysis (Chapter 3, page 55). The literature supports the idea that two physiologically different types of OFF-center RGCs exist among the large A-type RGCs (Pang et al, 2003; Margolis and Detwiler, 2007; Van Wyk et al, 2009). In my analysis

the response characteristic that served as the discriminating variable between these two populations was their peak firing rate to a stimulus matched to their RF center. OFF_{K1} RGCs have low peak firing rates and OFF_{K2} RGCs have high peak firing rates. In addition, OFF_{K1} RGCs are made up of nearly equal proportions of sustained and transient RGCs, whereas the majority of OFF_{K2} RGCs are sustained with response durations of ≥ 2 seconds. These clusters were similar across WT, *Gla2*^{-/-} and *Gla3*^{-/-} OFF-center RGCs (Chapter 3, page 59).

The maintained firing rate in *Gla2*^{-/-} OFF_{K1} RGCs is lower than WT. The direction of the effect and the lack of GlyR α 2-mediated currents in WT cone HBCs and OFF-center RGCs suggests GlyR α 2 participates either in cross-over inhibition from the On pathway or a glycine $\rightarrow$ glycine serial inhibitory circuit. The literature shows many examples of OFF inhibition from the On pathway, and vice versa, which is mediated by glycinergic NF-ACs (Roska et al, 2006; Molnar and Werblin, 2007; Hsueh et al, 2008; Molnar et al, 2009; Werblin, 2010). A role for GlyR α 2 in cross-over inhibition between On and Off pathways is supported by its expression and currents in a variety of NF-ACs with bi- or multi-stratified dendrites. However, my results argue against cross-over inhibition as a possible mechanism to explain lower maintained firing rates in *Gla2*^{-/-} OFF_{K1} RGCs. In WT retina a bi-stratified NF-AC that expresses GlyR α 2 is depolarized by a cone DBC in the On sublamina and releases glycine to a cone HBC or OFF RGC in the Off sublamina. In my experimental paradigm a dark spot presented to the RF center of an OFF RGC hyperpolarizes cone DBCs, decreasing glycine release from the NF-AC to cone HBCs or OFF RGCs. This would result in an increase, not a decrease in the maintained firing rate of OFF_{K1} RGCs. Therefore, my results argue that GlyR α 2 must

participate in a novel glycine (GlyR α 2) $\rightarrow$ glycine (GlyR α 1) serial inhibition. A NF-AC that expresses GlyR α 2 modulates the release of inhibition from a glycinergic AC. Since GlyR α 1 mediates chloride currents in WT cone HBCs and A-type OFF RGCs (Ivanova et al, 2006; Majumdar et al, 2007), the removal of GlyR α 2 will disinhibit the glycinergic NF-AC and increase glycine release, lowering the maintained firing rate of OFF_{K1} RGCs. In the WT retina then GlyR α 2 functions to reduce inhibitory inputs to the RF center to produce more sustained responses to light. GlyR α 2 mediates similar effects in all ON-center and OFF_{K1} RGCs. GlyR α 2 is widespread throughout all layers of the IPL and localized specifically to bi-stratified ACs, making it possible that the same AC expressing GlyR α 2 provides inputs to an ON- and OFF-center RGC. Although transgenic mouse lines expressing GFP in some ACs are available (Sarthy et al, 2007) the lack of specific markers for more than 20 types of ACs (MacNeil and Masland, 1999) makes it difficult to determine which AC provides inputs to different RGCs. In sum, the overall role of GlyR α 2 in both On and Off retinal pathways is to decrease inhibition to the RF center of RGCs.

In contrast, the peak firing rate is higher in *Gla3*^{-/-} OFF_{K2} RGCs compared to WT and indicates an increase in excitation in the absence of an inhibitory receptor. This effect can be accomplished most simply by the removal of a direct feedforward inhibition onto cone HBCs or OFF-center RGCs. Immunolabeling of GlyR α 3 is most dense in the Off sublamina where the dendrites of both transient and sustained OFF-center RGCs ramify (Van Wyk et al, 2009). Majumdar et al (2007) showed a very small number of GlyR α 3 immunoreactive puncta along the dendrites of OFF A-type RGCs. However, the small amount of puncta for GlyR α 3 (~5) paled in comparison to the ~27 GlyR α 1 puncta

along one dendrite of an OFF A-type RGC. Since chloride currents in cone HBCs and A-type RGCs are mediated GlyR α 1 (Ivanova et al, 2006; Majumdar et al, 2007) a simple removal of direct inhibition is unlikely and cannot explain increased excitation in *Gla3*^{-/-} OFF-center RGCs. Another possibility, given GlyR α 3 is expressed and mediates currents in AII ACs, relates to its newly discovered role in the cone pathway (Pang et al, 2007; Manookin et al, 2008). In this circuit, excitation in OFF-center RGCs is a combination of direct glutamate signaling from cone HBCs and their disinhibition that occurs from cone DBCs. A dark spot will hyperpolarize cone DBCs decreasing AII AC inhibition via bi-directional gap junctions and decrease glycine release (Trexler et al, 2001; Veruki and Hartveit, 2002, 2002a; Manookin et al, 2008). In the *Gla3*^{-/-} retina what is left to govern the AII AC polarization level is its connections to other AII ACs via gap junctions. The AII AC and cone DBCs that are inside the RF center will contribute but the small signal may be distributed throughout the network so that there is not net change in AII ACs. With no net current to the AII AC, glutamate signaling in the Off pathway cannot be modulated and OFF-center RGC peak firing rate increases. My data show that in the WT retina, GlyR α 3 increases inhibition to cone HBCs via a novel glycine (GlyR α 3) $\rightarrow$ glycine (GlyR α 1) serial inhibitory circuit that increases inhibition to attenuate the RF center peak response. Previous reports have only demonstrated GABA $\rightarrow$ glycine serial inhibition at the level of BCs (Eggers and Lukasiewicz, 2010), and GABA $\rightarrow$ glycine or glycine $\rightarrow$ GABA serial inhibition at the level of RGCs (Zhang et al, 1997; Russell and Werblin, 2010). Past reports would not have been able to parse out a glycine $\rightarrow$ glycine serial inhibitory connection using strychnine. Only through the use of GlyR KO mice is this conclusion possible.

The subpopulation of OFF-center RGCs affected by the absence of GlyR α 2 (OFF $_{K1}$) is not the same population of OFF-center RGCs affected by the absence of GlyR α 3 (OFF $_{K2}$). This suggests there may be two OFF circuits in the IPL defined by GlyR α 2- and GlyR α 3-mediated inhibition. There are four different morphological types of cone HBCs in the mammalian retina (Ghosh et al, 2004) with distinct temporal properties (Devries, 2000; Euler and Masland, 2000). Cone HBC output is shaped by GABA and glycinergic feedback inhibition from ACs to generate transient and sustained light responses in RGCs (Tachibana and Kaneko, 1987; Lukasiewicz and Werblin, 1994; Lukasiewicz and Shields, 1995; Dong and Werblin, 1998). My results are the first demonstration that GlyR subunit-specific inhibition modulates BC release in at least two types of cone HBCs. Given the kinetics of the GlyRs, serial inhibitory inputs mediated by GlyR α 2 most likely modulate sustained cone HBCs, whereas those mediated by GlyR α 3 most likely modulate transient cone HBCs. These specific glycinergic mechanisms contribute to additional functional asymmetries within the Off pathway that add to the already complex asymmetries previously characterized between the On and Off pathways (Chichilnisky and Kalmar, 2002).

At DA levels, GlyR α 3 but not GlyR α 2 mediates inputs to the RF center of OFF RGCs.

Similar to its effect on ON-center RGCs, GlyR α 2 does not affect visually-evoked responses of OFF-center RGCs at DA levels. This is consistent with the absence of GlyR α 2 expression and GlyR α 2-mediated currents in the components of the rod pathway. In contrast, the peak firing rate is lower in *GlyR α 3*^{-/-} OFF $_{K2}$ RGCs compared to WT and indicates a decrease in excitation in the absence of an inhibitory receptor. My results are

consistent with GlyR α 3 expression and currents in AII ACs and their role in the DA circuit and suggest again, a serial glycine (GlyR α 3) $\rightarrow$ glycine (GlyR α 1) inhibition to cone HBCs. In WT retina GlyR α 3 indirectly modulates the release of glycine from AII ACs through the primary rod pathway. The excitatory response initiated in the rod photoreceptors is relayed to OFF-center RGCs via rod DBCs and AII ACs which relay rod DBC signals to cone HBCs through a sign-inverting glycinergic synapse (Famiglietti and Kolb, 1976; Strettoi et al, 1992). In the absence of GlyR α 3, AII ACs are more depolarized and increase glycine release to cone HBCs, decreasing the peak firing rate in OFF-center RGCs. I interpret my results to suggest that in the WT retina GlyR α 3-mediated inhibition reduces inhibitory inputs to the RF center of OFF-center RGCs.

In OFF-center RGCs, GlyR α 2 but not GlyR α 3 increases suppression to the local RF surround via direct feedforward inhibition and decreases suppression to the lateral RF surround via serial inhibition.

Inhibitory networks of ACs in the inner retina modulate BC output and shape the magnitude and timing of inhibitory inputs to RGCs, ultimately spatially and temporally tuning their output (Roska et al, 1998; Eggers and Lukasiewicz, 2006; Eggers et al, 2007). The contribution of individual AC networks to RGC output is complicated given the diverse morphology of BCs (10 types) (Ghosh et al, 2004) and ACs (>20 types) (Masland, 2001). In addition, AC input to BCs and RGCs is mediated by classes of diverse inhibitory receptors that are disproportionately localized to specific BCs and RGCs (Sassoé-Pognetto et al, 1994; Pan and Lipton, 1995; Wässle et al, 1998; Euler and Masland, 2000; Lukasiewicz and Shields, 2003; Ivanova et al, 2006; Eggers et al, 2007;

Majumdar et al, 2007). Recently, it has been shown that serial inhibitory connections between ACs differentially affect local and lateral inhibition to BCs by decreasing or enhancing inhibitory inputs to a narrow or wide field light stimulus, respectively (Eggers and Lukasiewicz, 2010). They showed that serial connections between ACs limit the spatial extent of inhibition in BCs which subsequently contributes to spatial processing in RGCs.

I used an annulus whose inner diameter was optimized to isolate the local RF surround and an annulus whose inner diameter was larger than the optimal to isolate the lateral RF surround. The majority of ON-center RGCs (WT=93%, *Gla2*^{-/-}=98%, and *Gla3*^{-/-}=88%) and the majority of OFF-center RGCs (WT=96%, *Gla2*^{-/-}=11%, *Gla3*^{-/-}=86%) were suppressed during the presentation of annulus. Most NF-ACs are glycinergic and mediate inputs to the RF center whereas WF-ACs are GABAergic and mediate inputs to the RF surround. Given that GlyR α 2 is localized to GABAergic WF-ACs and GlyR α 3 is localized to NF-ACs, changes in the RF surround response in *Gla2*^{-/-} but not *Gla3*^{-/-} RGCs is an expected result. Specifically, GlyR α 2 differentially affects local and lateral RF surround responses in OFF- but not ON-center RGCs via two separate inhibitory circuits. GlyR α 2 increases inhibitory inputs to the local RF surround via a direct feedforward inhibition to RGCs and decreases inhibitory inputs to the lateral RF surround via serial inhibition with a GABAergic WF-AC. GlyR α 2 has the same effect in all OFF-center RGCs and therefore their responses were combined.

Local RF suppression is reduced in *Gla2*^{-/-} OFF-center RGCs compared to WT. Although GlyR α 2-mediated currents have not been localized to any type of RGC to date,

the only way to get a reduction in suppression in the absence of an inhibitory receptor is through the removal of a direct inhibitory input to the RF surround. My results suggest that a glycinergic WF-AC expressing GlyR α 2 must provide a direct input to the RF surround in OFF-center RGCs. If GlyR α 2 is eliminated, inhibitory inputs to the RF surround in *Glr α 2*^{-/-} OFF-center RGCs decreases, thus reducing the RF surround suppression. Eggers and Lukasiewicz (2010) showed that blocking serial AC connections did not affect suppression in the local surround of BCs, which supports a direct feedforward inhibition to local RF surround suppression.

In contrast, lateral RF suppression is greater in *Glr α 2*^{-/-} OFF-center RGCs. Annuli with larger inner diameters theoretically activate more of the AC network that generates lateral surround antagonism while at the same time activating BC input. The only way to get more suppression in the absence of an inhibitory receptor is via cross-over inhibition from the On pathway. GlyR α 2 expression and currents have been reported in bi- and multi-stratified GABAergic WF-ACs (Majumdar et al, 2009) suggesting they receive glycinergic inhibition but may also receive excitation within a separate sublamina. If GlyR α 2 is eliminated, these GABAergic WF-ACs do not receive inhibitory inputs and become more depolarized, increasing suppression to OFF RGC's lateral RF surround. Eggers and Lukasiewicz (2010) also showed that larger stimuli activate an extensive AC network and that GABAergic and glycinergic inputs to cone HBCs are both influenced by GABAergic WF-ACs. However in their circuit, GABA_AR inputs modulated glycinergic ACs and no serial glycine $\rightarrow$ GABA circuit was observed at the BC level. My results show GlyR α 2 inputs modulate a GABAergic AC and support a glycine $\rightarrow$ GABA cross-over inhibitory circuit at the level of RGCs. Glycine $\rightarrow$ GABA serial

inhibition has been reported in local edge detector RGCs in rabbit (Russell and Werblin, 2010). My data show that the effect I observe must be cross-over inhibition and cannot be serial inhibition within the Off pathway given cone HBCs would hyperpolarize to an annulus with a large, bright inner diameter and reduce suppression to the RF surround.

The mechanisms underlying inputs to the RF surround of RGCs are complex and are made up of multiple rectifying subunits that underlie nonlinear visual processes such as frequency-doubling and contrast-reversal grating responses (Enroth-Cugell and Robson, 1966; Hochstein and Shapely, 1976). These underlying subunits can either be inhibitory or excitatory depending on the temporal and spatial pattern of the stimulus (Passaglia et al, 2001; 2009). Reports have shown stimuli that exceed the RF surround, such as shifts in sensitivity during saccadic eye movements, will reverse suppression and increase RGC spiking (Barlow et al, 1977; Geffen et al, 2007). In my experiment WT OFF-center RGCs on average increase spiking to larger inner diameter annuli (refer to Figure 4-27). In contrast, *Gla2*^{-/-} OFF-center RGCs do not show a reversal in RF surround response to larger inner diameters but rather remain suppressed which most likely results from increased inhibition to the RF surround in these cells in the absence of GlyR α 2.

Multiple GlyR α subunits at post-synaptic clusters and up-regulation may contribute to the observed effects.

Synaptic GlyRs are composed of 2 α and 3 β subunits (Grudzinska et al, 2005). While the GlyRs are thought to contain only one type of α subunit, immunocytochemical studies showed ~28% coincident rate of α 3 and α 2 subunits (Haverkamp et al, 2003) and

~31% coincident rate of $\alpha 2$ and $\alpha 4$ subunits present at the same post-synaptic site (Heinze et al, 2007; reviewed by Wässle, 2009). The limitations imposed on the spatial resolution capacity of confocal microscopy cast doubts that these subunits are in perfect register with one another (Heinze et al, 2007). GlyRs consisting of $\alpha 1/\alpha 3/\beta$ for example, would further diversify the kinetics of glycinergic inhibition. The lack of selective agonists and antagonists that would distinguish different GlyR isoforms at post-synaptic sites are not available (Betz and Laube, 2006). Therefore as a general rule, post-synaptic clustering of GlyRs contain only one type of α subunit (Wässle, 2009).

The recent availability of GlyR subunit knock-out mice have proved to be useful tools in examining the role of glycinergic inhibition in different retinal cell types. A caveat to these genetically mutated mice however, is possible up-regulation of other subunits to compensate for the loss of a particular subunit (Heinze et al, 2007). For example, in the absence of GlyR $\alpha 3$ expression $\alpha 2$ is up-regulated, and in the absence of GlyR $\alpha 2$ expression $\alpha 4$ is up-regulated (Heinze et al, 2007). However, the effects I observed do not suggest up-regulation but rather GlyR $\alpha 2$ and GlyR $\alpha 3$ participate in very different retinal circuits and fulfill different roles in visual processing.

There is only one case where my results show that both GlyR $\alpha 2$ and GlyR $\alpha 3$ modulate a similar response and that is a lower maintained firing rate in ON-center RGCs. If $\alpha 2$ (slow) is up-regulated in the absence of GlyR $\alpha 3$ (medium-fast), I would expect an increase in the slow inhibitory current to the RF center of *Gla3*^{-/-} ON-center RGCs thereby prolonging their maintained component. However, in *Gla3*^{-/-} ON-center RGCs the maintained firing rate is lower and the temporal kinetics of the response is

more transient. Similarly, if $\alpha 4$ (very slow) is up-regulated in the absence of GlyR $\alpha 2$ (slow), I would expect an even more sustained inhibitory current to the RF center resulting in prolonged maintained firing rates. However, in *Gla2*^{-/-} ON- and OFF-center RGCs, the maintained firing rate is lower and the temporal kinetics of the response also is more transient. Therefore, up-regulation does not appear to be compensatory.

GlyR subunit-specific inhibition enhances RGC excitation via cross-over inhibition.

The serial inhibitory circuits that I proposed all reduce inhibition to the RF center. A reduction in inhibition suggests that glycine enhances rather than opposes RGC excitation, a phenomenon observed through cross-over inhibition (Werblin, 2010). Recent reports have shown that cross-over inhibition occurs at all levels of inner retinal processing including ACs, BCs and RGCs and is mediated by glycinergic NF-ACs (Roska et al, 2006; Molnar et al, 2007, 2009; Hsueh et al, 2008). The functional implication of cross-over inhibition is to linearize the non-linear rectification of signals inherent in synaptic transmission (Molnar et al, 2009; Werblin, 2010). Briefly, a pre-synaptic depolarization releases more transmitter than hyperpolarization. Once the pre-synaptic voltage is transmitted synaptically, it is presented as post-synaptic rectifying currents. Cross-over currents generated by glycinergic ACs are added and combined to produce a more linear post-synaptic voltage response (Werblin, 2010). While non-linearity is important for processing of motion (Barlow and Levick, 1965; Fried et al, 2002) and local edge detection (Van Wyk et al, 2006; Russell and Werblin, 2010) it can also compromise other forms of visual processing such as distinguishing between luminance and contrast (Werblin, 2010). Therefore, glycinergic-mediated cross-over

inhibition distorts the effects of synaptic rectification through synergistic changes in current such that inhibition decreases when excitation increases and combines to enhance, rather than oppose RGC excitatory responses (Roska et al, 2006). The effects I observe in the RF surround of OFF-center RGCs are the result of cross-over and not serial inhibition. Therefore, cross-over inhibition most likely serves to enhance the antagonistic effects of the RF surround and overall spatial tuning of OFF-center RGCs (Werblin, 2010).

Serial connections between interneurons are common across sensory systems as well (Schmidt et al, 2001; Pinaud et al, 2008; Raji et al, 2008). In addition, synaptic rectification is inherent in neurotransmission and therefore cross-over inhibition can be applied to all levels of sensory processing (Werblin, 2010). It is perhaps the combination of the two that is necessary to maintain balanced excitation and inhibition in the processing of sensory signals throughout the CNS.

GlyR subunit-specific inhibition plays a role in processing spatial-temporal filtering of visual information.

Intrinsic noise inherent in biological systems limits a neuron's ability to encode sensory signals (Srinivasan et al, 1982). In the early stages of visual processing, RF center/surround organization enables RGCs to encode meaningful spatial and temporal frequencies in a visual scene in a way that minimizes intrinsic noise (Barlow, 1961; Srinivasan et al, 1982; Tokutake and Freed, 2008). The processing of visual information in the RF surround lags behind the RF center mechanism in an attempt to filter temporal redundancies (Frishman et al, 1987; Tokutake and Freed, 2008). In addition, the

antagonistic surround computes the correlations between neighboring intensities in a visual image and takes the weighted sum to predict a signal at the RF center (Srinivasan et al, 1982). This predicted signal is then subtracted from the actual center in an attempt to minimize the range of signals the RGC needs to encode (Srinivasan et al, 1982). In this way, predictive coding eliminates spatial and temporal redundancies that are inherent in visual images (Srinivana et al, 1982; Tokutake and Freed, 2008). My results can be extended to include a role for GlyR α 2- and GlyR α 3-mediated inhibitory effects on RGCs' spike rate along different stages of information processing that affect the efficiency of RGC's neural coding to higher visual processing centers. Specifically, GlyR α 2 reduces inhibition to the RF center to produce more sustained responses to light. In the local RF surround, GlyR α 2 increases inhibition to enhance spatial tuning of RFs whereas in the lateral RF surround, GlyR α 2 increases sensitivity in the periphery. Finally, GlyR α 3 increases inhibitory inputs to the RF center to enhance the signal-to-noise ratio in the processing of visual information.

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Education

University of Louisville
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Ph.D. Experimental Psychology;
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Mentor: Dr. Paul DeMarco

M.A Psychology; August 2005

University of Louisville

B.A. Psychology; May 1999

Major: Psychology

Research Interests

My research interests include investigating the retinal circuitry and the synaptic mechanisms that contribute to the formation of receptive field center/surround organization in RGCs. I am particularly interested in the breakdown of signal transmission and the remodeling of synaptic connections that occur in retinal neurons during disease and degeneration.

Publications & Abstracts

Heflin, S.J.; Nobles, R.D., Sagdullaev, B.; Vessey, K.V.; Heath, K., Guido, W.; Gregg, R.G. and McCall, M.A. (manuscript submitted).

Receptive Field Spatial Organization of Mouse OFF and Bistratified Retinal Ganglion Cells Requires Normal Input from the On Retinal Pathway

Nobles, R.D.; Betz, H.; Cepko, C.; and McCall, M.A. (manuscript in preparation).
Glycine Receptor Subunit-Specific Inhibition Differentially Contributes to Receptive Field Center/Surround Organization in ON- and OFF-Center RGCs.

Nobles-Ralston, R.D., Bilotta, J., and Petry, H.M. (2004).
Circadian Cycles of Caspase-3 Activation in Zebrafish Cone Photoreceptors.
Poster session presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Ft. Lauderdale, Florida.

Nobles, R.D., Müller, U., Betz, H., and McCall, M.A. (2007).
Glycine Receptor α Subunit-Mediated Inhibition in the ON and OFF Pathways Differentially Contributes to Retinal Ganglion Cell Responses.
Poster session presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Ft. Lauderdale, Florida.

Nobles, R.D., Müller, U., Betz, H., and McCall, M.A. (2008).
Glycine Receptor α 3- and α 2 Subunit-Mediated Inhibition Differentially Contributes to the Responses OFF Retinal Ganglion Cells.

Poster session presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Ft. Lauderdale, Florida.

Thomas, A.A., Nobles, R.D., Howell, G.R., John, S.W., McCall, M.A. (2009).
Changes in Retinal Ganglion Cell Spontaneous and Visually-Evoked Responses in the DBA2J Mouse Model of Glaucoma.

Poster session presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Ft. Lauderdale, Florida.

Nobles, R.D., Müller U., Betz, H., McCall, M.A. (2010).
Glycine Receptor Subunits α 2 and α 3 Participate in Different Inhibitory Circuits that Alter the RF Organization of ON- and OFF-Center Retinal Ganglion Cells.

Poster session presented at the annual summer research conference for the Federation of American Societies for Experimental Biology, Saxtons River, Vermont.

Heflin, S.J.; Nobles, R.D., Heath, K.M., Gregg, R.G., McCall, M.A. (2010).
Altered Presynaptic Inputs of Ganglion and Displaced Amacrine Cells in No B Wave Mutants Result in Disrupted Receptive Field Organization.

Poster session presented at the annual summer research conference for the Federation of American Societies for Experimental Biology, Saxtons River, Vermont.

Grants

Sigma Xi Grant 2004

Project title: Caspase-3 Activation in Zebrafish Cone Photoreceptors.

Award: \$2000.00

Awards

Society for Neuroscience Louisville, Ky. Chapter: Graduate Poster Competition 2004

Project title: Circadian Cycles of Caspase-3 Activation in Zebrafish Cone Photoreceptors.

Award: \$250.00

The ARVO Foundation for Eye Research/Retina Research Foundation/Joseph M. and Eula C. Lawrence Travel Award 2007

Project title: Glycine Receptor α Subunit-Mediated Inhibition in the ON and OFF Pathways Differentially Contributes to Retinal Ganglion Cell Responses.

Award: \$750.00

CGeMM Travel Award

Project title: Glycine Receptor Subunits $-\alpha 2$ and $-\alpha 3$ Participate in Different Inhibitory Circuits that Alter the RF Organization of ON- and OFF-Center Retinal Ganglion Cells.

Award: \$1000.00

Grawmeyer Summer Fellowship 2004

Grawmeyer Summer Fellowship 2005

Grawmeyer Summer Fellowship 2006

Teaching Experience

Physiological Psychology, T.A.	Fall 2003
Physiological Psychology, T.A.	Spring 2004
Psychology 201, T.A.	Fall 2004
Psychology 201, T.A.	Spring 2005
Physiological Psychology, Guest Lecturer	Spring 2005
Psychology 201, T.A.	Fall 2005
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