**BCH 519**

**PROTEOMICS ASSIGNMENT**

**Research Paper chosen**: The Synaptic Proteome during Development and Plasticity of the Mouse Visual Cortex

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The significance of this paper, is that it induces a visual cortex plasticity by monocular deprivation during the critical period, by increasing the levels of kinases and proteins and regulating the actin-cytoskeleton and endocytosis. As the critical period with age is reaches or comes closer, the proteins associated with the transmitter vesicle release and the tubulin- and septin-cytoskeletons increases. The actin-regulators decreases in line with augmented synapse stability and efficacy.

In order to prepare protein extracts, an isolation technique was used where the binocular visual cortex was dissected and snap – frozen in liquid nitrogen and stored at 80 °C to be enriched for synaptic membranes. Bilateral binocular V1 and the binocular visual cortex contralateral was isolated. Pools of dissected visual cortex were randomized with regard to litter composition and were homogenized to remove the debris in ice-cold sucrose buffer with 5 mM HEPES at pH 7.4 and protease inhibitor (Roche), and then centrifuged. The supernatant was loaded on top of a dis continuous sucrose gradient. Ultracentrifugation was performed, and the synaptosomes were collected, resuspended, and pelleted. The pellet was subsequently resuspended in a hypotonic HEPES solution and lysed. For iTRAQ labeling, protein concentrations were determined by means of a Bradford assay (Bio-Rad) after which for each sample, a portion of the protein was transferred to a fresh tube and dried by SpeedVac.

The methods like relative absolute iTARQ and western blotting were used for the quantification and identification of the protein. Tandem MS from 5000 laser shots were taken into consideration here. Searches were performed with cysteine modification by methyl methanethiosulfonate as fixed modifications, oxidation of methionine as variable modification, a precursor mass tolerance of 150 ppm, and a fragment mass tolerance of 0.4 Da while allowing a single site of miscleavage

This study identified many novel candidate plasticity proteins and signaling pathways that allow the mediation synaptic plasticity during critical developmental periods or restrict it in adulthood. One of the major strengths of this paper is that multiple of proteins were identified for the experiments and changes in levels were quantified. The results and all the methods on each protein forms a really neat database. One of the backdrop of this research paper is that there are no knockout studies performed and no specific proten related mechanisms are elucidated. It also doesn’t specify the functionality of the proteins.