

# **NNPDF-Funded Research Grant # 23**

**TITLE: Analysis of Growth Factor Actions and Membrane Polarity in Neurons  
from NP-C Mice**

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**PERIOD: 8/15/2000 - 8/14/2003**

## **PROJECT DESCRIPTION**

(Project goals were expanded during funding period. See Final status report for details.)

## **FINAL STATUS REPORT**

**Dated 8/14/2003**

During the course of our research during the period of support by the National Niemann Pick Disease Foundation, we have developed new reagents to speed our research efforts. We have developed an adenovirus that expresses a GFP-tagged NPC1 protein that is functional and can correct abnormalities in cholesterol accumulation and movement in NPC neurons, astrocytes, and fibroblasts. We have also finished developing a new mouse model of NPC disease, in which the Purkinje neurons in the mouse express a green fluorescent protein (GFP). This not only facilitates the identification of these cells in brain slices and in cultures of cerebellar cells, but also allows these cells to be collected for biochemical analysis. These mice are proving to be extremely useful for our current studies. Using these new reagents, as well as other antibody labeling techniques and electrical recording, there are several issues we have made progress on to date, including:

1) In our initial studies, we found that striatal neurons from the brain of NPC mutant mice failed to respond to BDNF ("brain-derived neurotrophic factor"), a growth factor important for brain development and maintenance. Based on this initial finding, we hypothesized that this effect might be selective for some, but not all, neuronal growth factors, but would not be unique to just one type of neuron in the brain, and instead might also be true for other types of neurons. We tested another growth factor, neurotrophin-3 (NT-3), and found that the response of striatal neurons from NPC mice to this factor was normal. We have just recently tested another neuronal population, cerebellar Purkinje cells from our new hybrid subline of NPC mice, and find that, like the striatal neurons, they respond to NT-3, but not BDNF. Thus, it appears that the neurons from NPC mutant mice may respond normally to growth factors that play an important role in the early development of the nervous system (like NT-3), but not to factors like BDNF that are generally thought to play a more important role in the mature nervous system. If true, this might suggest the possibility of improving neuronal survival (including Purkinje cell survival) during NPC disease by supplying growth factors that the cells can still respond to but are not being provided to them endogenously any more.

2) We have constructed an adenovirus expressing a GFP-tagged NPC1 protein and shown that it is

functional, as evidenced by the ability of the expressed protein to dissipate cholesterol accumulation in neurons, glial cells, and human fibroblasts, and its ability to restore normal levels of cholesterol efflux from glial cells. As a “proof of principle”, we have shown that if we inject this adenovirus into the cerebellum of NPC mutant mice, that it appears to slow the progression of gait abnormalities and motor coordination in the mice. This supports our hypothesis that an introduced copy of the NPC1 gene could be expressed in the brain and have a beneficial effect on NPC disease. While these results are encouraging, the cerebellar expression did not prevent the progressive weight loss or extend the lifespan of the mice. This might be due to the limited spread of the virus in the brain in these initial experiments, which was limited to the cerebellum. Along with improving the spread of the virus in the brain, it may also be possible to combine this with other treatments which can affect the periphery, but do not access the brain.

3) We have made recordings of electrical activity from Purkinje neurons of NPC mutant mice using a technique known as patch clamp recording, and have found that these cells often exhibit abnormal electrical activity as early as 10-12 days after the mice are born. We have begun to analyze the connections between Purkinje cells and other neurons, with this analysis of “synapses” based on recent work that suggests cholesterol might be important for synapse formation and function. So far, it appears that at least the establishment of synapses on Purkinje cells during development occurs normally. Whether these synaptic interactions are maintained and/or function normally is still unknown, and is being addressed in our current experiments. However, a new and very surprising finding during the course of these experiments was that the Purkinje cells from NPC mice are different from wild type Purkinje cells with respect to their intrinsic electrical properties. In particular, they do not appear to develop a specific type of ionic current that is important for generating rapid electrical signals (action potentials) at high frequencies in these cells. Normally, this ionic current becomes evident during the course of development, but in the Purkinje cells from the NPC mutant mice, it never appears. This is very interesting, as it could be a possible explanation for the erratic, irregular electrical activity these cells eventually exhibit during NPC disease instead of the very regular, high frequency activity that normally occurs. If so, it might prove beneficial to find a treatment that could induce the appearance of this type of current and more appropriate levels of electrical activity in the Purkinje cells.

**PUBLICATIONS:**

<http://www.ncbi.nlm.nih.gov/pubmed/15465427>

<http://www3.interscience.wiley.com/journal/110568038/abstract>