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Elimination of synapses from injured motoneurons – a model for study of synaptic plasticity in the adult central nervous system

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ABSTRACT

Synapses are the contacts between nerve cells or between nerve and muscle cells. The integrity of these synapses is crucial for proper function. Several neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis and neurotrauma involve synaptic pathology. In this thesis we have used the nerve lesion models sciatic nerve transection (SNT) or sciatic nerve crush (SNC) to enable the study of events leading to synaptic stripping and subsequent reformation after lesion.

The aim of this thesis was to investigate the role of specific factors that mediate the response of the spinal cord to peripheral axotomy, particular emphasis was placed upon molecules and cell populations that could have an influence upon the synaptic stripping of lesioned motoneurons. Following axotomy glial cells – i.e. microglia and astrocytes surrounding the lesioned motoneurons – are activated and proliferate and interact intimately with lesioned neurons. Furthermore, these glial cells express and secrete complement factors that supposedly 'tag' synapses destined to be removed as suggested by Stevens et al. 2007. Simultaneously, motoneurons down-regulate the expression of several adhesion molecules important for the maintenance of structural integrity and this is followed by the removal of synapses.

In **paper I and II**, we studied the adhesion molecules SynCAM1, neuroligin 2 and -3 and Netrin G-2 ligand (NGL-2). *In vitro* these adhesion molecules can all induce synapse formation. They are all expressed by motoneurons and down-regulated after axotomy before synaptic stripping occurs. SynCAM1 expression correlates to loss and return of synapses in the SNT model. The expression levels of the neuroligins decreased to a smaller extent after SNC than SNT, suggesting that the contact with the distal nerve stump is important for the expression levels of the neuroligins and did not display as a clear correlation with synapse numbers as SynCAM1. NGL-2 displayed a lower general expression by motoneurons and was down-regulated to a similar extent both in the SNT and SNC model.

In **paper III**, we investigated the role of complement components C1q and C3 in the removal of synapses from axotomized motoneurons. In WT mice both C1q and C3 was clearly up-regulated after lesion. $C1q^{-/-}$ mice displayed the same degree of synaptic stripping as WT mice. In contrast, $C3^{-/-}$ mice displayed a hampered stripping response following axotomy that was associated with a preferential loss of inhibitory synapses and increased expression of the regenerative associated protein GAP-43. These effects were accompanied by faster functional recovery. We did not however, see any obvious signs of hampered inflammation at site of lesion. Complement IR was seen in close interaction with the lesioned motoneurons and its dendritic tree. Yet, we did not observe any clear evidence for a 'tagging' process as suggested by previous investigators. In **paper V**, we compared $C3^{-/-}$ and MHC class Ia deficient mice; two strains exhibiting contrasting responses to axotomy. The $C3^{-/-}$ mice exhibit a hampered stripping process compared to WT mice and MHC class Ia deficient mice have an augmented stripping response compared to WT mice. We asked whether variation in the expression of synaptic adhesion molecules previously studied in motoneurons (SynCAM1, neuroligin -2 and -3, and Netrin g-2 ligand) or changes in activation of microglia and astrocytes reflected the altered synaptic stripping that is seen in these mouse strains. We concluded that neither glia activation nor the down-regulation of synaptic adhesion molecules were correlated to variation in synaptic stripping observed in the two strains studied In **paper IV**, we examined the effects exerted by astrocytes on the stripping event by the usage of $GFAP^{-/-}VIM^{-/-}$ mice. We observed a marginally affected stripping response in these mice compared to WT mice and slower functional recovery. The delayed functional recovery was however, most likely due to effects on the lesion site and not in the spinal cord.

To summarize, complement C3 seems to be an important factor in the synaptic stripping event, especially for inhibitory synapses. The effects exerted by complement C3 do not seem to be linked to distorted glial up-regulation or by an affect on the down-regulation of the studied synaptic adhesion molecules. It remains to be unravelled via which pathways and receptors complement exert these effects and whether intervention aimed at the complement system could be used for therapeutic interventions in order to promote synapse preservation in neurodegenerative diseases.

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