CHAPTER 5

General discussion
The onset of the most common neurodegenerative diseases (NDs) is age-related, thus the world widely increased aging population enhanced the prevalence of NDs\(^1\). There is still no cure for NDs. Neuronal dysfunction and loss are related to the neuronal accumulation of toxic misfolded proteins, resulting in cell-autonomous neurodegeneration\(^2\). However, other cell types in the central nervous system (CNS), such as astrocytes and microglia, can also have effects on neurodegeneration, and influence neurons in a cell non-autonomous manner\(^3\).

Astrocytes are involved in maintaining neurotransmitter homeostasis, synaptic function, energy metabolism and inflammation in the CNS\(^4\). Astrocyte engages different molecules to exert these functions. For instance, they clear excessive extracellular glutamate, which is a neurotransmitter via the glutamate transporters (EAATs)\(^7\). For the regulation of immune responses in astrocytes, the NF-κB pathway plays a role\(^8\). However, astrocytes are activated in most NDs and can contribute to neurotoxicity, leading to neuronal damage and death\(^9\).

To obtain insight into cell non-autonomous contributions of astrocytes to neurodegeneration, we performed an RNAi candidate screen in a Drosophila ND model for Spinocerebellar ataxia type 3 (SCA3), targeting genes in astrocytes. We identified genes in astrocytes that may contribute to neurodegeneration in a cell non-autonomous manner. This highlights important cell non-autonomous roles for astrocytes in neurodegeneration. Our results suggest involvement of integrins and GABA-A receptors in astrocytes in neurodegeneration (Figure 1). We also identified intracellular signaling proteins in the RNAi screen, such as calcineurin, a calcium-dependent phosphatase as well as an NF-κB transcription factor (Relish).

Upon further exploration of the involvement of Relish in astrocytes, we showed that transcriptional targets of Relish, AMPs, contribute to neurodegeneration in SCA3 (Figure 1). In Chapter 3 we showed that expression the SCA3-associated protein in fly eyes or neurons resulted in an increased levels of NF-κB-dependent target genes in fly heads compared to control flies. Preliminary data show that activation of NF-κB (Relish) occurs in astrocytes.

While we identified candidate genes in astrocytes that can make a cell non-autonomous contribution to neurodegeneration, it still remains to be determined how astrocytes and neurons can mutually influence each other’s activity. One means that would provide insight into this would be to examine how gene expression in both astrocytes and neurons is altered upon expression of an aggregation-prone protein in neurons or astrocytes. This would provide further insight into the signaling that occurs between astrocytes and neurons in NDs, but may also result in the identification of candidate genes (in astrocytes but also in neurons) that can make contributions in NDs.
Figure 1. A model for the cell non-autonomous role of astrocytes in neurodegeneration. Our data suggest that neurons expressing diseased-associated misfolded proteins can result in the activation of astrocytes. Activated astrocytes are normally hypertrophic. Candidate molecules that can be released from neurons and signal to astrocytes include misfolded proteins (that can act as DAMPs), other DAMPs, cytokines, neurotransmitter and signals that remain to be identified (‘unknown signals’). We identified receptors in astrocytes, such as integrins and GABA-A receptors, which may contribute to neurodegeneration. We also identified intracellular signaling proteins such as NF-κB (Relish) and calcineurin. Activity of Relish and Relish-dependent target genes in astrocytes may have detrimental effects on neurons.
For future directions, using our SCA3 Drosophila model, we propose to examine gene expression in astrocytes and neurons. This will shed further light on the signaling events that occur in response to neurodegeneration and may result in the identification of genes that can be manipulated to attenuate or delay neurodegeneration. For this purpose, we will compare gene expression in Drosophila neurons that express GFP or GFP together with either SCA3polyQ78 or SCA3polyQ27. Simultaneously, RFP will be expressed in astrocytes. Neurons and astrocytes can be sorted by FACS and gene expression profiles can be determined, as has successfully been done before in Drosophila brains. Genes whose expression is altered in neurons expressing SCA3polyQ78 (and GFP) compared to the neurons only expressing GFP or expressing SCA3polyQ27 (and GFP) will be identified. Moreover, the corresponding gene expression signature of these samples in RFP-expressing astrocytes will provide insight in the signaling events that occur in astrocytes following neuronal expression of SCA3polyQ78. These data will provide insight into signaling in both astrocytes and neurons in response to neuron-specific expression of a disease-associated, misfolded protein. It would be of additional value to perform a similar experiment when expressing amyloid beta peptides in neurons, which are secreted, in contrast to SCA3polyQ78, which predominantly stays intracellular. Such an experiment would reveal potential differences, or similarities in signaling events between neurons and astrocytes that express an aggregation-prone protein that is extracellular or remains intracellular.

To further advance our understanding in the detrimental effect of NF-κB signaling in astrocytes on SCA3polyQ78-expressing neurons (Chapter 3), we could compare gene expression in control astrocytes to astrocytes in which expression of NF-κB is downregulated. Alteration of gene expression in these astrocytes may provide insight of the neurotoxic effects of NF-κB signaling in astrocytes.

In addition to astrocytes, microglia can contribute in neurodegenerative diseases. Activation of microglia results in changes in their morphology, ability of phagocytosis, and inflammatory responses (reviewed in), which can influence neuronal functioning. For instance, damaged neurons can activate microglia to produce an excessive amount of pro-inflammatory cytokines, which have been shown to be toxic to neurons (reviewed in). While gene expression profiling in microglia in NDs have been determined, the cell non-autonomous contribution of these genes to neurodegeneration still need to be determined. Similarly to what we did for astrocytes, our Drosophila SCA3 model can be used for studying the interaction between microglia (called ensheathing glia in Drosophila) and neurons that expressing disease-related misfolded proteins, using microglia-specific downregulation of genes. In order to downregulate individual genes in ensheathing glia, the ensheathing glia-specific driver line, mz0709-GAL4 can be used.
Using a similar experimental setup as described above, sorting out astrocytes and neurons, signaling events between microglia and neurons can be identified as well. Given the ease of genetic manipulation of flies and the reagents we already generated, establishing these fly lines will not be very time-consuming. Together, these experiments will shed light in the interplay between neurons, astrocytes and microglia. Whether altered gene expression of specific genes in either microglia or astrocytes is relevant for the pathogenesis of NDs can be subsequently established by testing knockdown of these genes specifically in microglia or astrocytes. This can initially be done in the SCA3 eye model, and can subsequently tested in lifespans in flies expressing an aggregation-prone protein (SCA3polyQ78 or amyloid beta peptides) in neurons.

Gene profiling in microglia in humans that suffer from NDs has been done\(^{14}\) and while a large number of genes have been identified, the relevance of these in the pathogenesis remains to be established. Comparison of these genes to the genes that we identified in the experiments proposed above would yield (1) insight into conserved genes that have altered expression in NDs (2) candidate genes that can be tested in our SCA3 or Alzheimer fly model. This would narrow down the number of genes of interest that can be further analyzed in other animal models like mice.

While beyond the scope of this thesis, similar experiments can also be done upon expression of a disease-associated, misfolded protein in either astrocytes or microglia and examine the resulting changes of gene expression in neurons, astrocytes and microglia. As aggregation-prone proteins in NDs are also found in astrocytes\(^{17}\) and microglia\(^{18}\), it would shed light on another aspect of NDs. The comprehensive studies for the specific contribution of microglia and astrocytes might benefit to develop a target-specific therapy for neurodegeneration.

Together, these future directions will open exciting avenues to explore, and hopefully advance the field of NDs.
REFERENCES:


Specificity of calcineurin signaling in *Drosophila* immunity