ENGLISH SUMMARY

Proteomic characterization of perisynaptic astrocytes in synaptic plasticity

Astrocytes are the most abundant cell type in the brain and mediate a myriad of functions, including neurogenesis, synaptogenesis, ion homeostasis, metabolic support, formation of the blood-brain barrier, clearance of toxic substances and responses to brain injuries. Fine astrocyte processes contacting synaptic elements, termed perisynaptic astrocyte processes (PAPs), express at their surface receptors and transporters to monitor synaptic activity and contain specialized protein machinery that allows them to respond to this activity by the release of signaling substances, called gliotransmitters. The capacity of astrocyte to actively regulate synaptic function has led to their formal recognition as active synaptic elements with the coining of the term “tripartite synapse”, a concept which includes the neuronal pre- and postsynaptic compartments as well as the PAP.

Astrocytes are able to respond to a variety of transmitters, including glutamate, acetylcholine, GABA, and ATP, indicating they are involved in a vast array of synaptic processes. Furthermore, alterations in tripartite synaptic function have been linked with neurological disorders such as epilepsy, depression, dementia, amyotrophic lateral sclerosis, and schizophrenia, and thus the continued study of astrocytic contributions to synaptic function are of clinical and societal relevance. However, progress in this important field has been hindered by a lack of existing tools that can reliably evaluate the function and content of PAPs due to their extremely small (<1 µm diameter) nature. Therefore, there is a need for additional techniques that can give insight into PAP function and be used for evaluation of PAPs in
experimental paradigms. In this thesis I have evaluated the potential utility of several preparations for the assessment of astrocyte proteins involved in the regulation of synaptic plasticity, and employed the most suitable of these preparations to measure regulation in astrocyte protein levels in models of synaptic plasticity.

In Chapter 2, I have developed an in vitro system for the analysis of genes induced in astrocytes by the presence of neurons, named cold jet. In this technique, application of an ice cold stream of phosphate-buffered saline to mature neuron-astrocyte co-cultures results in the preferential removal of neurons due to differences in adhesion characteristics between cell types. With this method, populations of astrocytes that had been exposed to neuronal secreted, adhesion, and electrical signals could be separated from neurons with approximately 90% purity. Microarray analysis of purified astrocytes from co-cultures, compared with astrocyte alone, indicated many genes involved in metabolic functions were regulated, among which Insig1, Hmgcs, and Dhcr7, genes involved in lipid metabolism, that we found down-regulation in astrocytes when cocultured with neurons.

As transcription-level changes do not necessarily correlate with protein expression levels and thus biological function, I proceeded to evaluate other biochemically isolated preparations using proteomics techniques. Gliosomes are a Percoll®-gradient isolated fraction that are enriched for several astrocyte marker proteins, including PAP proteins, and thus could potentially be of use for the identification of novel PAP proteins and to monitor PAP protein changes during experimental paradigms. In Chapter 3, I have used label-free TripleTOF mass spectrometry to conduct a more detailed evaluation of the proteins enriched in gliosomes.
For comparison, proteins enriched in Percoll®-isolated synaptosomes were also measured. Gliosomes were enriched for G-protein mediated signaling proteins whereas synaptosomes were enriched for proteins involved in vesicle-mediated transport. Surprisingly, although gliosomes were enriched for some PAP marker proteins, synaptosomes were enriched for more PAP proteins.

After evaluation of the appropriateness of these techniques for assessment of PAP properties, I proceeded to assess contributions of astrocytes to synaptic plasticity using several in vivo models. The supraoptic nucleus (SON) is well-known for its functional and structural neuronalglial plasticity changes induced by lactation and hyperosmosis. During these physiologically stimulating conditions, astrocyte processes around the synapses onto oxytocin magnocellular neurons are retracted, thereby altering the diffusion properties and gliotransmitter availability of the synaptic cleft. This structural plasticity results in altered synaptic plasticity that mediates the physiological changes needed in response to hyperosmosis and lactation.

In Chapter 4, I used Orbitrap label-free mass spectrometry to quantify changes in protein expression between virgin, lactating, and hyperosmotic animals in order to elucidate components of the mechanism underlying these plastic changes. Mass spectrometry identified 985 proteins significantly regulated by plasticity and subsequent filtering steps were employed to narrow this list down to the 86 proteins most likely to be present in astrocyte and involved in plasticity changes. Unfortunately, immunoblotting was unable to validate most of the measured regulations, although N-myc down-stream regulated gene 2 (Ndrg2) was significantly up-regulated by hyperosmosis and lactation by both mass spectrometry and
immunoblot analysis. I concluded that there were likely many sources of biological and experimental variation in this approach which resulted in the inability to validate the mass spectrometry results, but discussed several possibilities for methodological improvement to facilitate the proteomics-assisted detection of SON structural remodeling related proteins.

Another well-studied model of synaptic plasticity is contextual fear conditioning. Mice that are allowed to explore a conditioning chamber prior to receiving a foot shock learn to associate the aversive stimulus with the context in which it was received. Interestingly, mice that receive the foot shock before being given the opportunity to explore the context, do not form an aversive memory to the context and serve as a control for the stressful effects of the shock itself, thus effects on synaptic plasticity specific to fear memory consolidation can be determined. As such, in Chapter 5, we report that at 4 hours after training, 423 proteins were significantly regulated, with 164 regulated by the delayed shock and 273 regulated by the immediate shock. Regulations of astrocyte proteins were observed, including DS-specific down-regulations at 4 hours for glutamate transporters GLAST and GLT-1 and the astrocyte GABA transporter Slc6a11/GAT3. Together, this analysis demonstrated firstly that the IS alone is capable of inducing large protein expression changes, and secondly that astrocyte proteins are regulated by the IS and well as specifically by fear memory consolidation.

With the promising evidence that PAP proteins were regulated by fear memory consolidation, in Chapter 6 we further explored the levels of a selection of astrocyte proteins during different phases of contextual fear conditioning. Most proteins were found to be specifically regulated by fear memory consolidation at 4 hours. This work demonstrated the dynamic nature of
astrocyte protein expression over the course of consolidation and points to a role for astrocyte proteins in the modulation of memory consolidation.

Cumulatively, in this thesis I have characterized several preparations that can be used to evaluate astrocyte contributions to synaptic plasticity and identified numerous astrocyte-enriched proteins regulated by synaptic plasticity that can be targeted in future studies to elaborate upon the mechanisms of action of the tripartite synapse in both physiological and pathological contexts.