Chapter 17
DCX-Expressing Neurons Decrease in the Retrosplenial Cortex after Global Brain Ischemia

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Abstract Many studies have demonstrated cognitive function disorders including space learning disorders after global brain ischemia (GBI). Previous research on space perception and learning has indicated that the retrosplenial cortex (RS) is strongly involved. We performed immunostaining with doublecortin (DCX) for neurons with plasticity potential in the RS and investigated the neuronal numbers to assess the changes of plasticity in the RS following GBI. We employed male Sprague–Dawley rats and carried out bilateral carotid arterial occlusion for 10 min as a GBI model (control, $n=5$; GBI model, $n=5$). We counted the right and left hemispheres separately on two serial sections, for a total of four regions per animal to examine the differences in expression related to GBI. Additionally, we performed Fluoro-Jade B (FJB) staining to investigate the cause of any DCX-expressing neuron decrease. The total number of DCX-expressing neurons was 1,652 and 912 in the controls and GBI model, respectively. The mean number of DCX-expressing neurons per unit area was significantly lower in the GBI model than in the controls. FJB positive neurons were not found in the RS, while many were present in the hippocampus CA1 after GBI. The decrease of DCX-expressing neurons in the RS indicated a plasticity decrease following GBI. The lack of FJB positive neurons in the RS after GBI suggested that the decrease of DCX-expressing neurons in the RS was not due to neuronal cell death in contrast to the hippocampus CA1, while the FJB positive neurons in the hippocampus indicated a delayed neuronal cell death as observed in many previous studies.

Keyword Brain ischemia

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1 Introduction

The causes of global brain ischemia (GBI) include cardiac arrest, low blood pressure shock, and vascular disorders [1–3]. Many studies have demonstrated cognitive function disorders including space learning disorders after GBI. Most experimental investigations have focused on the hippocampus and revealed that delayed neuronal cell death in the CA1 and CA3 increased following GBI [4–6], but the association between cognitive impairment and cell death was unclear [7–10]. Research on space perception and learning has indicated that the retrosplenial cortex (RS) which forms the caudal cingulate cortex is strongly involved [11–14]. However, little work has been done on the RS after GBI, whereas the hippocampus has been well investigated.

In the developing brain, doublecortin (DCX) contributes to the radial migration of immature neurons from the paraventricle to cortex, nuclear translocation, and the extension of axons and dendrites [15]. Based on these functions, DCX-expressing neurons are considered immature and thought to contribute strongly to structural plasticity. It has been reported that neurons having DCX are present in the cerebral neocortex and allocortex of the mature individual, and it has been suggested that they are associated with the structural plasticity of the brain [16–18].

The RS has a synaptic plasticity potential [19]. We performed immunostaining with DCX for neurons with structural plasticity potential in the RS and investigated the neuronal numbers to assess the alterations of the neuronal circuits and plasticity in the RS following GBI.

2 Materials and Methods

2.1 GBI Model

We employed male Sprague–Dawley rats (body weight 250–300 g). The procedure to make transient global cerebral ischemia model has been reported previously [25]. Anesthesia was performed with pentobarbital (Somnopentyl, Kyoritsu Seiyaku; 15 mg/kg body weight) intraperitoneally, N_2O, and isoflurane. Obliteration time of bilateral internal carotid artery was 10 min (control, $n=5$; GBI model, $n=5$). The animals were purchased from Charles River Laboratories and bred at the animal housing facility of Nihon University. The colony was maintained at 22–23 °C on a 12-h light/dark cycle (lights on at 08:00). After 7 days, the rats were transcardially perfusion-fixed with lactated Ringer's solution, followed by perfusion of 4 % paraformaldehyde. Coronal serial brain sections (50 μm) were cut in the frontal plane on a vibratome and stored at 4 °C in PBS (pH 7.4) before being analyzed.

All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, National Academy Press, Washington, DC, 2003) and approved by the Animal Care and Use Committee of Nihon University.

Table 17.1 Primary antibodies

Antigen	Class of antibody	Dilution	Manufacturer
Doublecortin (DCX)	Polyclonal goat	1:2,000	Santa Cruz Biotechnology
PSA-NCAM	Monoclonal mouse IgG	1:500	Chemicon

2.2 Immunohistochemistry

We performed immunostaining and fluorescence immunostaining with DCX and polysialic acid-neural cell adhesion molecule (PSA-NCAM) to observe the neurons with plasticity. The primary antibodies used in this study are shown in Table 17.1. We counted the right and left hemispheres separately on two serial sections, for a total of four regions per animal to examine the differences in expression related to GBI.

2.3 Fluoro-Jade B Staining

Additionally we performed Fluoro-Jade B (FJB) staining to investigate the cause of any DCX-expressing neuron decrease. The FJB staining procedure was as reported previously [20].

2.4 Measurement and Analysis

We used Biozero (BZ-8000; Keyence, Japan) and a BZ-Analyzer (Keyence, Japan) to prepare the microphotographs, and a VH-Analyzer (Keyence, Japan) which had unbiased three-dimensional stereological software to count the numbers of immunostain-positive cells. We used Neurolucida (Version 3; MicroBrightField, USA) to analyze the area and cell counts without double counting of positive cells in the ACC and RS, and then estimated the cell counts per unit area in the control and GBI model. We employed the statistical software SPSS Statistics 17.0 for data analysis. We performed the Mann–Whitney test to compare unrelated groups.

3 Results

3.1 Number of DCX-Expressing Neurons and Colocalization with PSA-NCAM

We identified the RS by referring to the rat atlas of Paxinos and Watson (1982) [21] and measured the neuronal DCX expression in immunostained coronal sections subjected to DCX immunohistochemistry (Fig. 17.1).

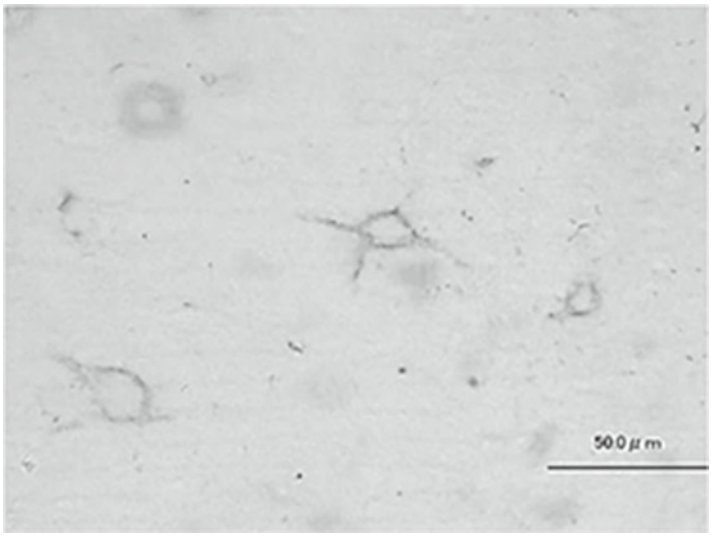

Fig. 17.1 Doublecortin (DCX)-expressing neurons in the retrosplenial cortex (RS) (diaminobenzidine reaction; scale bar, 50 μm; bregma posterior, 3.30 mm). This image was obtained from the RS of a global brain ischemia model (layer: III/IV). DCX-expressing neurons were similarly present in the RS of the control

The total number of DCX-expressing neurons was 1,652 in the controls and 912 in the GBI model. The mean number of DCX-expressing neurons per unit area (mean ± SD) was 67 ± 14.78 neurons/mm^2 in the controls and 34 ± 8.63 neurons/mm^2 in the GBI model. The mean number of DCX-expressing neurons was significantly lower in the GBI model than in the controls (Mann–Whitney test, $p < 0.001$) (Fig. 17.2).

PSA-NCAM represents one of the immature neuronal markers. We investigated the colocalization of DCX with PSA-NCAM in the controls and GBI model. The results showed that few DCX-expressing neurons expressed PSA-NCAM in the RS, while many DCX-expressing neurons were colocalized with PSA-NCAM within the hippocampus subgranular zone in which neurogenesis had occurred.

3.2 FJB Staining

FJB staining is used for the identification of neuronal cell death. As regards the RS, there were no positive cells in the controls and GBI model, respectively, while many positive neurons were present in the hippocampus CA1 of the GBI model. This number for the RS was not appropriate for the decreased number of DCX-expressing neurons.

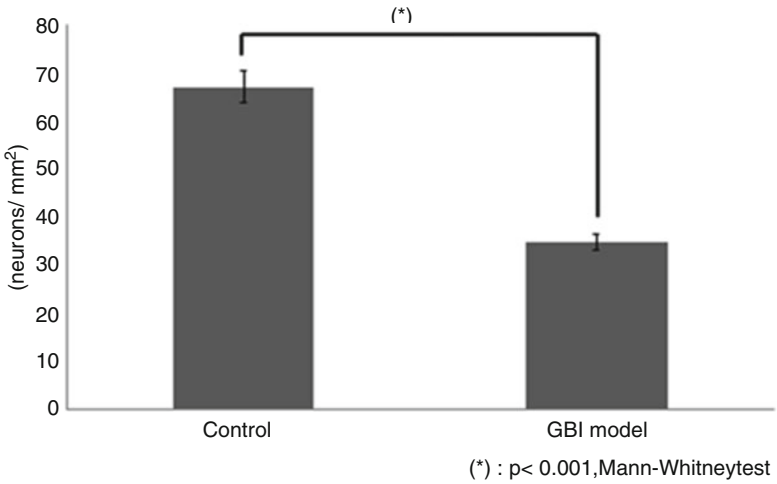

Fig. 17.2 In the global brain ischemia model ($n=5$), significantly less doublecortin (DCX)-expressing neurons were observed in the retrosplenial cortex (RS) than in the control ($n=5$) per unit area ($n=20$ samples/subregion; right and left hemispheres of two sections from each rat)

4 Discussion and Conclusion

Transient GBI could lead to neuronal differentiation of immature neurons in the RS followed by a decreased structural plasticity in the mature individual brain. The present data showed that the DCX-expressing neurons of the RS were significantly decreased after transient GBI. Following lethal ischemia, neuronal cell death occurs in the cortex [22]. The results for FJB staining revealed no FJB positive cells after GBI in the RS, indicating that neurons which fall into cell death are rare after GBI in the RS. Since cell death does not occur as the number of DCX-expressing neurons decreases, it is considered that DCX disappears from the neurons. In relation to the function of DCX, the neuron advances to a more mature form during the stages of neuronal development, and it is inferred that the neuron has a reduced function to grow dendrites and axons to form new synapses without neuronal cell death [15].

As described above, the RS plays an important role in space learning and memory. It has been suggested that information based on movement from the occipitoparietal cortex spreads in the space reference frame within the RS [23]. There are cells with a function to make cephalic presentation signals [23]. Also, the neurons make signals responding immediately to the animal body position and body movement as well as cephalic presentation [24]. The information associated with space navigation is unified within the RS.

We consider that many DCX-expressing neurons could respond to the stimulation of space perception and form local neuronal circuits according to new space information in the developed brain. A decrease of these neurons after GBI may lead

to a decrease in the RS plastic potential and, as a result, be associated with space learning failure and memory dysfunction.

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