Research Grants for Graduate Students

Departmental Evaluation Sheet

Please fill out an evaluation form for each RGGS application submitted by your department. The completed evaluation forms and RGGS proposals are due in the Graduate School by 4:30 PM, October 1, 2008, or February 4, 2009. Proposals should be evaluated according to the three primary criteria for the RGGS program:

1. The originality/creativity and significance of the student's proposed research.
2. The clarity and appropriateness of the student's research design and procedure.
3. The feasibility of the student's proposed research.

Also note that the RGGS research projects should be for work that is to be conducted. Proposals that describe projects where significant work has already been completed are ordinarily not funded. Please pay particular attention to the timeline of the proposal to see that it accurately reflects the status of the project. Please note that RGGS funds cannot be used to reimburse money spent prior to the award. If you have questions about the evaluation of proposals, please contact the Graduate School.

Student Name: ____________________

Project Title: Investigating the effects of autophagy...

This proposal was ranked ____ out of ____ proposals submitted by the department

In the space below, please provide your departmental evaluation of this proposal. If more than one proposal is submitted from your department, clearly explain the reasons for the relative ranking of this proposal. Attach additional sheet, if necessary.

Department: Biological Sciences

Signature of Chair: ____________________

Print Form

RESEARCH GRANTS FOR GRADUATE STUDENTS Application Cover Sheet		2009-2010 Deadlines 10/5/2009 OR 2/8/2010	
NAME	Student Number	Date:	Email Address
Home Town	Mailing Address		
Department Name		Dept. Campus Box	Requested Amount
Biology			$489

Project Title
Investigating the Effects of Autophagy Genes on Glutamate Receptors

Nature of Project (check one)	Is this a resubmission? (Check one)
☑ Thesis ☐ Other Research Project	☐ Yes. If yes, previous app: _____ ☑ No
Expected Date of Graduation:	Student's Signature:
May 2011	

Compliances (Please check if your project involves any of the following):

☑ Animal Care	☐ Biosafety	☐ Hazardous Waste	☐ Human Subjects	☐ Radiological Safety

Project Summary (No more than 300 words)

The central nervous system contains specialized cells called neurons that can communicate with one another via presynaptic cells releasing chemical neurotransmitters that bind to postsynaptic cells. Binding of the neurotransmitter to the postsynaptic cell can then initiate a response in the cell. This type of chemical transmission is dependent on the correct formation and localization of synaptic proteins, including postsynaptic receptors. Glutamate receptors (GluRs) are important in the central nervous system because they conduct a majority of fast, excitatory transmission between neurons. The trafficking, localization, and expression of glutamate receptors remains poorly understood even though it is known that several neurodegenerative diseases are the result of disrupting one or more of these pathways. It has been found that autophagy-specific

		2-1-10
Major Advisor (Printed Name)	Major Advisor Signature	Date
Dr. William Retzlaff	[signature]	2/4/10
Department Chair (Printed Name)	Department Chair Signature	Date

FOR GRADUATE SCHOOL USE

GPA: ______ **Earned Hours:** ______ **Reviewed** ______

Approved: ______ **Not Recommended:** _______

RPAB Chair Signature: ______________________________ **Date:** ______________

Project Begin Date: ____________________ **Final Report Due:** _______________

Project summary:

The central nervous system contains specialized cells called neurons that can communicate with one another via presynaptic cells releasing chemical neurotransmitters that bind to postsynaptic cells. Binding of the neurotransmitter to the postsynaptic cell can then initiate a response in the cell. This type of chemical transmission is dependent on the correct formation of synaptic proteins, including postsynaptic receptors. Glutamate receptors(GluRs) are important in the central nervous system because they conduct a majority of fast, excitatory transmission between neurons. The trafficking, localization, and expression of glutamate receptors remains poorly understood, although it is known that several neurodegenerative diseases are the result of disrupting one or more of these pathways. It has been found that autophagy-specific gene 1 (atg1) is required for GluR expression at the neuromuscular junction of Drosophila melanogaster. Animals with mutations affecting atg1 function show reduced GluR expression. There are several atg genes in addition to atg1, so it is important to determine if these genes affect GluRs. Completion of the experiments outlined in this proposal will help determine if (1) Atg1 is required in the postsynaptic muscle for normal GluR localization and expression and (2) Atg13 interacts with Atg1 and required in the postsynaptic muscle for normal GluR localization and expression. The results of these experiments will provide insight to the interaction of autophagy genes/proteins and glutamate receptors.

Background and Significance

The mammalian nervous system is vital for a variety of everyday functions and consists of a complex network of cells called neurons. Neurons communicate with one another at structures called synapses where the presynaptic neuron can release a chemical neurotransmitter across the synapse binding to postsynaptic receptors. Binding of the neurotransmitter to the postsynaptic receptor can initiate a change in the postsynaptic neuron, possibly leading to a muscle contraction or the addition of more receptors to the membrane to bind more neurotransmitter (Lynch, 2004). Glutamate is an excitatory neurotransmitter that can diffuse across the synapse and bind to glutamate receptors (GluRs) located on the postsynaptic cell. This type of communication is important for learning and memory (Hu et al., 2007; Matsuo et al., 2008). Mutations and factors affecting the proper function of GluRs can lead to numerous disorders including Parkinson's disease, amyotrophic lateral sclerosis, epilepsy, and many neurodegenerative diseases (Lai et al., 2006; Ossowska et al., 2007; Chapman, 2000).

The lab where I conduct my research uses the fruit fly, *Drosophila melanogaster*, to study expression and trafficking of GluRs. We look at the neuromuscular junction of the fruit fly, as it contains GluRs and synaptic proteins that are similar to mammalian central nervous system synapses (Collins and DiAntonio, 2007).

atg Gene Mutations Reduce Synaptic GluRs

Problem: Despite the importance of GluRs in a number of normal and pathological processes, the mechanisms that regulate the trafficking and expression of GluRs at the synapse are poorly understood. Previous work in the lab has used genetic screens to uncover novel genes that affect GluRs by identifying mutations that result in alterations of GluR expression at the synapse. One screen revealed that mutations in the autophagy-specific gene1 (*atg1*) produced a

reduction in synaptic GluRs (Liebl et al., 2006). The *atg1* gene is one of several genes required for autophagy, which is a degradative process where cellular components are broken down and released to supply the cell with essential nutrients (Codogno, 2005). Autophagy occurs during starvation when the cell is in need of extra nutrients and energy or when aged cellular components need to be broken down and new ones made (Levine and Klionsky, 2004).

Proper expression of GluRs at the synapse depends on the transcription of *glur* genes from DNA into RNA and subsequent translation of the *glur* RNA into protein. The GluR is then packaged into vesicles and transported along cellular tracts to the neuronal membrane (Fleck, 2006). Previous experiments in our lab indicate that Atg1 is not affecting the transcription of GluRs so it must be affecting translation or localization of the protein. Consistent with this, Atg1 has been suggested to interact with and phosphorylate an adaptor protein that helps transport vesicles along the axonal tract (Toda et al., 2008).

There are several autophagy genes in addition to *atg1* in *Drosophila* (Scott et al., 2004). Our lab has also observed reductions in synaptic GluRs correlated with mutations in *atg8a*. Based on our preliminary data, I will test the following hypotheses: (1) Atg1 is required in the postsynaptic muscle cell for normal GluR localization and expression and (2) Atg13 interacts with Atg1 for normal GluR localization and expression.

Procedure

Experiment 1: Determine if Atg1 is Required in the Postsynaptic Neuron for GluR Expression and Localization

I will perform rescue experiments to determine where Atg1 is required for proper GluR expression. In the rescue experiments, I will be restoring the *atg1* gene in the *atg1* mutants but

only in particular tissues. This can be done by mating the *atg1* mutant with an animal that carries the normal transgenic *atg1* gene. The progeny from this cross will then be crossed with other animals containing specific drivers that will allow *atg1* function to be restored to specific tissues such as postsynaptic muscle, glial cells, and presynaptic motor neurons. Immunolabeling techniques will be used to determine if GluR levels have been restored. Restoration is observed when GluR levels of the *atg1* mutant are comparable to the control animal and indicates that *atg1* is required in that tissue to regulate GluRs. If the mutant were to be rescued by restoring *atg1* expression in the postsynaptic muscle, we would observe synaptic GluR levels comparable to that of controls. If the restored mutant does not mimic control GluR levels, I would infer that *atg1* is not normally required in that tissue to regulate GluRs. Since GluRs are localized to the postsynaptic muscle, I expect that *atg1* is required in the postsynaptic muscle for normal GluR localization. It is possible, however, that presynaptic *atg1* is responsible for the proper localization of GluRs. Mislocalization of presynaptic proteins that help release glutamate are known to affect the number and location of postsynaptic GluRs (Wagh et al, 2006; Graf et al., 2009). Therefore, it will be important for me to test whether *atg1* is required in the presynaptic motor neuron, postsynaptic muscle, or glial cells for normal GluR expression.

Experiment 2: Determine if Atg13 is Required in the Postsynaptic Neuron for GluR Expression and Localization.

Atg13 is also required for autophagy. It has been previously suggested that Atg13 forms a complex with Atg1 and regulates its activity (Chang and Neufeld, 2004). Therefore, mutations in *atg13* can affect Atg1 and this may result in altered GluR expression or localization. To test this hypothesis, I will determine if synaptic GluR levels are affected in *atg13* mutants by immunolabeling. Synaptic GluR levels in controls and *atg13* mutant animals will be statistically

compared. GluR reduction in *atg13* mutants would suggest that Atg13 is required for normal GluR expression.

Changes in postsynaptic GluRs in *atg13* mutants would suggest that *atg1* and *atg13* are affecting GluRs via similar mechanisms. Therefore, I will observe GluR expression in *atg1, atg13* double mutants to help determine the pathway(s) by which these genes regulate the expression of GluRs. If GluR expression were further reduced in the double mutant, compared to the single *atg1* mutant or *atg13* mutant, then these genes would be using separate pathways to regulate GluRs. It would be a separate pathway because the effect, or the reduction in GluRs, is additive with the two separate mutations.

Collectively, the experiments described above will give us a greater understanding of the signaling pathways used and factors that can affect GluR expression and localization.

Timeline

March 2010-May 2010: Immunolabel and image *atg1* and *atg13* mutants

May 2010-August 2010: Construct *Drosophila* lines for rescue experiments. Immunolabel and image *atg1, atg13* double mutants.

August 2010-October 2010: Verify that recombination necessary for the rescue experiments has occurred by performing PCR .

October 2010-January 2011: Immunolabel and image the rescued mutants

January 2010-Febuary 2010: Analyze data

Budget

I request funding for the immunlabeling experiments described in experiments 1 and 2. I'm also requesting funds to purchase reagents necessary for rearing the flies including food, vials, and cotton.

Bibliography

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RESEARCH GRANTS FOR GRADUATE STUDENTS (RGGS)
BUDGET REQUEST

	Requested Amount	Department Recommendation
COMMODITIES *(Supplies, etc.):*		
1. Jazz-Mix Drosophila Food	$94.00	
2. Drosophila vials	$78.00	
3. Anti-mouse FITC	$92.00	
4. Microscope Slides	$119.00	
5. Vectashield Mounting Medium	$106.00	
Commodities Sub-Total:	$489.00	
TRAVEL:		
1.		
2.		
3.		
4.		
Travel Sub-Total:	$0.00	
CONTRACTUAL SERVICES *(Postage, photocopying, etc.)*		
1.		
2.		
3.		
4.		
Contractual Services Sub-Total:	$0.00	
EQUIPMENT:		
Equipment Sub-Total:	$0.00	
TOTAL REQUEST:	$489.00	