5. CONCLUSIONS

A major goal of this thesis was to examine the spectral profiles of scrapie infected animals at pre-clinical time points, with the aim of determining molecular differences which might guide to the direction of future research. Therefore, a time course study of scrapie infected dorsal root ganglia starting at 100 dpi as well as the investigation of 70 dpi brain sections was performed. These experiments showed that dramatic protein-related changes occur at the pre-clinical stages of scrapie, beyond the transformation of PrP^{C} to PrP^{Sc}. The detected alterations probably reflect first responses of the host, possibly by or as a result of activation of pro-apoptotic and deactivation of anti-apoptotic enzymes and other molecular events induced by the presence of misfolded prion protein. In both ganglia and the brain, spectral differences were seen not only in the protein, but also in lipid composition and in the content and composition of nucleic acids, phospholipids and carbohydrates. Spectral alterations in the region between 1100 – 1000 cm^{-1}, indicative of an altered composition and/or structure of carbohydrates of nucleic acids or a changed content of metabolic sugar molecules in the cell such as glucose could partly reflect microvacuolation (Marsh & Kimberlin, 1975), membrane proliferation, and the structural and functional damage of mitochondria (Choi *et al.*, 1998), which were reported as early scrapie induced events. A decrease in glycolysis prior to apoptosis has been shown to be the primary cause of the decline in mitochondrial membrane potential, an early universal event of apoptosis (Mignotte & Vayssiere, 1998) and of matrix condensation. In turn, a decreased glycolysis could lead to an increase in glucose, which could partly be the reason for the increased peak absorbance between 1100 – 1000 cm^{-1} in 70 dpi brain and 100 dpi DRG. Finally, membrane proliferation, mitochondrial damage and alterations in the constitution of membrane attached proteins, for example PrP^{C} and others, could mirror the detected alteration in CH_3/CH_2 ratio. To investigate the possible role of trace metals at the initial events of scrapie, a longitudinal study examining different time points of the disease was performed. While it was shown that compared to controls at pre-clinical stages, differences in biomolecules such as proteins, lipids, nucleic acids and carbohydrates were already prominent, perturbation of metal homeostasis could only be observed for copper and calcium. However, intracellular relocation of elements would not influence

the total metal concentration and changes in metal homeostasis could therefore remain undetected here.

Current available diagnostic tests for TSE rely on detection of the disease through identification of PrP^{Sc} only. In this case, protease treatment is essential to eliminate all other proteins including PrP^{C} and therefore, as generally agreed, TSE tests to date are limited in their sensitivity, emphasizing the need of better or non-prion markers (Parveen *et al.*, 2005). In addition, especially at very early time points during the disease, PrP^{Sc} might not be present in sufficiently high amounts to be detected. The findings of molecular alterations in content and composition of all major biomolecules and trace elements in pre-clinical scrapie presented in this thesis strongly support the necessity of identifying the molecules and metals involved in early pathogenesis, in order to improve the understanding of prion pathogenesis and design new diagnostic tests. The goal would be to test for TSE before symptoms occur, ideally in tissue or body fluids that can be easily collected.

At the terminal stage of the disease, spectral changes in DRG were present in the amide I band, where an increase in proteins high in β-sheet could be detected. This could partly be explained by the disease related increasing numbers of PrP^{Sc} molecules. However, other β-sheet rich proteins most likely contribute to the detected changes too. A relatively lower content of total protein, as detected in the terminal stage suggests protein degradation and a reduction in protein synthesis in the affected cells. Changes were also seen in other spectral ranges, for example the fingerprint region. Here, alterations represent the sum of molecular changes in nucleic acids, phospholipids and carbohydrates. Results of the XRF microprobe experiments suggested an increase of copper, zinc, iron, calcium and manganese levels in scrapie infected animals at the terminal stage of the disease, while phosphorus levels decreased. The triangle between misfolded prion protein, trace metals and apoptosis is a complex network raising the question of which came first. The results obtained here suggest that calcium and copper might play an important role in scrapie pathogenesis at pre-clinical time points. PrP^{Sc} on the one hand induces changes in the homeostasis of intracellular trace metals, which trigger apoptosis. In return, metals may influence prion protein conformation and protein aggregation, triggering greater imbalances of intracellular levels of the elements. PrP^{Sc} on the other hand induces

apoptosis directly which influence metal concentrations, which in turn increase the turnover from PrP^C to PrP^{Sc}. XRF microprobe successfully provided information about subcellular content and distribution of trace elements, even those that are present in extremely low amounts in the tissue. For example copper, which has been shown to bind PrP^C in vitro, and has been suggested to play a role in Cu homeostasis. However, future experiments would need to investigate larger numbers of animals to compensate for individual differences. Incorporation of other techniques, such as XANES to study the oxidation state of trace elements on the one hand and a focal plane array based synchrotron coupled spectrometer or UV-Resonance Raman to achieve higher spatial resolutions one the other hand would allow to extend the current knowledge of prion diseases. In contrast to for example circular dichroism, gas chromatography or mass spectrometry, all these methods would still provide spatial resolution.

A second major goal of this thesis was to estimate the specificity of scrapie induced spectral changes detected in the fingerprint region in earlier studies (Kneipp *et al.*, 2000; Kneipp *et al.*, 2002; Kneipp, 2001). This was attempted by comparing reovirus induced spectral differences in the DMNV of the brain relative to age matched mock-infected control animals to those induced by scrapie (also relative to age matched controls). One spectral feature only exhibited in scrapie infected animals was the appearance of a shoulder in original spectra at ~1050 cm^{-1}, indicative of alterations in complex sugar ring vibrations of carbohydrates possibly only occurring in scrapie. However, it can not be completely ruled out that the spectral differences observed in scrapie are found in an other diseases as well. In the future, comparison with other encephalitides, which were shown to affect the DMNV, could be performed. Herpes simplex virus 1 and pseudorabies viruses were shown to follow the same pathway after oral uptake as reovirus T3C9 and the scrapie agent (Card *et al.*, 1990; Krinke & Dietrich, 1990). This would also provide the possibility of comparing adult animals in both diseases, since it was shown that age related differences in the DMNV are greater than those induced by scrapie before 150 days post-infection. Control animals at different ages revealed alterations in the same spectral regions as induced by both scrapie and reovirus, indicating age related differences in phospholipids, nucleic acids and carbohydrates. The same spectral regions are affected by reovirus and scrapie induced encephalitis, and at least at pre-clinical time points, less prominent than

differences due to different ages. Nevertheless, after the onset of clinical signs, i.e. at 150 dpi and later, scrapie induced differences in the fingerprint region exceed those due to aging. Since FTIRMS is not capable of determining the causation of the spectral differences, care must be taken in interpreting the observed spectral alterations. However, the detected increase in β-sheet in scrapie-infected DRG over the course of the disease might likely be a specific feature for prion diseases and/or other protein misfolding diseases.

By comparing spectra of DRG from terminally diseased animals of 263K scrapie to those of another scrapie strain, ME7, no differences in the secondary structure of proteins were observed. Since PrP^{Sc} accounts for only a fraction of all proteins in the neuron, detection of protein structural changes of the prion protein are diluted with those of all other proteins in the tissue. However, spectra from 263K and ME7 infected animals differed remarkably in the region between 1300 – 1000 cm^{-1}, suggesting differences in phosphates, nucleic acids and carbohydrates. It is therefore possible that the determined spectral patterns can even be specific on a strain level.