1. SUMMARY

The diffusion properties of the extracellular space (ECS), which govern the movement of neuroactive substances through its volume and thus extrasynaptic transmission, are profoundly affected in states associated with cell swelling. In this work we compared ECS diffusion parameter changes during acute cell swelling *in vivo* in models of pilocarpine-induced status epilepticus (SE) and ischemia/anoxia evoked by cardiac arrest. To elucidate mechanisms involving the aquaporin 4 (AQP4) water channel in pathological cell swelling, we used models of hypotonic stress and elevated K^+ in coronal slices of genetically modified mice. The ECS diffusion parameters volume fraction α (α = ECS volume/total tissue volume), tortuosity factor λ (λ^2 = apparent diffusion coefficient/free diffusion coefficient) and non-specific uptake (k') were determined by the real-time iontophoretic method, which was the primary method used in my thesis work. Changes in the activity-related extracellular K^+ concentration ($[K^+]_e$) were measured using ion-selective microelectrodes. The apparent diffusion coefficient of water (ADC_W) was determined by diffusion-weighted MRI.

In both models *in vivo*, ECS volume fraction and ADC_W decreased; these changes were more profound and their time course was faster during terminal ischemia/anoxia in comparison to the SE model. The shrunken ECS volume fraction in the initial phase of epileptiform activity further increased $[K^+]_e$, which influenced neuronal activity and triggered the onset of the first ictal discharges 30 minutes after the pilocarpine injection. A more pronounced increase in the discharge amplitude started when the ECS volume was approximately 30% smaller than its initial value, showing the possible contribution of ECS volume reduction to the initiation of SE. The reduced ECS also affected the extracellular concentration of tissue metabolites such as glucose, lactate and glutamate. Maximum increase in $[K^+]_e$ during SE did not exceed 15 mM and no significant change in λ was detected. On the contrary, an increase in $[K^+]_e$ above 50 mM during terminal ischemia/anoxia was accompanied by a dramatic increase in tortuosity values (above 2.00).

During resting conditions, higher values of ADC_W and α were found in α-syn-negative (α-syn -/-) compared to α-syn-positive (α-syn +/+) mice, however, no significant differences were observed in values of λ or k'. The deletion of α-syn resulted in a significantly smaller relative decrease in α during elevated K^+ (10 mM) and severe hypotonic stress (-100 mOsmol/l), but not during mild hypotonic stress (-50 mOsmol/l). After the induction of terminal ischemia/anoxia, the final values of ADC_W and ECS volume fraction indicated less cell swelling in α-syn -/- in comparison with α-syn +/+ mice. Shortly after terminal ischemia/anoxia induction, the onset of a steep rise in the extracellular potassium concentration and an increase in λ was faster in α-syn -/- mice, however, the final values did not differ between the animal groups.

Our data indicates that the removal of the perivascular pool of AQP4, due to α-syntrophin deletion, reduces edema formation. This is especially the case under severe pathological conditions and during states associated with elevated K^+, which may be related to altered K^+ transport in these animals. A larger initial extracellular volume could also serve as a protective factor by buffering any increase in the concentration of potentially neurotoxic substances, slowing down the process of cell swelling. On the other hand, impaired water movement across cell membranes may delay the recovery and normalization of the ECS volume.

Changes in tortuosity, reflecting the number and extent of diffusion barriers, and in the extracellular volume fraction affect the movement of neuroactive substances as well as trophic factors and thus may modulate the extent of the damaged area, the recovery processes and/or drug distribution. Understanding the detailed mechanisms underlying the movement of water and ions (especially K^+) across the cell membrane could reveal new targets for potential therapeutic intervention during serious human pathologies associated with cell swelling, such as stroke or blood-brain barrier damage.