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New Jersey Commission on Brain Injury Research
Annual Narrative Report

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1. Original aims of the project

Brain injury from trauma or ischemia (oxygen deprivation) can result in the loss of mobility, sensation, memory, cognition, and autonomic function. The initial events are restricted to a small region of the brain; however, damaged neurons release large quantities of the neurotransmitter glutamate into surrounding brain tissue. High levels of glutamate overactivate glutamate receptors present on surrounding neurons, resulting in calcium influx and subsequent cell death (excitotoxicity). We are studying excitotoxicity using a genetic approach in the nematode *C. elegans*, which uses glutamate receptors in sensory circuits that are strikingly similar to the circuits found in the human brain. My lab studies genes that regulate these receptors. The overactivation of these same receptors in *C. elegans* leads to excitotoxic neural death, and provides an excellent model system with which to study the process of neuronal injury. Our proposal aimed to identify undiscovered mediators of excitotoxicity using a genetic approach in the nematode *C. elegans*. *C. elegans* uses the AMPA-type glutamate receptors GLR-1 and GLR-2 in a mechanosensory circuit. The aberrant activity of these receptors leads to excitotoxic neuronal death, and there are currently two genetic models for glutamate-mediated excitotoxicity in *C. elegans*. In the first model, expression of a *GLR-2(R)* transgene (GLR-2 with an editing site mutation) results in excitotoxicity. In the second model, expression of activated $G\alpha_s$ results in an excitotoxicity that depends on glutamate release from presynaptic cells and proper calcium homeostasis in postsynaptic cells. We proposed to test whether mutations in genes that regulate glutamate receptor trafficking in turn affect excitotoxicity. We proposed to screen for and identify at least two new genes that, when mutated, suppress excitotoxic death. These were our specific aims:

1. To determine whether defects in receptor localization influence excitotoxicity.

We previously identified several genes that regulate (both positively and negatively) glutamate receptor abundance at synaptic membranes. We proposed to determine whether excitotoxicity is suppressed or enhanced in loss of function mutants for these regulators.

2. To identify novel genes that mediate excitotoxicity.

We proposed a screen for suppressors/enhancers of GLR-2(R)-mediated excitotoxicity. We proposed to use several criteria to prioritize two suppressor/enhancer genes for intense study.

3. To clone and characterize at least two genes that mediate excitotoxicity.

We proposed to genetically map and clone two suppressor/enhancer genes. We proposed to determine the tissue expression and subcellular localization of their gene products,

and to test for functional interactions between these modifier genes and genes known to regulate glutamatergic signaling and calcium homeostasis. We have successfully completed all aims.

2. Project successes

We previously proposed to search for new genes that participate in the excitotoxic death of neurons in *C. elegans*. First, we tested the hypothesis that the genes that regulate the location of glutamate receptors within neurons would also participate in excitotoxicity. We found that mutations that impaired the ability of synapses to accumulate glutamate receptors also partially blocked the excitotoxic death of neurons expressing those receptors. We also found that mutations in the two *C. elegans* glutamate receptors themselves suppress excitotoxicity. Our findings suggest that the factors that regulate glutamate receptor accumulation within neurons could be important targets for minimizing excitotoxic damage.

Second, we tested the hypothesis that the genes that regulate the ability of neurons to repair oxidative stress damage would also protect against excitotoxicity. We found that mutations in two genes known to regulate the oxidative stress response had different effects on excitotoxic death. These results suggest that by the appropriate buffering of reactive oxidative species, excitotoxicity can be suppressed.

Third, we tested the hypothesis that an activated adenylate cyclase pathway, which results in excitotoxicity in *C. elegans*, does so by overactivating nicotinic acetylcholine channels. We found that mutations in an acetylcholine co-receptor partially block excitotoxicity. These results suggest that acetylcholine channels are substrates for activation by adenylate cyclase signaling pathways. Moreover, they highlight that such channels can contribute to excitotoxic death, and are potential targets for minimizing such neural death.

Finally, we performed a forward genetic screen for modifiers of excitotoxicity. We found seven new mutations that alter the effects of excitotoxicity. One of these, EGL-9, is a protein known to function in mammals as a part of an emergency response to hypoxia (oxygen deprivation). The gene is of particular interest to us, as hypoxia has an important role in excitotoxicity during both traumatic injury and stroke. Indeed, we have recently shown that treating *C. elegans* with hypoxic conditions can mimic the effects of the mutation in the gene that we identified in the hypoxia response pathway. Another one of the genes that we identified is SOD-1, which is involved in detoxifying reactive oxygen species and is linked with the disease ALS (Lou Gehrig's Disease). In addition to their effects on excitotoxicity, we have found that hypoxia, EGL-9, and SOD-1 can modify glutamate receptor trafficking. This has opened up a

whole new avenue of study for my lab, and we are extending the aims of our grant to follow this avenue to its logical next steps – the effects of hypoxia and reactive oxygen species on neuronal necrosis and glutamate receptor function.

Interestingly, one of the genes that we identified as a regulator of GLR-1 trafficking is RPM-1, which contains a protein domain of unknown function called a PHR domain. Our screen identified a mutation in the rpm-1 PHR domain, and one of my postdocs (Eunchan Park) supported by the NJCBIR grant collaborated with Parthasarathy Sampathkumar at Eli Lilly to examine the structure of the PHR domain and the consequences of the mutation that we identified. Our results identify a key surface of the PHR protein-protein interaction domain that are disrupted by the mutation, and should prove useful for other researchers exploring the function PHR domain proteins.

3. Project challenges

We originally proposed to model excitotoxicity using a transgene that expresses GLR-2(R), a glutamate receptor subunit with an editing mutation. In addition to causing excitotoxicity, animals expressing GLR-2(R) exhibited a temperature-sensitive lethality, which he had hoped to exploit in our suppression screen. Unfortunately, we found a significant amount of sample variation in both the lethality and the excitotoxicity caused by GLR-2(R). While we do not understand the source of this variation, we deemed that moving the proposal forward using $G\alpha_s$, which does not demonstrate such variation, was the best course of action.

The strong penetrance of PVC killing combined with the weak penetrance of RIG killing in adults expressing $G\alpha_s$ allowed us to perform a forward genetic screen that could identify both suppressors and enhancers of killing. We crossed an integrated transgene that expresses mRFP via the *glr-1* promoter into animals expressing $G\alpha_s$ to more easily facilitate the scoring of dead PVC and RIG cells. Less than 1% of these animals have both PVC neurons intact at adulthood. About 35% of them have at least one RIG intact. We found that the false-positive rate for PVC (about 1% of total F2) was low enough to allow us to identify true suppressors in the second generation without undue added work.

4. Implications for future research and/or clinical treatment

The most important finding from our research is that the PHD protein EGL-9 regulates the trafficking of GLR-1 glutamate receptors through a novel mechanism involving the protein LIN-10. We find that impairment of EGL-9 or LIN-10 activity results in glutamate receptor internalization and neural protection from excitotoxicity. Our results suggest that neuronal

damage could be minimized by the application of agents that block the activity of these two proteins. Future research should focus on whether these proteins are performing a similar role in mammalian brain. Future clinical research should focus on the development of pharmacological drugs that interfere with the function of these proteins; such drugs could reduce or prevent brain damage during or just after TBI or stroke.

5. Plans to continue research

The EGL-9 protein is a prolyl hydroxylase that is known to covalently modify the transcription factor HIF-1 at key proline residues. The EGL-9 catalytic domain binds to the PDZ/PTB domain protein LIN-10 at its proline-rich N-terminus. We will continue our studies by testing whether EGL-9 hydroxylates LIN-10 directly. We will identify the key prolines on LIN-10 that are hydroxylated, and determine the effect of such hydroxylation on LIN-10 function. We will also expand our studies to determine the broader effects of hypoxia on neuronal physiology. We are using our initial findings supported by the NJCBIR grant to apply for federal funding for these studies. Indeed, an R01 grant application is currently under review at NIH.

In addition, we will examine how ROS detoxifying enzymes like SOD-1 are able to affect GLR-1 trafficking. As p38 MAPKs are known to respond to high levels of ROS, we suspect that *sod-1* mutants are unable to detoxify ROS, triggering the activity of p38 MAPK pathways like PMK-1 and PMK-3. Thus, we will test whether these pathways are activated in *sod-1* mutants and required for the changes in GLR-1 subcellular localization in these mutants.

6. Leveraging NJCBIR funding

As described above, we have used the findings from our NJCBIR grant as preliminary data for an NIH R01 application. In addition, one of the graduate students (Piya Ghose) working on the NJCBIR project recently applied for and received an NIH F31 predoctoral fellowship using her NJCBIR data as preliminary data for the application. Finally, one of the postdocs (Eunchan Park) working on the NJCBIR project recently applied for and received a postdoctoral fellowship from the NJ Commission on Spinal Cord Research.

domains from Mus musculus Phr1 (Mycbp2) explain the loss of function mutation (Gly1092Glu) of the *C. elegans* ortholog RPM-1. *Journal of Molecular Biology* 397(4):883-892

We are currently preparing two additional manuscripts for publication:

(2) Park, E.C., Ghose, P., and Rongo, C. (2009) The p38 MAPK PMK-1 regulates AMPA receptor trafficking.

(3) Park, E.C., Ghose, P., Shao, Z., Kang, L., Xu, S., Powell-Coffman, J.A., and Rongo, C. (2009) Hypoxia regulates AMPA receptor trafficking via a novel PHD-mediated pathway.

8. Financial Summary

Our actual spending during the first 18 months of the grant was less than we anticipated. The original budget requested funds for a postdoc (Dr. Doreen Glodowski) and two graduate students (Eunchan Park and Daiying Chen). Dr. Glodowski ended up leaving my laboratory. Eunchan Park was awarded a predoctoral fellowship on a separate project. Daiying Chen was awarded a training fellowship from our Institute, and thus did not require funds for her stipend. Last year she left my lab after graduating with an M.S. degree. I recruited two graduate students: Gang Liu and Piya Ghose. Ms. Ghose was already being supported by an internal fellowship (through 1/31/08), and we therefore did not draw funds for her salary from the NJCBIR grant until 2/1/08. We did not anticipate these funding and personnel changes at the time of our NJCBIR application. Importantly, these changes did not impact our ability to accomplish our research aims. Indeed, the success of our initial screen during the first year of the grant led to more projects in our second year than we had anticipated, including the new hypoxia findings; thus, by carrying over the unspent funds, we were able to pursue all of these novel avenues of research in year 2.

Approximately $16K was still committed to salary and wages through the end of the original grant period (4/14/09), and an additional $36K remained uncommitted. Because of the novel finding of the hypoxia effect and the *egl-9* gene, we reasoned that we would require additional time to pursue experiments that fully explored these novel findings. As the hypoxia effect only came to light after identifying the *egl-9* gene from our screen, we could not have anticipated these additional experiments when we first wrote the proposal. We therefore