**Final Summary of Research report:**

The grant focuses on a gene we identified called, serum and glucocorticoid- induced kinase (SGK), during a previously funded NASA project. The abundance of SGK messenger RNA and protein is increased in CNS tissues from animals reared in microgravity in comparison with 1G reared animals. In the funded proposal we had three aims: 1) characterize the distribution of SGK mRNA in the developing and adult rat CNS, 2) determine if expression of enzymatically active or inactive forms of SGK in cells influenced cell morphology (neurite growth), and 2) determine if SGK is a CREB kinase – that is, a protein kinase that adds phosphate groups to the transcription factor CREB. Over the past year we have made strong progress in the 2 most difficult parts of the project, namely specific aims 2 and 3.

In specific aim #2 we planned to express a dominant negative or a constitutively active form of SGK in PC12 cells and assay the effects on neurite growth. Several methods are available for examining the effects of a transgene on PC12 neurite growth. Relevant variables include the performance of the assay +/- serum, +/- NGF, substratum for growth, timing between transfection and assay. Over the past 8 months we have customized the assay to enable us to most readily determine the effects of transgene expression on neurite growth. We have also compared the relative utility of transfecting DNA as opposed to protein itself. We are now well positioned study the effects of SGK on neurite growth.

We have also made progress in parallel studies in primary neurons. We have made constructs which will lead to transgene expression in cultures of spinal cord neurons. Co-transfection of a reporter and the SGK constructs can now be performed.

In specific aim #3 we wanted to determine if SGK phosphorylates CREB. A number of constructs were engineered: His-tagged wild type CREB and His-tagged S133A CREB (this is a version of CREB that can not be phosphorylated), tagged versions of wild type, dominant

negative and constitutively active SGK. Two types of experiments followed the creation of these constructs. First, we wanted to know if SGK led to an increase in phosphoCREB when these proteins were co-expressed in cells. We found in co-transfected HEK 293 cells, active SGK leads to the phosphorylation of CREB while the kinase-dead construct reduces baseline phosphoCREB levels. The second type of experiment is performed entirely free of the cellular environment. We inquired whether recombinant SGK can phosphorylate recombinant CREB. An important piece of background workwas the demonstration that recombinant SGK is enzymatically active. We show that a small peptide fragment of CREB (SGKtide) can be phosphorylated by recombinant SGK. Experiments performed this week indicate that enzymatically active recombinant SGK can , in fact phosphorylated CREB. We need to repeat this and also show that the S133A mutant is not phosphorylated, but I do not anticipate these control experiments to be problematic.

Thus we have made very significant progress on the most difficult portions of the grant and expect that movement forward will continue.

Future Research Plans:

The Kalb lab laboratory will continue with the planned experiments as described in the original proposal in the new location – Children's Hospital of Philadelphia. There is no necessity or indication for modification of initially proposed and approved research agenda.

Complete list of all subject inventions that resulted forom the work of this grant at Yale:

None

Final inventory of Federally-own property

None