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Immediate early genes regulation in rat cerebellar cortex during long-term synaptic plasticity induction

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The cerebellum is one of the brain areas involved in learning and memory formation. Long-term synaptic plasticity is thought to play a pivotal role in supporting these functions. Moreover Immediate Early Genes (IEGs) expression and *de novo* protein synthesis and/or modification have been strictly associated with maintenance of Long-Term Potentiation (LTP) as well as memory consolidation and storage. Two highly conserved signalling cascades, PKA and MAPK, seem to be involved in early- to late-LTP conversion; both pathway can activate CREB transcription factor through phosphorylation and P-CREB has been suggested to initiate the protein synthesis leading to late-LTP induction. The transcription factor c-fos is known to be rapidly and transiently induced in the Nervous System by a variety of stimuli and is thought to be directly involved in processes of neuronal plasticity including LTP.

We used rat parasagittal cerebellar slices as a model system in which specific patterns of stimulation delivered to the mossy fibers can induce both Long-Term Potentiation and Long-Term Depression (LTD), depending on local inhibition and other regulating factors. Using Voltage Sensitive Dye (VSD) imaging we obtained high-resolution maps of the spatial distribution of LTP/LTD induced from a Teta Burst Stimulus (TBS) application. Control and stimulated slices were fixed at different times from the TBS application and processed for in situ hybridization or immunohystochemistry in order to detect IEGs mRNA expression patterns and protein expression/modifications.

The expression pattern of c-fos and CREB mRNAs and their protein distribution and/or phosphorylation were then correlated with LTP/LTD maps generated by VSD imaging.

Preliminary data indicate a significant increase of P-CREB in the granular layer suggesting that CREB phosphorylation is induced as early as 15 minutes post TBS application. In situ hybridization experiments indicate a good correlation between c-fos and CREB mRNAs up-regulation and LTP distribution at 120 minutes post TBS. At the protein level, the comparison of immunofluorescence signals and VSD immaging data indicate a clear correlation between c-Fos and P-CREB distribution and synaptic plasticity patterns.

We are planning further experiments to confirm these data and to test our experimental system in the presence of drugs that could interfere with the transcription, translation or post-translational protein regulation.

Keywords: Immediate early genes, long-term synaptic plasticity, cerebellar cortex, VSD imaging, in situ hybridization, immunohystochemistry