The goal of this project is to determine the effects of chemical modifications of melanin occurring with aging in the human brain and eye on protective and antioxidant action of this unusual pigment. It is postulated that significant oxidative changes of melanin in dopaminergic neurons of the midbrain and retinal pigment epithelium of the eye, modify in adverse way redox and metal ion-binding properties of the melanin pigments reducing their antioxidant and protective abilities. To achieve the major goal of the project, extensive research is planned, involving natural melanin pigments isolated from post-mortem brains and eyes of donors of different age, synthetic models of natural melanins, obtained from selected melanin precursors, and in vitro cultured retinal pigment epithelium cells and astrocytes, which in the presence and absence of melanin will be subjected to oxidative stress employing different stressors. The protective or toxic effect of melanin with different degree of oxidative modifications dependent on age or induced under in vitro conditions, will be determined based on the analysis of selected functional parameters of the cells and measurements of their survival. Particular attention will be focused on expected changes in electron-exchange and ion-exchange properties of the brain and eye melanin that occur with aging as a result of oxidative modifications of the melanins. To analyze key physicochemical properties of natural and synthetic melanins, an array of advanced techniques and methods will be used including electron paramagnetic resonance (EPR), infrared and Raman spectroscopies, atomic force microscopy and spectroscopy, selected spectroscopic and analytical methods for detection and identification of reactive oxygen species, and chemical identification of specific products of melanin degradation and their verification using quantum chemical calculations. The main reason for undertaking the specific research topic of this project is the postulated role of melanin in protection of pigment cells against oxidative stress induced by different agents. However, it turns out that melanin, particularly in post-mitotic cells, such as dopaminergic neurons of the midbrain and retinal pigment epithelium of the eye, undergoes chemical modifications aggravated by aging, which may reduce the protective and antioxidant properties of the melanin. Even pro-oxidant action of strongly modified melanin is considered, and as a result, such a melanin may enhance oxidative stress in Substantia Nigra of the brain and outer retina of the eye, thus contributing to development of Parkinson's disease (PD) and age-related macular degeneration (AMD). Prevalence of these diseases is constantly on a rise and there are no effective treatments and no preventive strategies for the diseases. Determination of the physicochemical nature of the melanin modifications responsible for critical changes of antioxidant properties of the melanin, could help in understanding of the action of one of the agents postulated to be involved in the pathogenesis of PD and AMD. It is expected that the most important outcome of the project will be the elucidation how age-related oxidative modifications of the brain and eye melanin, modulate protective and toxic effects of the pigment in cells in vitro.