The main goal of the proposed research is to assess the ability of selected histone deacetylase inhibitors (sodium butyrate and trichostatin A) to stimulate endogenous neurogenesis in an experimental model of neonatal brain damage. Perinatal hypoxic-ischemic states, known as asphyxia, often lead to the death of the newborn, whereas 25% of infants, which survive these episodes, display permanent structural and functional disabilities (such as cerebral palsy, epilepsy, pseudobulbar and extrapyramidal palsy as well as spastic paresis) known as postischemic encephalopathy. Despite significant progress made towards understanding the pathomechanism of the insult development, current therapies are still not capable of dealing with the effects of brain asphyxia. Attempts to provide neuroprotection leading to decrease in psychomotor dysfunctions do not bring the expected beneficial results and cause undesirable side effects which additionally impair functioning of nerve cells (Gardoni and Di Luca 2006, Zalewska et al. 2015). During the intensive search for new neuroprotective agents, researchers focused their attention on histone deacetylase inhibitors (HDACi). In the course of this research not only a neuroprotective action of these compounds was observed but also their ability to stimulate neurogenesis. Their administration after experimental stroke, leads to increased proliferation of progenitors in neurogenic regions (in the subgranular cell layer of the dentate gyrus of the hippocampus and subventricular zone of lateral ventricles), their migration to damaged structures and differentiation to a neuronal phenotype. A noted reduction in the lesion size and weakened neurological deficits might suggest a regeneration of the damaged nerve tissue through direct repopulation (Montgomery et al. 2009, Kramer 2009, Kim et al. 2009, 2010, Ren et al. 2004). The mechanism of the neurogenic effect of histone deacetylase inhibitors is not yet fully understood. It is well known that acetylation/deacetylation of histones is a crucial posttranslational modification of proteins. An increase in acetylation of histone proteins and transcriptional factors might stimulate the expression of a number of genes, which protein products take part in neuroprotection, neuronal plasticity and memory.

The analysis of published data, that concerns brain damage in adult animals, encourages checking the effects of histone deacetylase inhibitors in the context of early postnatal brain injury, especially with reference to neonatal asphyxia, which constitutes a serious social and economic problem. The assessment of the influence of HDAC inhibitors on neuroprotection/neurogenesis in this experimental model has not been a subject of systematic research yet. The few published data are fragmentary and were based on experiments conducted under different protocols. Therefore, the obtained results do not allow drawing a reasonable conclusion (Fleiss et al. 2012, George et al. 2013).

The object of our research will be to determine the impact of selected histone deacetylase inhibitors on endogenous neurogenesis in the subventricular zone (SVZ) after hypoxic-ischemic brain damage.

Experiments will be conducted on an established, widely used in the world, model of hypoxic-ischemic brain injury, induced in 7-day old rats (Levine model adopted for young animals described by Rice et al. (1981)). Based on comparison of various parameters describing and defining the level of development of the brain it is accepted that a 7-day old rat matches the level of brain development of a full-term newborn. Hypoxia-ischemia results in a unilateral brain damage (ipsilateral hemisphere) by blocking blood flow and oxygen and represents the encephalopathy that develops after neonatal asphyxia. The opposite hemisphere (contralateral) will be a control hemisphere.

To assess the influence of tested agents on the process of endogenous neurogenesis in the early postnatal period, some experiments will be conducted on naïve animals. The subject of our interest will be to investigate the effect of two histone deacetylase inhibitors, representing a distinct chemical structure: sodium butyrate (SB) – a fatty acid derivative and trichostatin A (TSA) – a hydroxamic acid derivative. Realization of the first step of the proposed experiments will include assessment of the influence of the chosen inhibitors on the level of brain damage (morphological analysis) and on the proliferation of progenitor/stem cells in the subventricular zone, their migration towards the injured area and differentiation to a neuronal and glia phenotype.

The material for analysis will be obtained in the 3, 6, 7, 9, 11, 14, 21 and 28 day after the induction of hypoxia ischemia. Immunohistochemical labelling will be done on brain sections and the received images analyzed in a confocal microscope.

The ability of cells to proliferate will be determined by the level of BrdU incorporation into DNA during its replication in the S phase of cell cycle.

In accordance with published data, active extracellular matrix metalloproteinases from the MMP family are involved in the migration of cells. Therefore we will label their activity and localization. Identification of cell phenotypes will be conducted using an immunohistochemical method on the basis of positive reactions with specific neuronal and glia antibodies.

T he next step will include the analysis of potential crucial factors engaged in physiological neurogenesis, to determine their role in the mechanism regulating the expected regeneration processes after neonatal asphyxia. The analysis will involve selected growth factors – brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) as well as their receptors (TrkA, TrkB, p75) and the transcription factor phospho-CREB. We will also assess the expression/activity of MAPK/ERK1/2 kinase and PI3K/Akt kinase.

The realization of the proposed project will have research purposes. Obtained results will explain if the tested histone deacetylase inhibitors stimulate neurogenesis after an experimental brain insult in the early postnatal period. A positive outcome can contribute to the development of new therapeutic strategies which will provide replacement for the damaged neurons with newly generated neuronal cells after neonatal asphyxia.