

P070 Real-time single cell analysis of Bid cleavage and translocation to the mitochondria in neurons during excitotoxic neurodegeneration

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The mechanisms underlying the injury associated with the extensive over activation of the glutamate receptors during excitotoxic neurodegeneration still remains unclear. We show that the over activation of glutamate receptor results in the propagation of neuronal injury, with the extent and type (necrotic v apoptotic) of injury induced dependent on the duration of the glutamate insult. Apoptotic neuronal injury is associated with an extensive hyper-polarization of the mitochondrial membrane potential following the termination of the glutamate stimulus. The Bcl-2 only protein Bid has been identified as having a significant role in the injury associated with glutamate receptor overactivation. We transiently transfected a vector that encoded Bid fused with yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP) and employed fluorescence resonance energy transfer (FRET) to monitor the cleavage of Bid following prolonged and transient glutamate excitation in cerebellar granule neurons. We establish that Bid is cleaved and translocates to the mitochondria in neurons undergoing apoptosis, but not necrosis. This study is the first to identify that the cleavage and translocation of Bid to the mitochondria is a major regulatory step in glutamate induced apoptosis in primary cerebellar granule neurons.