

Neuronal nuclear calcium signalling suppression of microglial reactivity is mediated by osteoprotegerin after traumatic brain injury

Introduction: The Acute effects of a traumatic brain injury (TBI) entail a strong neuronal excitation/inhibition imbalance and a severe immune response mediated among others by microglia. So far a connection between the neuronal activity upon TBI and the immune response have been shown but not yet elucidated in full detail. In fact, nuclear calcium signalling, an activity dependant program regulating transcriptional responses of an overall neuroprotective nature, is a possible pathway controlling this neuron to microglia signalling. In this study, we investigate the effects neuronal nuclear calcium signalling has on the microglial response during TBI in detail and embark on a search for new mediators able to shape neuroprotection upon TBI.

Methods: To control the nuclear calcium signalling, Parvalbumin, a nuclear-tagged calcium-buffer, was expressed in neurons of the somatosensory cortex in mice, buffering nuclear calcium signalling. After a mild TBI or Sham injury treatment, microglial response was assessed together with the neuronal and excitatory synaptic density using immunohistochemistry and transcriptome analysis at 3hpi, 1dpi and 7dpi. Behavioural effects of buffering nuclear calcium signalling during TBI were measured by quantifying spontaneous whisking of mice after the injury between 1dpi and 7dpi. Activity-dependent neuronal osteoprotegerin (OPG) expression was verified via in situ hybridisation in nuclear calcium buffered mice (modelling decreased neuronal activity) and after chemogenetic inhibition of parvalbumin interneurons using a PSAM/PSEM system (modelling increased neuronal activity). Finally, the function of OPG was validated using a rescue experiment overexpressing OPG together with the nuclear calcium buffer and assessing the effects on excitatory synapses via SHANK2/3 density and on the microglial reaction at 1dpi.

Results: Buffering neuronal nuclear calcium signalling during trauma persistently increased microglia density, expressing phagocytic and Disease-Associated-Microglia (DAM) markers at 3hpi and 1dpi. Furthermore, in this mild TBI model whisking activity and excitatory synaptic density were significantly reduced after TBI. Buffering nuclear calcium signalling worsened the behavioural and synaptic phenotype. Transcription analysis showed that on one hand neuronal nuclear calcium buffering upregulates inflammatory responses (complement factors, chemokines, DAM markers, interferon response) while on the other hand epigenetic regulators and synaptic genes are downregulated during TBI. OPG is in fact expressed specifically in neurons, its expression is altered by neuronal activity and has been shown to be nuclear calcium dependent. Overexpression of OPG in mice being affected by a nuclear calcium buffer is able to decrease microglial reactivity and synaptic loss in the acute and subacute phase of TBI. Furthermore, OPG is a highly translational target with therapeutic potential due to its upregulation in the CSF of human TBI patients.

Conclusion: Neuronal nuclear calcium signalling is able to regulate Neuron-Microglia crosstalk and the neuroinflammatory cascade during TBI. This activity dependent crosstalk is in parts mediated through OPG, which is expressed by neurons in an activity dependent manner and able to restrict microglial infiltration and synaptic loss. In total, this study highlights neuronal activity dependent alterations in microglia and synaptic density together with the neuronal expression of OPG, a new player in the TBI field with high translational and therapeutic potential.