Focal ischemia induces enhancement of neurogenesis/gliogenesis in the subventricular zone (SVZ) of the lateral ventricle and it also leads to glial scar formation in the vicinity of the ischemic lesion. The gliotic scar is mainly formed by reactive astrocytes that express glial fibrilarly acidic protein (GFAP), nevertheless this protein is also expressed in adult multipotent neural stem cells (NSCs). Therefore, we have used the strain of transgenic mice (GFAP/EGFP mice), in which the enhanced green fluorescent protein (EGFP) is expressed under human GFAP promoter in astrocytes as well as in NSCs, thus allowing us an immediate vizualization of these cells, and to estimate the effect of ischemic injury on their fate during proliferation and differentiation *in vitro*. Focal ischemia was induced by the occlusion of the middle cerebral artery (MCAO) and 3 days post injury, an immunohistochemical analysis was carried out. Furthermore, the cell isolation from SVZ and the region of gliotic scar was performed, followed by their cultivation under proliferative conditions (as neurospheres) and their differentiation for 7-10 days. The differentiation potential of these cells was studied using immunocytochemical analyses and patch clamp technique was employed to estimate their membrane properties.

Based on increased proliferation and changes in the expression of specific neuronal/glial markers we have confirmed that a marked enhancement in SVZ neurogenesis and an increase in gliogenesis in the region of cortical lession occur in response to MCAO. Furthermore, we have showed that in neurospheres, the proliferation of NSCs and progenitor cells (PCs) isolated from SVZ of ischemic animals is markedly increased. The average diameter of neurosphers obtained from sham-operated (control) mice was 176.8 μm, while in those obtained from SVZ of ischemic mice was significantly higher (222,1 μm). Moreover, the number of EGFP-positive ($EGFP^+$) neurospheres also significantly increased; 17.9% of $EGFP^+$ neurospheres were found in controls, while 32.5 % neurospheres was EGFP positive in those obtained after MCAO. This suggests an increase in the number of proliferative NSCs in response to ischemia. On the other hand, the number of neurospheres that were obtained from the region of gliotic scar was very low, none of the neurospheres expressed EGFP and they expressed NG2 proteoglycan. This suggests that reactive astrocytes in gliotic scar do not have the properties of NSCs, despite the fact that they express their specific markers, such as vimentin, RC2 or nestin. Furthermore, after neurosphere dissociation and cell platting onto poly-lysine coated coverslips we have studied the membrane properties of differentiated cells using immunocytochemistry and the patch-clamp method in the whole-cell configuration. The cell culture obtained from SVZ after ischemia

contained higher number of NSCs/ astrocytes (64.2 %) and lower number of neural precursors (20.9 %), when compared to that obtained from SVZ of control mice (42.7 % NSCs/astrocytes and 40.9 % neural precursors). Additionally, in response to ischemia the current densities of inwardly rectifying K^+ channels were significantly increased in NSCs/astrocytes, from 0.4 pA/pF in controls to 2 pA/pF, while in PCs and the neural precursors the current densities of fast activating/inactivating outwardly rectifying K^+ (K_A) channels were significantly lowered. In controls the average K_A current density of PCs was 26.2 pA/pF , while in the culture obtained from ischemic SVZ the K_A current density was 3.8 pA/pF. Similarly , neural precursors of controls had the K_A current density of 88.1 pA/pF and after ischemia only 9.4 pA/pF. In summary, the ischemic injury affects proliferative potential of NSCs/PCs, however it affects also the differentiation potential of cells isolated from SVZ, namely their membrane properties.