VOLTAGE GATED CURRENTS AND GLUTAMATERGIC SYNAPTIC TRANSMISSION IN THE RETINAL SLICE OF THE LITTLE SKATE, RAJA ERINACEA, AND SPINY DOGFISH, SQUALUS ACANTHIAS.

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The vertebrate retina is an extension of the brain into the eye and is capable of transforming the light signal imaged on the back of the eye into a high order biological signal within the intricate neural circuitry of the retina. Fast, excitatory synaptic transmission in the intricate neural circuitry of the vertebrate retina is largely mediated by a variety of glutamatergic synapse types, whose underlying differences may be the consequence of the differential expression at the synapses of some combination of the at least 6 known ionotropic glutamate receptor subunits and 6 metabatropic glutamate receptor subunits expressed in the retina. The postsynaptic neurons at the glutamatergic synapses possess voltage gated sodium, potassium, and calcium channels which enable the glutamate mediated signal to be further processed (e.g. Maguire, G. J. Gen. Physiol. 95:775, 1990). It is now thought that different glutamate receptor subtypes mediate rod versus cone photoreceptor synaptic transmission to their postsynaptic neurons (Nawy, S., and Copenhagen, D. Nature 325:56. ; Maguire, G. and Werblin, F. Biophysical J. 59:448, 1991). In order to better define the glutamate receptor subtypes involved in processing the rod photoreceptor signal, and the consequential activation of the postsynaptic neuron through activation of a concert of voltage gated channels by depolarization at the rod mediated glutamatergic synapse , study of the all rod retina of the little skate, and of the rod dominated retina of dogfish was undertaken.

Whole cell patch recording of bipolar and amacrine cells in the retinal slice of both skates and dogfish were made using animals obtained at MDIBL. The whole cell method is an approximation to the voltage clamp method; The interior of the micropipette is made contiguous, and approximately isopotential, with the interior of the cell. The two eyes were enucleated and one was used immediately for making slices, while the other was immersed in saline solution and refrigerated for later use. Briefly, the tissue surrounding the orbit of the eye was removed using fine iris scissors, and then the front portion of the eye was cut away leaving an eyecup preparation. Using fine iris scissors, a small chip of retina ($< 1cm^2$) was cut away from the eyecup. The chip of retina, still attached to sclera, was laid retina side down onto a piece of millipore filter. The millipore was attached to the bottom of a recording chamber by pressing it firmly into two strips of petroleum jelly at the bottom of the dish. The sclera was then grasped with fine forceps and removed from the retinal chip. The retina remains tightly and flatly adhered to the millipore which serves as mechanical support for the retinal chip. Physiological saline was quickly added to the chamber, and then small slices (100-200 um thick) were made using carbon surgical razor blades. Each slice was separated, seated in the two petroleum jelly strips, and rotated 90 degrees so that the layering of the retina was facing upwards, under view of the microscope objective (Hoffman modulation contrast, water immersion, 40X, 0.8 mm working distance). Patch recordings were made using an Axopatch 200 amplifier with an TL-1 interface and P-clamp software for data acquisition and analysis. Recording pipettes were filled

with Lucifer yellow which diffused into the cells within several minutes, and was viewed under UV epifluorescence. Bathing solution was (in mM): NaCl, 120; KCl, 2; CaCl, 3; MgCl, 2; HEPES, 4, glucose, 3 brought to pH 7.5 with NaOH. Pipettes were filled with: KCl, 12; K-gluconate, 104; EGTA, 1; HEPES, 4; CaCl, 0.1 brought to pH 7.4 with KOH. Input resistances of the cells varied between 500 Mohm and 2 gigaohms.

Lucifer yellow filling revealed a variety of bipolar and amacrine cell types in these two species. Whole cell patch recordings of voltage gated channels revealed a complex potassium current in all of the cells studied, which was probably carried by a combination of many potassium channel types. Calcium channel currents were revealed in both cell types by blocking K+ channels with extracellular TEA, and TTX sensitive sodium currents were observed in most amacrine cells. Rapid and focal application of glutamate and its analogues onto the processes of the two cell types revealed that a variety of glutamate receptor types are present in these retinae, including rapidly desensitizing glutamate receptors in amacrine cells and a conductance decreasing type glutamate receptor in bipolar cells. Current-voltage relations for a typical voltage gated sodium, and glutamate gated current taken from an amacrine cell are presented in Figure 1.

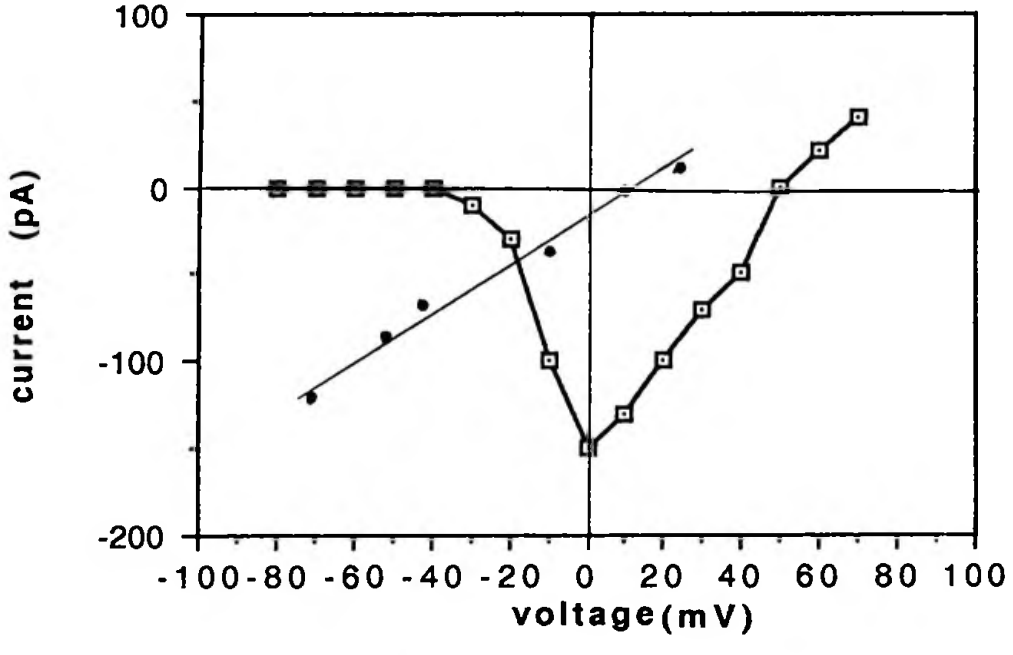

Figure 1. I-V curves for a voltage gated sodium current (■), and for a glutamate gated current (●) in retinal amacrine cells.

Future work will concentrate on defining the glutamate receptor subtypes, and voltage gated channel types located in identified cells in these two retinae. The methods developed here will allow for a clearer understanding of the rod photoreceptor neural circuitry of the vertebrate retina, and can provide a model system for defining the side effects of cardiovascular drugs on the retina, as well as precisely defining the site and modes of actions of neurotoxins on the rod pathways of the neural retina.

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