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Neurogenesis in adult human brain after hemorrhage and ischemic stroke

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Abstract

Introduction: Adult neurogenesis includes proliferation and differentiation of progenitor cells as well as their migration and maturation. In the adult human brain, two neurogenic regions, the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of lateral ventricles, have been identified. In the dentate gyrus, three types of transcriptionally active cells and in the subventricular zone, four types of transcriptionally active cells, including GFAP-positive neural stem cells (NSCs), have been differentiated.

Material and methods: The aim of the study was to identify and compare density of neurogenic cells between two study groups of patients (7 men, 7 women, mean age 70 ± 6.03) with ischemic stroke and with hemorrhage (6 men, 2 women, mean age 64.75 ± 12.23) and the control group of patients (6 men, 2 women, mean age 64 ± 10.95) free of neuropathologic changes who died suddenly within less than 10 min.

Results: In both groups, in the hippocampal dentate gyrus and in the subventricular zone of lateral ventricles, the presence of single GFAP-positive neural stem cells and the transcriptionally active cells labelled with phosphorylated histone $H3_{Ser-10}$ (p-Histone $H3_{Ser-10}$)/neural progenitor cells (NPCs), was observed. The quantitative analysis of cells with p-Histone $H3_{Ser-10}$ expression in the hippocampal DG revealed significant differences between the hemorrhage and control groups (p = 0.001, test t). However, in the SVZ, it showed a statistically significant decrease in the density of transcriptionally active cells in the group of patients with ischemic stroke (p = 0.001, test t). A distinct decrease in the density of transcriptionally active cells, proportional to the length of the patients' hospitalization, was observed.

Conclusions: Hypoxia belongs to pathomechanic factors responsible for ischemic stroke, which can induce neurogenesis. However, hypoxia along with ischemia and other factors implicated in ischemic stroke, such as the patient's age or duration of ischemia can have a decisive influence on the decrease in the density of transcriptionally active cells in this pathologic process.

Key words: adult neurogenesis, ischemic stroke, p-histone $H3_{Ser-10}$.

Introduction

Neurogenesis is the process by which new neurons are generated. It includes proliferation and differentiation of progenitor cells as well as their migration and maturation. Initially it was thought that neurogenesis occurs only during embryonic and perinatal stages in mammals. The first direct evidence of the occurrence of the neurogenic process also in the adult human brain was announced in 1998. Ericsson revealed the generation of new neurons in the granular layer of the hippocampal dentate gyrus (DG) throughout the human life [10]. In

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the adult human brain, two locations of adult neurogenesis, the dentate gyrus (DG) and the subventricular zone (SVZ), have been identified [5,22]. Newborn neurons migrate from the SVZ towards the olfactory bulb (OB). The fate of newborn neurons in the DG is strictly determined topographically and they do not show a tendency to migrate towards the brain neocortical areas. They only participate in the constant supplementation of the pool of new granule cells in the dentate. In the DG three types of transcriptionally active cells have been identified. They are glia-like neural stem cells (NSCs, type-I cells), cells without processes (NSCs, progenitor cells, type-II cells) and neuroblasts [3,8]. In the SVZ three types of transcriptionally active cells have been distinguished, namely GFAP-positive neural stem cells (NSCs, type-B1 cells), progenitor cells (NSCs, type-C cells) and neuroblasts (type-A cells) [20].

Cells with an expression of astrocyte-like acidic filaments in the dentate gyrus generate new neurons [25]. Neural stem cells (NSCs) are probably contained within the lineage: "neuroepithelium-radial glia-astrocytes" [1]. This hypothesis is particularly appealing to astrocytes in hippocampal dentate gyrus, because their origin from radial glia has been shown in the developing primate brain [9]. Generation of new neurons and the stages of their maturation are determined by the appearance of specific proteins. An analysis of the protein markers facilitates the identification of neurogenesis phases. Markers of early neurogenesis phases comprise DCX (microtubule-associated protein expressed during neuronal migration) and NeuN (neuronal nuclear antigen) that labels migrating neuroblasts and immature neurons as well as GFAP (glial fibrillary antigen protein) that labels neural stem cells [11,27]. Phosphorylated histone H3$_{Ser-10}$ (p-Histone H3$_{Ser-10}$) is more precise and contrary to DCX, its expression occurs only in newborn neurons (neuronal progenitor cells, NPCs) [23]. Phosphorylation of N-terminal domain of histone H3 in position Ser-10 and/or Ser-28 destabilizes chromatin, directly preceding replication and transcription [6,7,17].

The aim of our study was to identify transcriptionally active cells and perform a quantitative analysis of neurogenesis cell density as well as to compare three groups of patients with ischemic stroke and with hemorrhage and the control group.

Hypertension, cerebrovascular and cardiovascular diseases are the major causes of ischemic and hemorrhage stroke. Myocardial ischemia and hypoxia are responsible for the loss of neuronal and glial cells,

leading to functional impairments and cognitive deficits. Hypoxia belongs to factors that induce neurogenesis. Therefore, an analysis of the involvement of transcriptionally active cells may play a key role in the development of therapeutical procedures [15,16,19].

Material and methods

The study material was collected from the archives of the First Polish Brain Bank at the Institute of Psychiatry and Neurology, Warsaw, Poland. It comprised two structures – the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricles. The first study group was composed of the brains derived from the patients (7 men, 7 women, mean age 70 ± 6.03) with diagnosed ischemic stroke without focal secondary hemorrhagic transformation. The duration of their hospitalization ranged from 8 h to 78 days after the occurrence of the ischemic episode. Ischemic changes were located in the left hemisphere in 6 cases, the right hemisphere in 3 cases and in both hemispheres in 5 cases.

The second study group was composed of the brains derived from the patients (6 men, 2 women, mean age 64.75 ± 12.23) with hemorrhage. The length of their hospitalization ranged from 9 h to 22 days after the occurrence of hemorrhage. In brains derived from 6 patients with intracerebral hemorrhage (ICH) we observed blood in the ventricular system and/or damage of the basal ganglia.

The control group of brains had no significant neuropathological lesions. They were derived from the patients (6 men, 2 women, mean age 64 ± 10.95) whose death occurred suddenly (within less than 10 min).

Immunohistochemistry

The study material, embedded in paraffin, was cut serially at 5 µm slices on the rotary microtone (Leica) into silane-coated glass slides (Silan Sigma A36648), deparaffinized in xylene 3 × 10 min, hydrated in an ethyl alcohol series, 100%, 96% and 70% for 10 min and rinsed in distilled water for 10 min. Epitopes were exposed to temperature of 100ºC 3 × 5 min in citrate buffer (pH 6.0), cooled at room temperature for 10 min and rinsed in distilled water for 10 min. These were followed by blocking of endogenous peroxidase with 0.3% TRIS + H$_2$O$_2$ (TrisBase T1503, pH 7.4-7.6) for 10 min and rinsed in TRIS for 10 min and blocked with 2.5% normal horse

serum (Vector PK-7200) for 1 h. Specimens were incubated with GFAP antibody (1 : 700) (MCA 9733 G-1, Bio-RAD) or with p-Histone antibody $H3_{Ser-10}$ (1 : 50) (sc-8656-R, Santa Cruz Biotechnology) for 50 min in 2% normal horse serum. Then rinsing in TRIS for minimum 40 min took place, incubation with chromogen DAB (Sigma, D5637-SG) for 0-3 min. Rinsing again in distilled water for 10 min, dehydration for 10 min in two ethyl alcohols, 96% and 100%, respectively, rinsing in xylene 2 × 10 min and sizing of Histokitt specimens (MaeFour 1025/500).

Quantitative analysis

The quantitative analysis was performed using the CellSens software. Determined cells were counted in two structures, the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of lateral ventricles.

Statistical analysis

The results were analyzed with use of STATISTICA 12 software.

Results

In all the three groups the presence of single GFAP-positive neural stem cells and the presence of transcriptionally active cells, labelled with p-Histone $H3_{Ser-10}$, were observed in both the hippocampal dentate gyrus and the subventricular zone. In the control group, GFAP-positive oval cell bodies with visible ample cytoplasm and processes were observed in the DG (Fig. 1A-C) and in the SVZ, while in the groups of patients with ischemic stroke and hemorrhage, astroglial processes were generally not observed (Fig. 3A-C).

In all groups, cells positively labelled with p-Histone $H3_{Ser-10}$ showed in the DG a clear point reaction in round cell nuclei (Fig. 2A-C). Whereas, in the SVZ, positive point reaction with phosphorylated histone $H3_{Ser-10}$ occurred in more oval or longitudinal nuclei (Fig. 4A-C).

The quantitative analysis of cells with p-Histone $H3_{Ser-10}$ expression in the SVZ of lateral ventricles revealed a statistically significant decrease in the
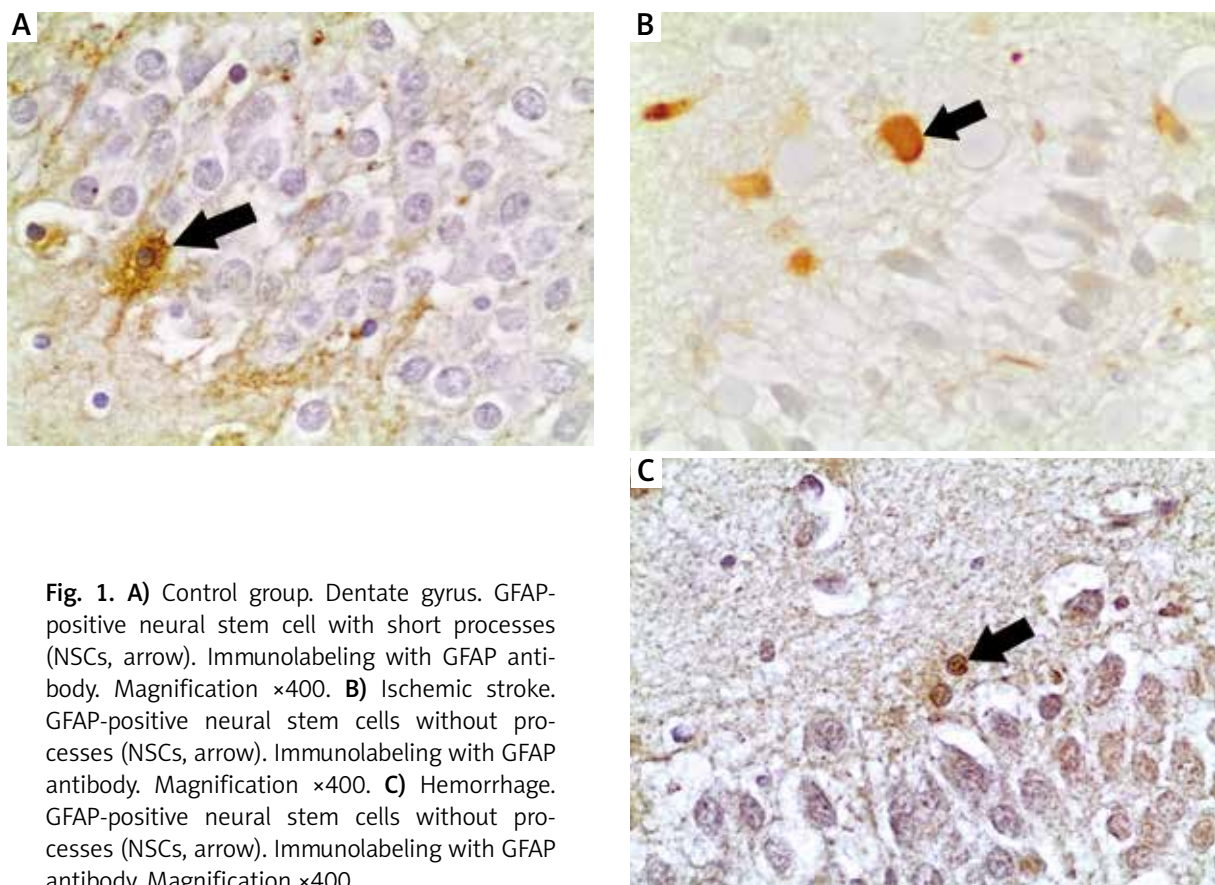

Fig. 1. A) Control group. Dentate gyrus. GFAP-positive neural stem cell with short processes (NSCs, arrow). Immunolabeling with GFAP antibody. Magnification ×400. **B)** Ischemic stroke. GFAP-positive neural stem cells without processes (NSCs, arrow). Immunolabeling with GFAP antibody. Magnification ×400. **C)** Hemorrhage. GFAP-positive neural stem cells without processes (NSCs, arrow). Immunolabeling with GFAP antibody. Magnification ×400.
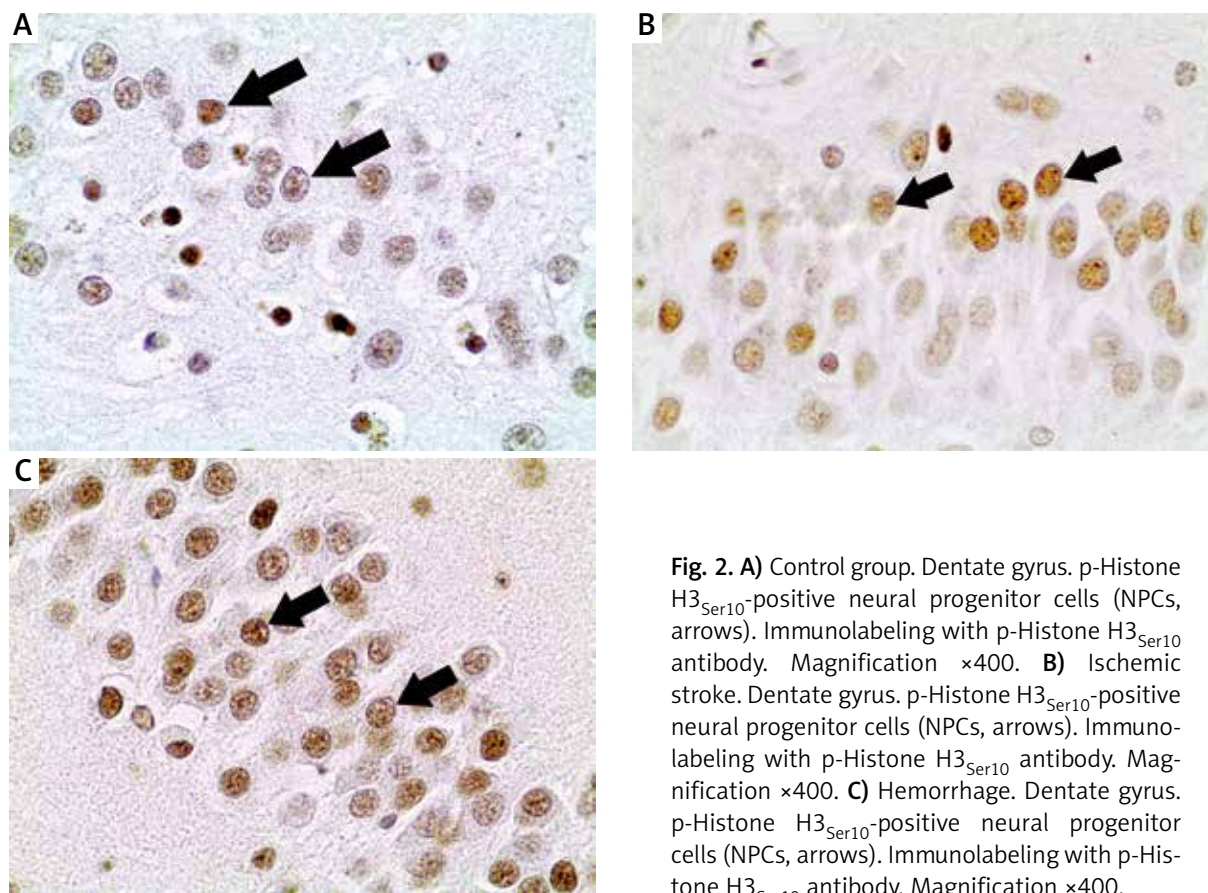
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Fig. 2. A) Control group. Dentate gyrus. p-Histone $H3_{Ser10}$-positive neural progenitor cells (NPCs, arrows). Immunolabeling with p-Histone $H3_{Ser10}$ antibody. Magnification ×400. **B)** Ischemic stroke. Dentate gyrus. p-Histone $H3_{Ser10}$-positive neural progenitor cells (NPCs, arrows). Immuno-labeling with p-Histone $H3_{Ser10}$ antibody. Magnification ×400. **C)** Hemorrhage. Dentate gyrus. p-Histone $H3_{Ser10}$-positive neural progenitor cells (NPCs, arrows). Immunolabeling with p-Histone $H3_{Ser10}$ antibody. Magnification ×400.

density of transcriptionally active cells in the group of patients with ischemic stroke (442 NPCs/mm^2), compared to the control group (856 NPCs/mm^2). The same analysis in the hippocampal DG showed significant differences between the hemorrhage (1616 NPCs/mm^2) and the control group (424 NPCs/mm^2) ($p \leq 0.001$, test t, Fig. 5). No significant differences concerning the density of neural progenitor cells were displayed between ipsilateral and contralateral SVZ hemispheres.

Discussion

Stroke is one of the most common causes of death and disability of people over 40 years old. Fast symptom recognition can protect against severe consequences, however, we can do nothing if neurons are already damaged. The research has shown that transplantation of NPCs into the brain has improved the neurobehavioral function of subjects in an animal model of intracerebral hemorrhage (ICH) [14].

Ischemic stroke occurs when blood vessels become clogged with atheromatous plaque or atheroembolism and it is one of the most frequent causes of death or disability among older people. Except for thrombectomy and thrombolytic therapy within the first few hours after ischemia onset, other therapeutic methods for preventing loss of neurons and glial cells, leading to functional disorders, have not as yet been developed. The process of adult neurogenesis supplementing the pool of neurons and neuroglia cells (astroglia, oligodendroglia) in the damaged brain could become an expected predictor of the injured brain repair and functional recovery.

Our study carried out in both the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) in the study groups showed the presence of GFAP-positive neural stem cells and the presence of cells labelled with p-Histone $H3_{Ser-10}$ transcriptionally active cells of neurogenesis.

The increase in proliferation and migration of progenitor cells towards damaged sites has also been
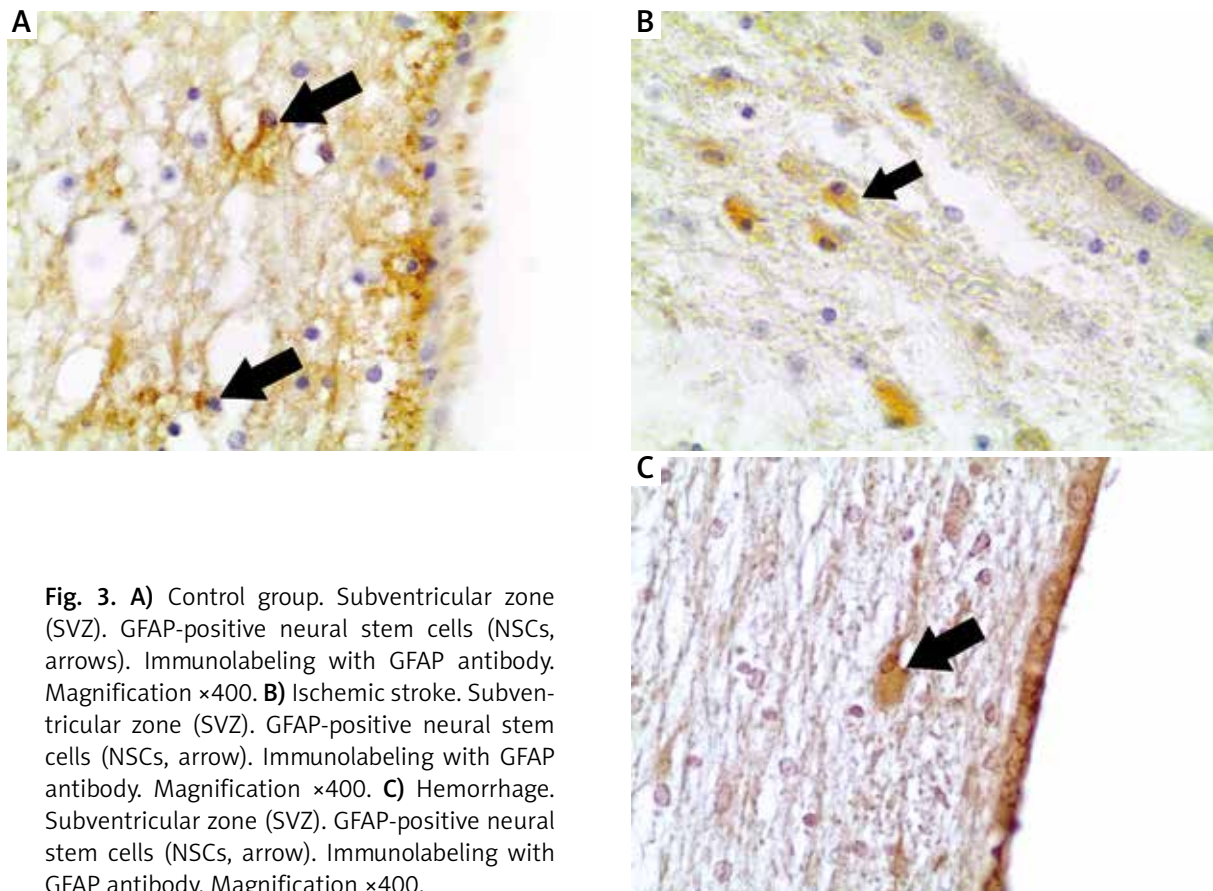
Fig. 3. A) Control group. Subventricular zone (SVZ). GFAP-positive neural stem cells (NSCs, arrows). Immunolabeling with GFAP antibody. Magnification ×400. **B)** Ischemic stroke. Subventricular zone (SVZ). GFAP-positive neural stem cells (NSCs, arrow). Immunolabeling with GFAP antibody. Magnification ×400. **C)** Hemorrhage. Subventricular zone (SVZ). GFAP-positive neural stem cells (NSCs, arrow). Immunolabeling with GFAP antibody. Magnification ×400.

observed in focal ischemia and hypoxia [2,31,32]. However, stroke-induced neurogenesis is limited [32]. The quantitative analysis of cells with p-Histone $H3_{Ser-10}$ expression in the hippocampal dentate gyrus reveal statistically significant differences between the group of patients with hemorrhage stroke and the control group. The quantitative analysis of cells with p-Histone $H3_{Ser-10}$ expression in the subventricular zone revealed a statistically significant decrease in the density of neural progenitor cells ($p \leq 0.001$, test t), which suggests the predominance of factors that inhibit the cell division in the process of neurogenesis in patients with ischemic stroke. The patients' age (mean age 70 years ± 6.033) could be one of the unquestionable inhibiting modulators, the age in which the concentration of growth factors decreases. There is strong evidence in humans that hippocampal neurogenesis is reduced across the lifespan [13].

There are many molecular candidates for regulating neurogenesis, including the growth factors.

Brain-derived neurotrophic factor (BDNF) whose decrease has a significant impact on the diminished pool of newborn neurons may serve as an example [33]. Disorder of the hypothalamic-pituitary-adrenal (HPA) axis able to increase the cortisol concentration and to reduce neurogenesis is another age-related neurogenesis inhibitor [18]. An evident decrease in the density of transcriptionally active cells, proportional to the length of the patients' hospitalization, inevitably indicating one of the pathogenic factors that inhibits neurogenesis, as well as hypertension medications were also observed [29].

Some studies have suggested that hemorrhage induced neurogenesis, but the majority of the newborn cells did not survive more than 3 weeks due to apoptosis [21]. Our results indicate that intracerebral hemorrhage induced significant proliferation of neural progenitor cells only in dentate gyrus.

We did not observe inducing of neurogenesis in the subventricular zone. It is likely that this observation was associated with the location of cerebral
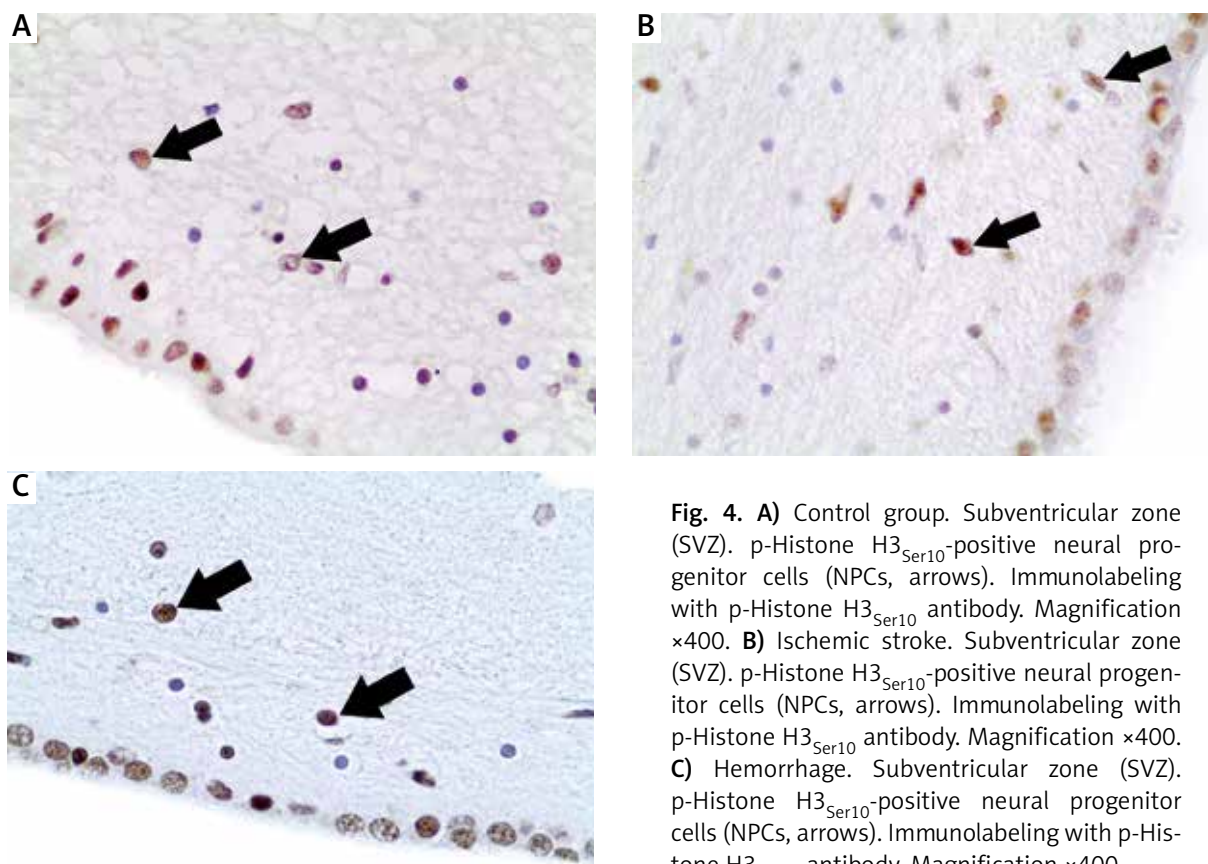
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Fig. 4. A) Control group. Subventricular zone (SVZ). p-Histone $H3_{Ser10}$-positive neural progenitor cells (NPCs, arrows). Immunolabeling with p-Histone $H3_{Ser10}$ antibody. Magnification ×400. **B)** Ischemic stroke. Subventricular zone (SVZ). p-Histone $H3_{Ser10}$-positive neural progenitor cells (NPCs, arrows). Immunolabeling with p-Histone $H3_{Ser10}$ antibody. Magnification ×400. **C)** Hemorrhage. Subventricular zone (SVZ). p-Histone $H3_{Ser10}$-positive neural progenitor cells (NPCs, arrows). Immunolabeling with p-Histone $H3_{Ser10}$ antibody. Magnification ×400.
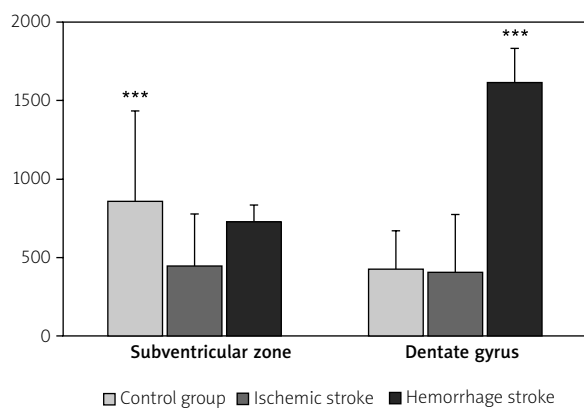

Fig. 5. Density of p-Histone $H3_{Ser10}$-positive neural progenitor cells (NPCs) in dentate gyrus and subventricular zone in control and ischemic stroke groups. ***$p \leq 0.001$.

ischemic foci. Our study material included 3 cases where necrotic foci also slightly embraced a part of temporal lobes, while in other cases they were primarily located in frontal lobes and remaining cere-

bral structures. In addition to hypoxia in intracerebral hemorrhage (ICH), there is also the destructive effect of extravasated blood and the effect of mass induced by it. Almost all patients in the hemorrhage group had an extravasated blood in the ventricular system.

In contrast to ischemic stroke, the number of cells lost after hemorrhage is not large, arguing that cell loss is less likely to provide the signal for increased neurogenesis. One possibility is that neurogenesis increases after hemorrhage in response to increased intracranial pressure, which could be detectable at sites, such as SVZ or DG, that are remote from the lesion. Although the effect of increased intracranial pressure on adult neurogenesis has not been examined directly, neurogenesis is enhanced in experimental models of traumatic brain injury [30], which also elevates intracranial pressure [24]. The functional significance of injury-induced neurogenesis remains unclear, but neurogenesis may contribute to brain repair and functional recovery, either through cell replacement or by indirect mechanisms. Some

findings indicate that the inflammatory process evoked by ICH may increase neurogenesis [28].

However, the latest research suggests that the neurogenesis in dentate gyrus (DG) from fetal development to adulthood a marked decrease, and does not continue, or is extremely rare in adult humans [26]. Similar observations were made by examining the stem/progenitor cells in the heart, liver, kidney and progenitor cells from bone marrow during cardiovascular, cerebral stroke, acute kidney injury and other damage, caused an increase in quality of stem/progenitor cells [12]. A vast number of factors able to modulate the process of neurogenesis have been described. Trophic growth factors (BDNF, FGF, VEGF, TGF, VEGF), serotonin and antidepressants may exert their influence on the growth rate of cell division through affecting specific receptors [4,16].

The identification and elimination of adverse factors affecting the generation of newborn neurons and neuroglial cells in the process of neurogenesis, ischemic stroke and hemorrhage could greatly contribute to the development of new therapeutic methods.

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Disclosure

The authors report no conflict of interest.

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