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FY97 TASK PROGRESS

Dr. Haig S. Keshishian, PhD

Effects of Spaceflight on *Drosophila* Neural Development

General goals and accomplishments: The project being performed for Neurolab has moved forward excellently during the last year. We now have two lines of flies which we are ready to use for our designated flight of October 1998. Two key tasks remain to be solved prior to flight. First we need to test the behavior of these animals in the designated hardware for the flight, namely the BRIC canisters maintained in CRIM incubators. Secondly, we need to finely calibrate the timing shifts for the experiment, in light of the results from the temperature shift experiments performed with BRIC canisters. Both tasks seem readily accomplishable, and we are confident that we can complete necessary ground preparations for next year's flight opportunity.

Recent discussions with NASA Ames has led to the plan for them to do preliminary testing with BRIC canisters. Among our key concerns is the time it takes for petri dishes transferred between BRIC's to equilibrate at a new temperature. This will directly affect the timing of the planned temperature shifts.

The major goal from the animal side, however, has been achieved, namely to develop Drosophila lines where we can assay individual neuromuscular endings directly without dissection. This was achieved by means of using the GAL4-UAS system, where we have succeeded in establishing stocks of flies where the key neuromuscular connections can be assayed directly in undissected larvae by means of the expression of endogenously fluorescent reporters in the specific motor endings. The green fluorescent protein (GFP) as a reporter allows scoring of neural anatomy en-masse in whole mount using fluorescent microscopy without the need for either dissection or specific labeling. Two stocks have been developed. The first, which we developed first, uses the S65T mutant form, which has a dramatically brighter expression than the native protein. This animal will use GAL4 drivers with expression under the control of the elav gene, and which will ensure expression in all neurons of the embryo and larva. The second transgenic animal we have developed is of a novel kind, and makes use of *dicistronic* design, so that two copies of the protein will be expressed per insert. We have also developed a tricistronic form, but this has not yet been transformed into flies, and we do not imagine that this third line will be ready in time for the flight.

The new lines have extraordinary GFP expression, and allow for all analysis of motor endings in *undissected, whole mount* animals. This will vastly simplify our analysis of the data, and will easily increase our data base dramatically. We previously feared that the rate limiting step in doing this project would be our ability to handle all of the needed tissue processing upon the end of the project. We have reduced the project to nearly no tissue processing.

Background Information on GFP Reporter Constructs: There is no difficulty in obtaining excellent images from undissected embryos at the developmental stages when the mesoderm and nervous system are undergoing their differentiation. The problem is to identify the relevant cells conveniently, and to do so in whole mount after stage 17 of embryogenesis is a daunting task. As noted in the original proposal, we had planned to avoid dissection, as this will be a major rate-limiting step in the analysis of the embryos and larvae. A goal of the ground based studies for Neurolab is to develop robust cellular reporters to make this possible in whole mount embryos *and* larvae. We now have the tools needed to image neurons in undissected animals and get high resolution images through the cuticle in larvae. This has been made possible by the development of GFP probes (Green Fluorescent Protein) of the jellyfish *Aequorea victoria* (Chalfie et al., 1994; Wang and Hazelrigg, 1994; Heim et al., 1994; Marshall et al., 1995). GFP is intensely fluorescent and shows relatively little photoinactivation.

A route to create fluorescently marked precursors is the GAL4/UAS expression system developed by Brand and Perrimon (1993). This technique allows one to use a regulatory element of interest to drive expression of the transcriptional activator GAL4. GAL4 in turn binds to UAS sequences fused to the coding region of a reporter of interest, driving expression. For our work we used a *Drosophila* line where the regulatory UAS sequences have been fused to the coding region of GFP. We are currently focusing our efforts on a GAL4-elav driver with strong neuronal expression during embryonic and larval development. As a reporter we are using a UAS-(S65T) mutant form of GFP, as well as the double mutant *distronic* form we have developed.

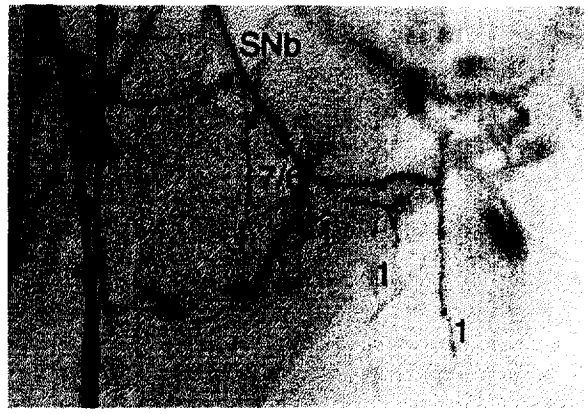

Figure 1. GFP-labeled motor endings, located on the ventral longitudinal muscles of a live, undissected, third instar larva. All the branches and motor endings of SNb are visible, as are the individual presynaptic boutons. Negative image, 16X 0.5NA neofluar objective.

This line is especially advantageous, because it gives excellent whole animal expression in larvae. Using it we have succeeded in examining fluorescently neuromuscular projections as late as the third instar in undissected live animals. All central and peripheral neurons are intensely fluorescent, and they can be examined in situ through the cuticle (Figure 1). A second line which we are exploring makes use of expression in subsets of ventral neurons, but at present we are confident that the goals of the project can be met with the GAL4 drivers we presently have.

Drosophila strains expressing GFP in neuromuscular endings greatly simplify anatomical screens to identify neuromuscular innervation phenotypes caused by hypoactivity regimes such as microgravity. In effect, synapses can now be considered to be externally visible structures. As seen in Fig. 1, in larvae of the C155 -

GAL4/UAS-GFP stock, all of the motor endings of the SNb nerve can be clearly imaged through the cuticle in live larvae. We have found that it is possible to line up larvae on a compound fluorescent microscope using low power optics (16X 0.5NA neofluars) to obtain excellent detail of the motor endings, including the presence of the appropriate motor ending arbor types (types 1b , Is, and II), the branching patterns on the muscle fibers, as well as the presence of ectopic motor endings. These can be done both on an upright or on an inverted fluorescence microscope (either available for this project). The larvae examined in this fashion are unharmed, and will develop to adults. This makes it possible to perform screens for the effects of microgravity on synaptic structures.

Polycicstronic GFP Expression Systems: This is being carried out by Dr. Marc Halfon of the lab. The new constructs represent a "third generation" GFP line, where we have made the double mutant GFP that is approximately 6 fold brighter than the S65T single mutant form. In addition, by employing the IRES system, we will be able to express multiple copies of GFP with a single promoter. We have created four vectors to date, and have successfully done transformations with three of them. The first was a proof of concept, where a metallothionein promoter was used to drive GFP expression in single and dicistronic forms in S2 culture cells. Fluorescence expression following induction was examined using a FACS cell sorter. The dicistronic form yielded cells with an approximately two fold enhancement of expression over the conventionally transformed cell lines. In addition we created a UAS-diGFP dicistronic vector and successfully obtained *Drosophila* transformants. The transformed flied express UAS-GFP with a single copy insertion at roughly the same fluorescence and homozygotes with the conventional form. We are now preparing to inject for transformation a tricistronic form. When homozygosed this fly will express six copies of GFP, each in a mutant form with 6 fold brighter fluorescence that the S65T forms we currently are using. We do not expect that these flies will be ready for ground testing in time for the flight. Nevertheless they will be of outstanding value for future missions using *Drosophila*.

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FY97 EARTH BENEFITS

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Effects of Spaceflight on *Drosophila* Neural Development

a. *Benefits to space life science research:* Studies on *Drosophila* have already demonstrated that it is an excellent model system for studying synaptogenesis at the cellular and molecular level. If plans exist for long-term human exposure to reduced gravity, it is essential that all consequences to normal development and plasticity be understood at the cellular and molecular level. Vertebrate somatosensory and motor systems undergo extensive plasticity throughout life (including the adult), and therefore microgravity may potentially cause long-term changes or injury to the CNS and peripheral synapses of humans. If prolonged exposure to microgravity is anticipated (as in the case of the space station or related missions), then these studies using a model genetic system will prove valuable for identifying the kinds of changes in nervous system connectivity which may occur in humans.

b. *General benefits:* Two general benefits will result from these studies: 1. The reporter constructs will be of great value to all researchers interested in examining nervous system development in *Drosophila*, both for mutagenesis studies and for examining normal development. Thus, the *Drosophila* lines being developed specifically for the Neurolab mission will be of wide utility to the research community for other studies. 2. Insights into the role of alterations in neuromuscular activity will be of considerable value in examining the problem of synaptic plasticity.

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