Comprehensive Synaptome Proteomics Targeting Protein Expression and PTMs in HD

https://neurodegenerationresearch.eu/survey/comprehensive-synaptome-proteomics-targeting-protein-expression-and-ptms-in-hd/

Principal Investigators

ELLERBY, LISA M

Institution

BUCK INSTITUTE FOR RESEARCH ON AGING

Contact information of lead PI
Country

USA

Title of project or programme

Comprehensive Synaptome Proteomics Targeting Protein Expression and PTMs in HD

Source of funding information

NIH (NINDS)

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€ 531,740.37

Start date of award

30/09/2015

Total duration of award in years

1

The project/programme is most relevant to:

Huntington's disease

Keywords

Huntington gene, Synaptosomes, syntaxin 1A, Huntington Disease, protein expression

Research Abstract

? DESCRIPTION (provided by applicant): An essential goal of quantitative proteomics is to understand how proteins in cells and tissues change in their expression levels and

posttranslational modification (PTM) status, ideally with knowledge of their spatial and temporal reorganization, protein interaction networks and functional status. These attributes are currently impossible to obtain in any single experiment, and thus require the development and implementation of multiple technologies. A critical complicating factor in mass spectrometry-based proteomic methods is how to sample tissues, especially ones that are inherently heterogeneous. The brain is particularly challenging as there are dozens of defined subregions, and where each region contains combinations of neuronal, glial and other cell types. This underlying heterogeneity can greatly complicate and obscure data interpretation in tissue proteomics, especially when one is tasked with identifying neuronal dysfunction in diseases such as Parkinson's, Alzheimer's and Huntington's disease. Methods for preparing a well-defined fraction from brain synapses, the synaptosome, has been available for some time, although improvements have been made in recent years with regards to increased efficiency and purity. Synaptosomes contain components of both the pre-and post-synaptic terminal, including mitochondria, vesicles, cytoplasm and post-synaptic density, and are therefore still a relatively complex cellular/synaptic fraction. In this application, we propose to tackle the inherent complexity of synaptosomes using mass spectrometry-based proteomic methods that will improve sampling efficiency and quantitative methodology, as well as provide information on temporal dynamics and PTMs. Proteomic sampling efficiency of the synaptome will be increased by generating a comprehensive spectral library and then using a new SWATH acquisition approach to obtain fragment and primary ion data on all ionizable analytes. For improved quantitation, novel label-free methods will integrate both primary scan (MS1 Filtering) and collisional induced fragment (MS2) ion intensity data. Affinity enrichment methods will target phosphorylation and lysine-acetylation, both important in synapse function and regulation. As we have extensive experience in mouse models of Huntington's disease (HD), we propose to use an Htt-polyQ expanded knockin mouse, zQ175 compared to littermate controls (B6/J background), as our model system. Synaptosomes isolated from the cortex and striatum will be examined using these proteomic strategies, as these brain regions are known to be effected in HD. The striatal and cortical proteome will lay the groundwork for the defining the subtypes of synaptosomes that will be purified from reporter zQ175 mice (genetic crosses). We will also use this data set to identify modifiers of HD disease progression using induced pluripotent stem cell models of HD and siRNA knockdown strategies.

Lay Summary

PUBLIC HEALTH RELEVANCE: New mass spectrometry and affinity selection methods will be developed that will significantly increase our ability to sample the synaptosomal proteome and provide critical information of changes in proteins expression and posttranslational modifications. A Huntington's disease mouse model will be used to develop and test these new approaches, and at the same time provide new information on synaptic dysfunction in this neurodegenerative disease that could be used to develop and test new therapies.

Further information available at:

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Investments > €500k

Member States:
United States of America

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Huntington's disease

Years:
2016

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