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Hippocampal Neurogenesis in the New Model of Global Cerebral Ischemia

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Abstract. The study aimed to evaluate the changes of hippocampal neurogenesis in a new model of global transient cerebral ischemia which was performed by the occlusion of the three main vessels (tr. brachiocephalicus, a. subclavia sinistra, and a. carotis communis sinistra) branching from the aortic arch and supplying the brain. Global transitory cerebral ischemia was modeled on male rats (weight = 250–300 g) under chloral hydrate with artificial lung ventilation. Animals after the same surgical operation without vessel occlusion served as sham-operated controls. The number of DCX-positive (doublecortin, the marker of immature neurons) cells in dentate gyrus (DG) and CA1-CA3 fields of hippocampus was counted at the 31st day after ischemia modeling. It was revealed that global cerebral ischemia decreased neurogenesis in dentate gyrus in comparison with the sham-operated group ($P<0.05$) while neurogenesis in CA1-CA3 fields was increased as compared to the control ($P<0.05$).

INTRODUCTION

Neurogenesis in adult mammals takes place during the whole life in two specific neurogenic regions, such as subventricular zone near lateral ventricles (SVZ, subventricular zone) and the subgranular layer in dentate gyrus of the hippocampus (DG, dentate gyrus) (Cayre et al., 2009; Yagita et al., 2001). When several studies revealed the significant changes of neurogenesis in pathological conditions the interest in the study of this process under ischemic conditions increased (Cayre et al., 2009; Kee et al., 2001; Keilhoff et al., 2010; Yagita et al., 2001). The findings offered promise for the use of endogenous processes to recover the brain after ischemic injury.

The described changes in neurogenesis depend on the model of cerebral ischemia. There are two main groups of animal models of cerebral ischemia, which causes changes of neurogenesis in two neurogenic niches. The well standardized and widely used model of middle cerebral artery occlusion (MCAO) leads to an increase of neurogenesis and the migration of a large number of neuron progenitors from subventricular zone to the damaged area where they pass the stage of maturation and even organize new neural networks (Cayre et al., 2009; Yagita et al., 2001). The model of global ischemia is less standardized and is found in a relatively smaller number of studies of neurogenesis after ischemic injury. Among the models of global transient cerebral ischemia, a 2-vessel occlusion model of forebrain ischemia in Mongolian gerbils (Liu et al., 1998; Salazar-Colocho et al., 2008) and a 2-vessel occlusion model of cerebral ischemia in rats with hypotension (Kee et al, 2001; Pforte et al., 2005) are the most frequent. The main disadvantage of the forebrain ischemia model in gerbils is spasms in the postischemic period, but it is known that seizures may stimulate neurogenesis (Salazar-Colocho et al., 2008). The model of 2-vessel occlusion with hypotension in rats (Kee et al, 2001; Pforte et al., 2005) or mice (Kim et al., 2015; Tian et al., 2014) is performed by the occlusion of both common carotid arteries with an optional simultaneous hypotension and subsequent reperfusion. However, this model requires the use of special drugs, which may complicate the interpretation of the results (Myron et al., 1989).

Studies of neurogenesis in the model of global transient cerebral ischemia (occlusion of the four major vessels supplying the brain) are rare [15].

The aim of the study was to evaluate changes of neurogenesis in the new model of global transient cerebral ischemia which was simulated by the occlusion of three main vessels supplying the brain (Chernysheva et al., 2014).

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MATERIALS AND METHODS

Animals

Male Wistar rats (weight = 250–300 g) were obtained from the clinic of laboratory animals of Goldberg Institute of Pharmacology and Regenerative Medicine (Tomsk, Russia). Animals were housed nine or ten per cage and allowed access to water and food *ad libitum*. The cages were maintained at a constant temperature (23.5±0.5 °C) and relative humidity (55±5%) under a 12-h light/dark cycle (lights on from 08:00 to 20:00). The experiments were performed in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Surgery

Modeling of transient global cerebral ischemia was performed by the occlusion of the main branches of the aortal arch providing blood supply to the brain as was described elsewhere (Chernysheva et al., 2014). Briefly, under chloral hydrate (450 mg/kg i.p.) brachiocephalic aortic trunk, left common carotid, and left subclavian arteries were exposed and occluded with microsurgical clips for 6 min. The rats after the same surgical operation without vessel occlusion served as sham-operated controls. Respiration was maintained automatically with an artificial lung ventilation apparatus during the occlusion. Circulation was restored by removing clips. After reperfusion, the rats were returned to their home cage. The survival of animals was controlled on the 11th and 31st days after the surgery.

Tissue preparation

On the 31st day after surgery the rats were anesthetized and perfused transcardially with phosphate buffer followed by 4% paraformaldehyde and then decapitated. The brains were removed and postfixed in phosphate buffer containing 4% paraformaldehyde overnight and then immersed successively in the 10% and 20% sucrose solution (in PBS) at 4 °C. After that the brains were frozen in liquid nitrogen gas and stored at -80 °C until sectioning. Cryosections were prepared in the coronal plane (10 µm thick, -2.64 mm and -3.60 mm from bregma) using a Microm HM 525 cryostat (Thermo Scientific, Germany) at -25 °C. 10–20 sections at 20-section intervals (200 µm) per brain were used for immunohistochemical analysis.

Immunohistochemistry

Neurogenesis in hippocampus was detected by indirect immunofluorescent labeling of doublecortin (DCX), namely, the marker of immature neurons. For this purpose the sections were incubated with a blocking solution for 1 h and then incubated with primary antibodies (goat anti-DCX (C-18): sc-8066, 1:100, Santa Cruz) overnight at room temperature. After washing in PBS, the sections were incubated with secondary fluorescence-conjugated antibodies (donkey anti-goat Alexa Fluor 488 (705-545-147), 1:500, Jackson Immunoresearch) for 3 h at room temperature, washed in PBS and coverslipped with Vectashield mounting medium containing DAPI (4',6-diamidino-2-phenylindole) (Vector Laboratories Inc., Burlingame, CA, USA).

Microscopy

From each animal, 10–22 microphotographs of brain sections of both left and right hemispheres were obtained. Microscopy was performed with an Axio Imager Z2 (Carl Zeiss, Germany) fluorescent microscope and AxioVision Rel. 4.8 (Carl Zeiss, Germany) software with a MozaiX module, which allow creating images of about 11 mm^2 by stitching 20–28 smaller images (depending on the size of the hippocampus). Immature neurons were determined as DCX-positive (DCX+) cells in subgranular zone of dentate gyrus in hippocampus. Besides, DCX-positive cells were counted for in CA1-CA3 fields of the hippocampus. DCX-positive cells were counted using ImageJ software.

Statistics

Data were analyzed with a mixed-ANOVA design (Variance Components, Mixed Models), for which the following three factors were used: the group (a fixed factor, 2 levels), the number of rats (a fixed factor, 10 levels), and the number of the photo (a random factor, 10–22 levels, depending on the number of images). When the number of measurements for each subject is different, the use of this particular ANOVA model is preferable as compared to the repeated measures (Gonen et al., 2001). Statistical significance was set at $P<0.05$ versus the sham-operated group.

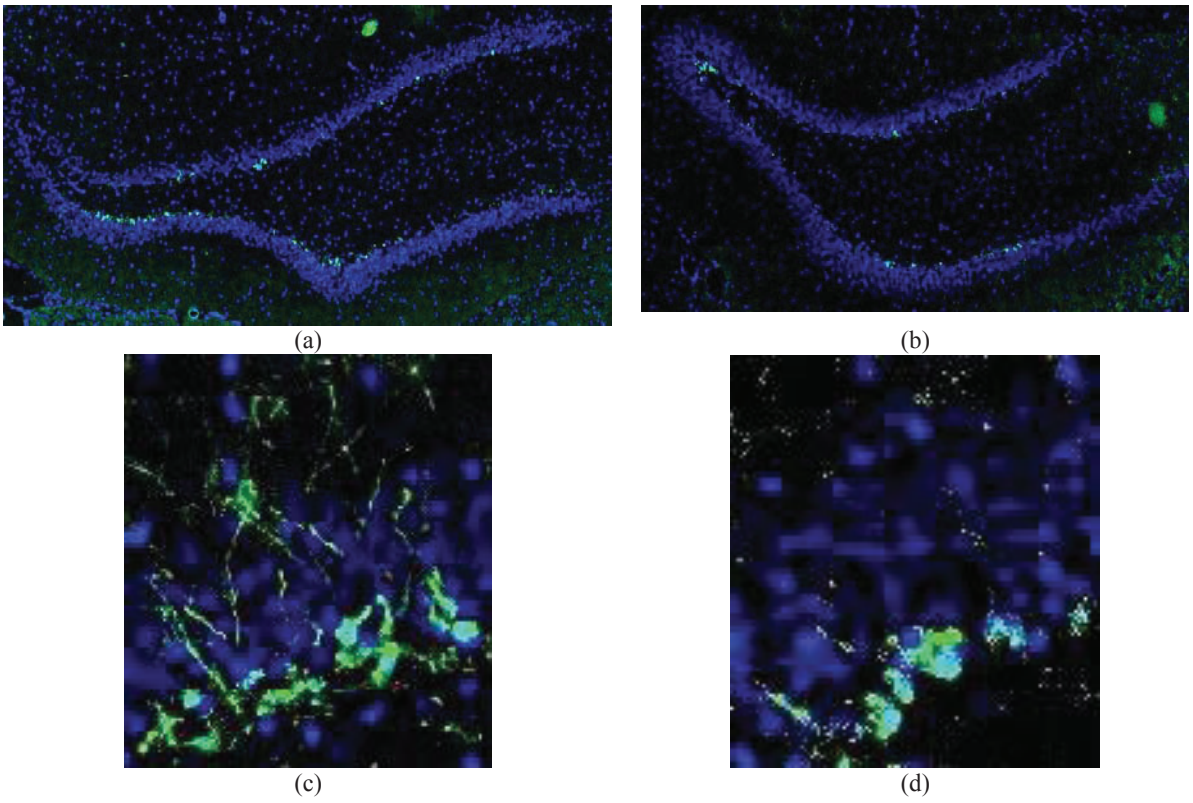
RESULTS

23 rats underwent surgery and 10 animals survived (Tab. 1). These data agree with the previous one (Chernysheva et al., 2014) and suggest ischemic injury. The majority of the animals died within the 1st day after ischemia (the sham-operated animals did not die). The highest mortality rate was observed during first hours after the modelled ischemia, and the surviving animals showed a severe CNS deficit, such as areflexia, spastic paralysis, and the tonic tension of body muscles.

The considerable quantitative (Figs. 1a, 1b, 2) and qualitative (Figs. 1c, 1d) differences were revealed in dentate gyrus neurogenesis in the ischemic group, compared to the sham-operated control. Immature neurons in dentate gyrus in the sham-operated rats exhibited well developed axons forming neural networks while in the ischemic group no significant neurite outgrowth was observed. In addition, global ischemia reduced the amount of DCX-positive cells approximately by 17 percent ($P<0.05$) as compared to the sham-operated animals (Figs. 1, 2a). However, the number of DCX-positive cells were increased approximately by 43 percent ($P<0.05$) in CA1-CA3 fields of hippocampus (Fig. 2b).

TABLE 1. Survival rate after surgery

Group	11th day after surgery	31st day after surgery
Sham-operated (n=5)	100%	100%
Ischemia (n=18)	33.3%	27.8%



(a) (b) (c) (d)

FIGURE 1. Representative photographs showing immunofluorescence staining of DCX in the dentate gyrus of the sham-operated ((A) and (C)), and ischemia ((B) and (D)) groups. DCX – green, DAPI – blue. In the dentate gyrus of the ischemia group DCX-positive cells were decreased on 31st day after transient global ischemia. Immature neurons in dentate gyrus in the sham-operated rats (C) exhibited well-developed axons forming neural networks while in the ischemic group no significant neurite outgrowth was observed (D).

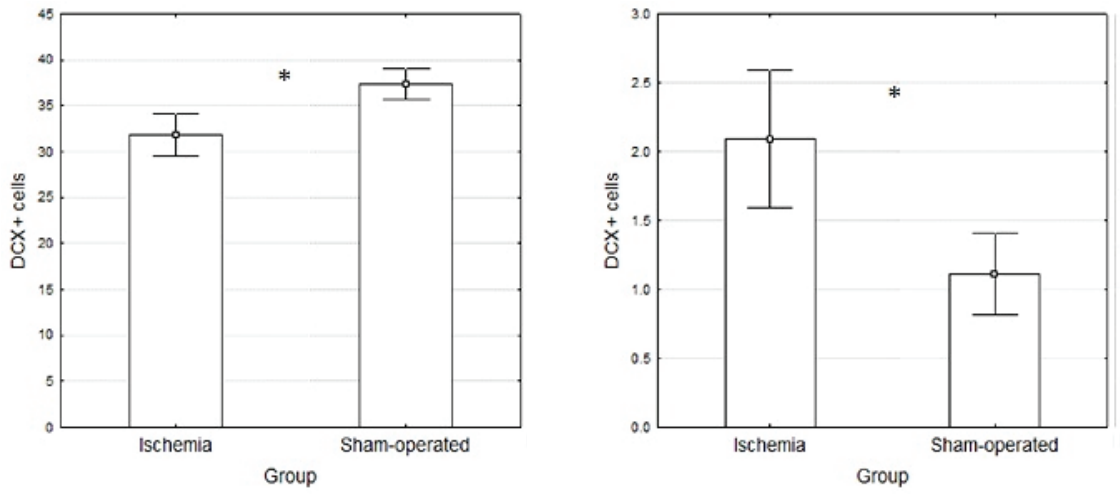


FIGURE 2. Neurogenesis in hippocampus after surgery in dentate gyrus (a) and in CA1-CA3 fields of hippocampus (b). The quantitative analysis of DCX-positive cells is expressed as the mean ± SEM. *P<0.05.

DISCUSSION

Our results demonstrate significant changes of neurogenesis in a month after transient global cerebral ischemia. A decline of neurogenesis in the dentate gyrus was described in some studies (Pforte et al., 2005; Kim et al., 2015). However, this decline was observed soon after global ischemia (within the 1st week). In other studies (Schiavon et al., 2010; Tian et al., 2014; Schmidt and Reymann, 2002; Spaccapelo et al., 2013) neurogenesis in the dentate gyrus the hippocampus was increased in ischemic groups compared to control and time points were different, i.e. on the 14th, 28th days after ischemia. The data of neurogenesis in CA1-CA3 fields of the hippocampus obtained in our study agree with the results obtained in the other studies (Schmidt and Reymann, 2002; Bingham et al., 2005).

The majority of works using the global transient cerebral ischemia model with a 2-vessel occlusion in gerbils (Liu et al., 1998; Salazar-Colocho et al., 2008), mice, and rats (Kee et al., 2001; Yagita et al., 2001) demonstrated an increased proliferation in the dentate gyrus in 3–5 days after surgery. It peaks in 8–10 days and returns to the control level in 3–5 weeks after ischemia (Wiltrout et al., 2007; Cayre et al., 2009). Using double labelling staining (Brdu+/NeuN+ cells) Salazar-Colocho with colleagues found that a significant percentage of cells in the dentate gyrus which had been born in 7–10 days after surgery exhibited a phenotype of mature neurons in a month after surgery (Salazar-Colocho et al., 2008). At the same time the number of DCX-positive cells was significantly reduced, as compared to sham-operated animals in the early period after the ischemia (Pforte et al., 2005) indicating an extremely negative impact of ischemic damage on the survival of immature neurons.

Preferential usage of the 2-vessel occlusion model is predetermined by anatomy of animal brain vessels (Myron et al., 1989). In these circumstances 2-vessel occlusion in gerbils is more suitable than 2-vessel or 4-vessel occlusion in rats or mice because it produces full ischemia and full reperfusion. The main disadvantage of the forebrain ischemia model in gerbils is that the implementation of brain ischemia is characterized by spasms in the postischemic period, but it is known that seizures may stimulate neurogenesis (Salazar-Colocho et al., 2008).

The 3-vessel occlusion model of global cerebral ischemia is a modification of 4-vessel occlusion (Chernysheva et al., 2014). It allows a full reperfusion which is absent in the original model and does not cause seizures like in the 2-vessel model in gerbils. Moreover, the new model initiates full global ischemia as compared to 2-vessel occlusion with hypotension in rats or mice where residual blood flow is kept by arterial ring. This study is the first study of neurogenesis in the 3-vessel occlusion model of global cerebral ischemia so results can differ from the results of similar studies employing another global ischemia model. The inversion of the ischemia effect on neurogenesis is probably due to the severity of ischemic damage in this model as compared to the occlusion of only common carotid arteries. The dependence of neurogenesis on the extent of damage in the global ischemia model was demonstrated by Liu with colleagues (Liu et al., 1998).

CONCLUSIONS

This study demonstrated changes of hippocampal neurogenesis in the new model of global cerebral ischemia. An increase of immature neurons appears in CA1-CA3 fields and gets place with a significant decrease of neurogenesis in dentate gyrus within a month after stroke. Moreover, the data suggest the dependence of neurogenesis on global ischemia model in similar studies.

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