The purpose of investigations planned in this project is discovering the molecular mechanisms acting during brain development that guide elongation of cortical axons forming interhemispheric neocortical connections. In monotreme and marsupial mammals these connections pass through the anterior commissure, while in eutherians they form a new, much shorter fiber pathway, the corpus callosum. Investigation of the mechanisms of development of that youngest structure of the mammalian brain may shed a new light into mechanisms of mammalian evolution. Moreover, in humans disturbances of development of the corpus callosum may lead to cognitive deficits and disorders in social communication. Therefore, results of our investigations may contribute to elaborating the ways of prevention of such developmental malformations and compensating their effects.

All mammals have the six-layered brain neocortex. Its structure and connections are different from those of the three-layered cortex present in the same place in other vertebrates. Increased number of neurons and different connections of the neocortex allow mammals for more complex and faster processing of information than in the cortex of other vertebrates. However, stimuli acting on the left and right sides of the body and from the left and right side of the visual space are processed by cortical areas of the contralateral hemispheres and integrated mainly by interhemispheric connections. Without that integration, perception and reaction of an organism would be inconsistent and learning would be disturbed.

In both monotremes and marsupials the interhemispheric connections of the neocortex pass via the anterior commissure. The corpus callosum is a brain structure connecting the neocortex of both hemispheres only in eurherians, while their anterior commissure connects mainly the olfactory structures. The interhemispheric connections travelling via the corpus callosum are much shorter than those passing via the anterior commissure, which enables faster analysis, integration and evaluation of the stimuli and in consequence shorter reaction time. Therefore, development of the corpus callosum, together with changed ways of reproduction and higher metabolism are listed as the main modifications that had led to supremacy of eutherians that at present encompass 90% of the extant mammalian species.

The corpus callosum takes an unusual path, as it penetrates the external surface of both hemispheres in the place where they adhere medially. The interhemispheric connections develop in both marsupials and eutherians at the same stage of brain development, when axons of neurons of the upper cortical layers elongate. In marsupials they are guided by molecular signals in such way, that they grow rostrally, reaching the anterior commissure, while in eutherians they grow medially and towards the cortical surface. At the place where the two hemispheres touch each other along midline, their cells die by apoptosis and axons cross the midline, growing into another hemisphere.

The object of our planned investigations are differences in systems of molecular signals guiding elongating interhemispheric neocortical axons in marsupials and eutherians. We hypothesize that during evolution resulting in emergence of eutherian mammals, the system of molecular signaling acting during development of the interhemispheric connections had been changed. Changed were the genes coding receptor molecules expressed on growing axons or signaling molecules present in the environment of growing axons, or the expression of those genes. As a result, axons of the upper neocortical layers changed their trajectory and formed the corpus callosum. We would like to discover those differences in the molecular system of guidance of the interhemispheric axons in the Monodelphis opossum and mouse (representing marsupials and eurtherians) that lead to formation of new interhemispheric connections.

The system of molecular signals acting during development of the interhemispheric connections is poorly understood, in both marsupials and eutherians. In the mouse, knockout of the SATB2 gene coding a chromatin-remodeling protein, results in the absence of the corpus callosum. In these mouse mutants interhemispheric axons pass via the anterior commissure, like in marsupials. However, results of our preliminary investigations showed that the Satb2 protein is expressed in the opossum neocortex at that stage of development. This suggests a different interaction of this protein with the growth cones of interhemispheric axons in the opossum and mouse. Reasons of that difference remain unknown. Therefore, the present state of knowledge does not allow for explanation of the evolutionary changes in the mechanism of brain development during evolution leading to emergence of the eutherian mammals. We plan to perform microarray analysis and in situ hybridization using probes specific for opossum homologues of mouse genes, which are known to control the growth direction of cortical axons. Examine the differences and similarities in the expression profile of genes that control interhemispheric cortical connections in the mouse and opossum will allow to understand the mechanism of formation of the corpus callosum.

The next step, which is an innovative and original element of this project will be attempt to change the expression of selected genes (reducing their expression using shRNA, or increasing the expression with adenovirus), aimed at production of the cortical interhemispheric connections homologous to the placental corpus callosum in the opossum brain.

Moreover the better understanding of the similarities and differences in the genes and their expression guiding development of cerebral commissures, first of all controlling the direction of axonal growth in the neocortex of placental and marsupial mammals could significantly increase our knowledge of the mechanisms of the mammalian brain formation.