Diabetic Complications Consortium

Application Title: Synaptic changes in the outer retina of a diabetic zebrafish model

Principal Investigator: Salvatore L. Stella, Jr.

Subaward (Grant) 30835-13 2 U24 DK076169-11

1. Project Accomplishments:

We are still in the process of acquiring, analyzing data, and carrying out many of the originally planned experiments as they relate to this pilot project. The award for this project officially started in 01/01/2017 at Penn State University, College of Medicine, Hershey. The project award letter arrived on 10/4/2016, with a completion date of 07/31/2017. Due to unforeseen administrative delays the IACUC protocol was pending upon notification of the award letter and was approved on 02/08/2017. This significant delay precluded my lab for starting any experiments associated with this project until 02/08/2017. The project end date was originally set for 07/31/2017, however, this was not sufficient time to compete the proposed studies. We requested a no cost extension that was approved and extended one year to 07/31/2018.

In addition, we made a major discovery, this diverted from our originally proposed strategy as originally described in a study by *Gleeson et al., Acta Diabetol. 44:157-63, 2007*. The authors oscillated the glucose concentration daily and the tank underwent complete water changes from 2% glucose to 0% daily for at least 30 days. This original approach severely slowed the progress of our project down. This method resulted in significant mortality of zebrafish (60-80% death in our hands). After consultation with our IACUC, Zebrafish Core Facility Manager, and other external PIs working with hyperglycemic zebrafish models, we have pioneered a newer more stable method for inducing hyperglycemia that involves graded changes in the external glucose environment starting 0.5% through 3%. This method prevents any significant mortality, and affords up the opportunity of maintaining zebrafish for at least 30 days at 3.0% glucose. In addition, the zebrafish under these conditions maintain a serum glucose level of at least 180 mg/dL for the duration of the studies. This method utilizes younger adult zebrafish $\geq$ 6 months. In low glucose water starting at 0.5% for 48hrs, changing the solution every two days at 0.5% glucose increase every 48 hours. This approach maintains the necessary elevated glucose level without impacting zebrafish survival.

A diagram is shown below:

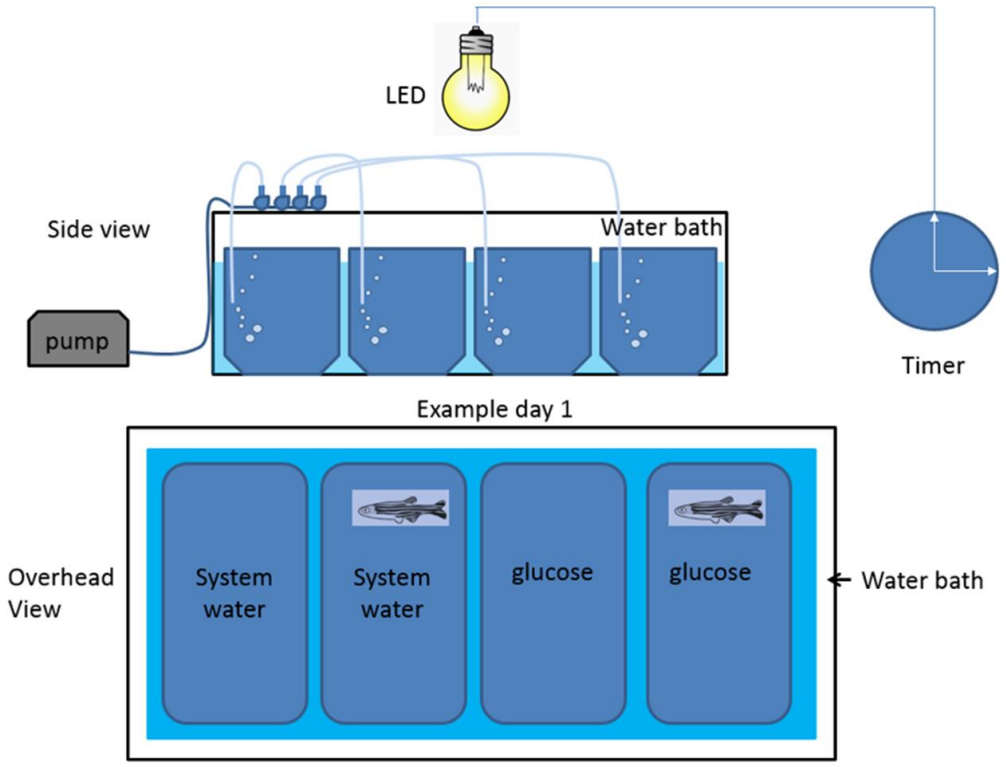

Fig.1 Aquarium set up. Tanks are located in a 28°C water both and fish are moved to a new tank every 24 hours.

The water is changed daily for glucose-treated and non-diabetic aged-match controls, there will be no need to measure nitrates or nitrites or provide water filtration or measure conductivity. Water will be tested with a standardized colormetric pH test in the morning before the water change occurs and in the evening in all tanks. All tanks will be bubbled continuously with air derived from an aquarium pump in each tank separately. Typically fish will experience increasing glucose levels every 48 hrs until they reach 3% glucose. Once 3% is achieved the fish are kept in that level of glucose, with water changes occurring evry 24 hours.

2. Specific Aims:

Aim 1: Test whether hyperglycemia alters presynaptic properties and synaptic architecture in neurotransmission at the first synapse in visual processing.

Results: We have mapped and characterized the Cav1.4 calcium channel and CtBP1, a component of the photoreceptor synaptic ribbon, in diabetic zebrafish treated in 3% glucose water for 30 days and compared that to control conditions. We tested whether critical components like the voltage-gated calcium channels (CaV1.4), which are responsible for transmitter release that are found at the synapse are altered and the expression of Cav 1.4 is reduced in diabetic zebrafish after 30 days in 3% glucose (see Fig. 2). This figure shows that the expression of the CaV 1.4, calcium channel is reduced in glucose treated or hyperglycemic zebrafish. Decreased expression of the voltage dependent calcium channel can greatly alter transmitter release form cone photoreceptors in the outer retina. Our next step is to record Ca^{2+} currents, and perform Ca^{2+} imaging

from photoreceptors looking at the magnitude of the calcium responses in hyperglycemic zebrafish versus untreated controls. In addition, we plan on measuring exocytosis, using activity-dependent dyes like FM 1-43 to test whether there is a significant difference in the level of exocytosis in hyperglycemic zebrafish and see if that correlates with decreased expression of CaV1.4 in cone photoreceptors.

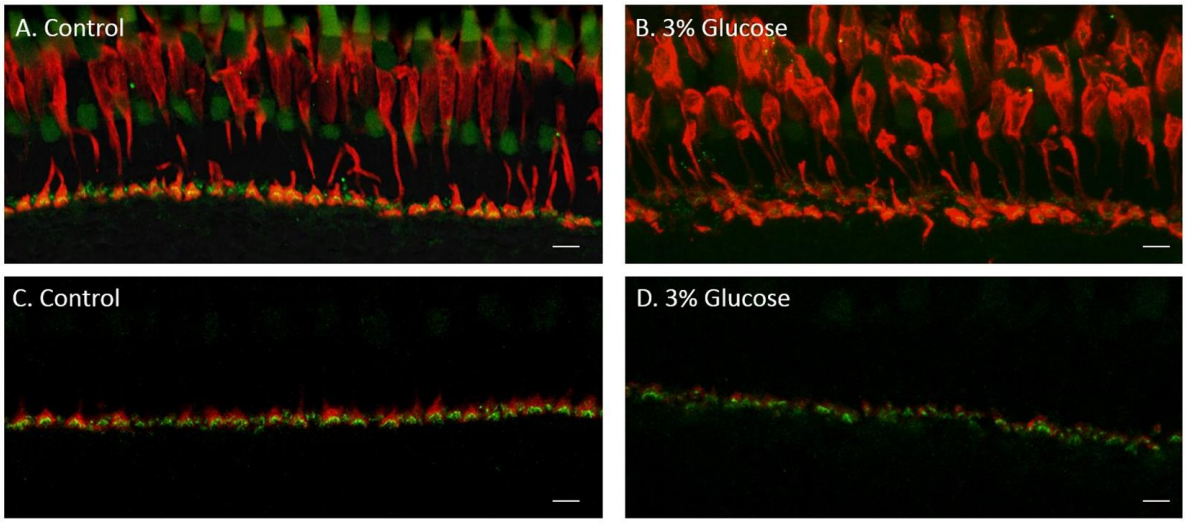

Fig. 2. Outer retinal presynaptic protein laebling in hyperglycemic (3% glucose) versus untreated zebrafish. A. Untreated (control) retina and B. Hyperglycemic (3% glucose treated). A and B illustrates immunolabeling of CaV1.4 (voltage-dependent calcium channel, green) and zpr-1 (a cone photoreceptor marker, red). C. Untreated (control) retina and D. Hyperglycemic (3% glucose treated). C and D illustrates immunolabeling of CaV1.4 (green) and CTBP1 (a synaptic ribbon marker expressed in photoreceptor terminals, red). Arrow indicate disorganized synaptic terminals and decreased expression of synaptic proteins in the outer plexiform layer. Scale bar is 10 microns.

Specific Aim 2: Test whether hyperglycemia alters post-synaptic responses and receptors involved in neurotransmission at the first synapse in visual processing.

Results: Our working hypothesis for this aim is that hyperglycemia-induces changes that alter either the structure or the receptors at this outer retinal synapse, and disrupt post-signaling from photoreceptors to second-order neurons (post-synaptic). Currently, we using whole-cell patch clamp electrophysiology and immunohistochemistry address these questions. So far we have mapped the key proteins and examined if there are any significant structural changes that we observe between presynaptic photoreceptors and post-synaptic connections. We find that since the outer retina is disorganized following hyperglycemic changes we see changes in the contacts that are made between Zpr-1 positive photoreceptors and PKC alpha positive Mb1 bipolar cells (see Figure 3). In addion we are testing the differences in expression of mGluR6 expressed at all ON bipolar cells, along with the commensurate Go signaling G-protein, and TRPM1, cation channel found at all ON bipolar cells. We predict that this relationship will be altered because we find structural difference among glucose treated and untreated zebrafish, (see Fig. 3).

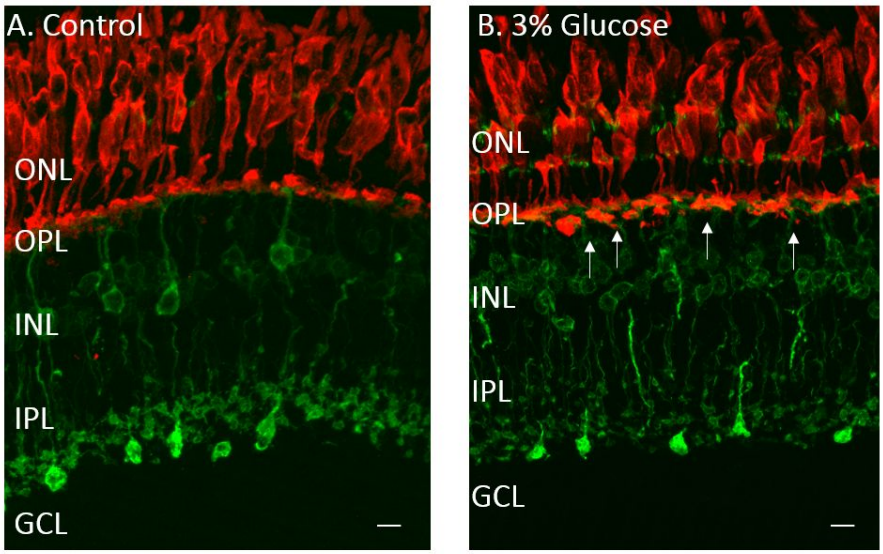

Fig. 3. Connectivity among photoreceptors and second-order neurons. A. Control (untreated) zebrafish retina immunolabled with Zpr-1(red) and PKC alpha (green), an Mb1 ON bipolar cell marker. The OPL is well organized and the connections between the photoreceptor and PKC alpha positive bipolar cells are organized. B. 3% Glucose treated zebrafish retina immunolabeled with Zpr-1(red) and PKC alpha (green), arrowheads indicate disorganized synapses among photoreceptors and PKC alpha positive bipolar cells. Scale bar is 10 microns.

3. Publications:

To date there are no publications from this study, however, we have a manuscript in preparation that we plan to submit to a zebrafish focused journal, e.g., Zebrafish, outlining the utility and our findings using the graded glucose approach as described above. This novel strategy affords easy generation of hyperglycemic zebrafish that maintain a total blood glucose > 180 mg/dL. This submission is planned for Jan 2018.