Long-term changes in the ipsilateral substantia nigra after transient focal cerebral ischaemia in rats

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Summary

Transient focal cerebral ischaemia can cause neuronal damage in remote areas, including the ipsilateral thalamus and substantia nigra, as well as in the ischaemic core. In the present study, we investigated long-term changes in the ipsilateral substantia nigra from 1 up to 20 weeks after 90 min of transient focal cerebral ischaemia in rats, using tyrosine hydroxylase (TH), neuronal nuclei (NeuN), Iba-1, glial fibrillary acidic protein (GFAP) and brain-derived neurotrophic factor (BDNF) immunostaining. These results show that transient focal cerebral ischaemia in rats can cause a severe and prolonged neuronal damage in the ipsilateral striatum. Our results with TH and NeuN immunostaining also demonstrate that the atrophy of the ipsilateral substantia nigra after transient focal cerebral ischaemia was not static but progressive. Furthermore, our double-labelled immunohistochemical study suggests that BDNF released by GFAP-positive astrocytes may play a key role in the survival of dopaminergic neurones in the ipsilateral substantia nigra at the chronic stage after transient focal cerebral ischaemia, although the areas of the ipsilateral substantia nigra are decreased progressively after ischaemia. Thus our study provides further valuable information for the pathogenesis of neuronal damage after transient focal cerebral ischaemia.

Keywords

atrophy, focal ischaemia, glia, neurotrophic factor, remote area, substantia nigra

The most commonly encountered type of brain stroke in human is cerebral ischaemia. Occlusion of a single trunk, particularly the middle cerebral artery is the most frequent of the many causes of cerebral ischaemia. Many cerebral ischaemic models in non-human primates (Hudgins & Garcia 1970), cats (O’Brien & Waltz 1973) and rats (Robinson 1981; Abe et al. 1988; Longa et al. 1989) have been developed in order to investigate the pathophysiology of cerebral ischaemia and the efficacy of various drug treatments. Especially, the reperfusion of ischaemic brain can lead to extensive cell injury and death (Aronowski et al. 1997).

Transient focal cerebral ischaemia leads to neuronal damage not only in the ischaemic core, but also in the non-ischaemic area distant from the ischaemic core (Nagasawa &
Changes of remote area after focal cerebral ischaemia

Kogure 1990; Tamura et al. 1990). In transient focal cerebral ischaemia, neuronal cells in the ischaemic core are damaged by a rapid cell loss process such as necrosis and apoptosis (Sakuma et al. 2008; Matsuda et al. 2009). However, it is well known that neuronal damage in the remote area is not an immediate process but a delayed one. Several previous studies reported that permanent occlusion of the middle cerebral artery in rats can cause delayed atrophy of the ipsilateral thalamus and substantia nigra (Fujie et al. 1990; Tamura et al. 1990). Especially, neuronal damage, gliosis and atrophy were observed in the substantia nigra 2 weeks after focal cerebral ischaemia (Nagasawa & Kogure 1990; Tamura et al. 1990; Yamada et al. 1996). Therefore, the changes of remote areas are of particular interest in relation to the pathogenesis of secondary neuronal damage after transient focal cerebral ischaemia. However, little is known about long-term changes in the remote areas after transient focal cerebral ischaemia in rats.

In the present study, therefore, we investigated long-term changes in the ipsilateral substantia nigra after transient focal cerebral ischaemia in rats using immunohistochemical study.

Methods

Experimental animals

Male adult SD rats, weighting 200–250 g (Nihon SLC Co., Shizuoka, Japan) were used. The animals were housed in a controlled environment (23 ± 1 °C, 50 ± 5% humidity) and were allowed food and tap water ad libitum. The room lights were on between 8:00 and 20:00. The animals were anaesthetized with 2% halothane in a mixture of 30% oxygen and 70% nitrous oxide. Focal cerebral ischaemia was induced with a silicone-coated 4-0 monofilament into the internal carotid artery and advancing it. By so doing, the middle cerebral artery was occluded. Ninety minutes later, the animals were briefly reanaesthetized and filament completely withdrawn to allow reperfusion, and the animals were allowed to survive for 1 week (n = 4), 2 weeks (n = 4), 6 weeks (n = 4), and 20 weeks (n = 4) after focal ischaemia. Sham-operated animals (n = 5) were treated in the same manner, except that the middle cerebral artery was not occluded, as described previously (Abe et al. 1988; Araki et al. 1998; Sakuma et al. 2008; Matsuda et al. 2009). Body temperature was maintained at 37–38 °C with a heating pad equipped with a thermostat until the animals started moving. Survival of animals was >90% up to 20 weeks after focal cerebral ischaemia. All experiments were performed in accordance with the Guidelines for Animal Experiments of the Tokushima University School of Medicine.

Immunohistochemistry

The animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) 1, 2, 6, and 20 weeks after focal cerebral ischaemia, and the brains were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) following a heparinized saline flush. The brains were removed 1 h after perfusion fixation at 4 °C and were immersed in the same fixative until they were embedded in paraffin. Paraffin sections, 5 μm in thickness, were stained with Cresyl violet and used for immunohistochemistry.

For immunohistochemical studies, a Vectastain elite ABC kit (Vector Labs. Burlingame, CA, USA), anti-neuronal nuclei (NeuN) antibody (Chemicon International Inc., Temecula, CA, USA, 1:200), anti-tyrosine hydroxylase (TH) antibody (Chemicon International, Inc., 1:200), anti-glial fibrillary acidic protein (GFAP) antibody (Chemicon International Inc., 1:400), and anti-Iba-1 antibody (Wako Pure Chemicals, Osaka, Japan, 1:4000) were performed as described previously (Sakuma et al. 2008; Matsuda et al. 2009). Negative control sections were treated in the same way except that each primary antibody or secondary antibody was omitted. Immunoreactions were visualized using Vector DAB (3’3’-diaminobenzidine) substrate kit (Vector Labs) as described previously (Kurosaki et al. 2005; Sakuma et al. 2008). Furthermore, double-labelled immunostainings with anti-brain-derived neurotrophic factor (BDNF, Chemicon International, Inc., 1:200) and anti-GFAP antibodies were performed in substantia nigra 1 and 20 weeks after focal ischaemia, as described previously (Himeda et al. 2006, 2007). Immunoreactions were visualized using Vector

Figure 1 Representative photograph of in the rat fresh brain 20 weeks after transient focal cerebral ischaemia. Left side; the arrow indicates the ipsilateral region (atrophy). Right side; the arrow indicates the contralateral region.
Nova Red substrate kit (red) and Vector SG substrate kit (grey) respectively.

For immunochemical stainings, changes of densities of these immunoreactivity, changes of areas of these immunoreactivity or changes in the number of these immunopositive cells in stained sections were evaluated under a light microscope at a magnification of ×400 without the examiner knowing the experimental protocol, using a computer-associated image analyzer (WinRoof Version 5; Mitani Corporation, Fukui, Japan), as described previously (Sakuma et al. 2008; Matsuda et al. 2009). Values were expressed means ± SD. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Fisher’s PLSD multiple comparison test or Student’s t-test (Statview 5.0J; SAS Institute Inc., Cary, NC, USA). The P values less than 0.05 was considered statistically significance. Each group consisted of four to five rats.

Results

Brain damage after focal cerebral ischaemia

Representative photograph of fresh brain 20 weeks after focal cerebral ischaemia is shown in Figure 1. Severe atrophy was observed within the territories of the occluded middle cerebral artery, i.e. in the ipsilateral cerebral cortex and striatum. Especially, the ipsilateral cerebral cortex supplied by the anterior cerebral artery showed a severe atrophy. In the contralateral side, the atrophy was not observed in the cerebral cortex and striatum.

NeuN immunostaining in the ipsilateral striatum after focal cerebral ischaemia

Representative microphotographs of NeuN immunostaining in the striatum are shown in Figure 2 (a). In sham-operated rats, NeuN immunoreactivity was clearly observed in neurones of the dorsolateral striatum. No damage of NeuN immunopositive neurones was detected in the sham-operated rats. In contrast, severe damage of NeuN immunopositive neurones was observed in the ipsilateral striatum from 1 up to 20 weeks after focal ischaemia, as shown in Figure 2 (b).

TH immunostaining in the ipsilateral substantia nigra after focal cerebral ischaemia

Representative microphotographs of TH immunostaining in the substantia nigra are shown in Figure 3 (a). In sham-operated rats, TH immunoreactivity was clearly observed in neurones of the substantia nigra. In contrast, a significant decrease in the number of TH immunopositive neurones was observed in the ipsilateral substantia nigra 2 weeks after focal cerebral ischaemia. Thereafter, a conspicuous decrease

Figure 2 (a) Representative microphotographs of NeuN immunostaining in the ipsilateral rat striatum after transient focal cerebral ischaemia. (A) sham-operated; (B) 1 week after ischaemia; (C) 2 weeks after ischaemia; (D) 6 weeks after ischaemia; (E) 20 weeks after ischaemia. Bar (A–E) = 20 μm. (b) NeuN immunopositive cell number in the ipsilateral striatum of rats after transient focal cerebral ischaemia. Total number of NeuN immunopositive cells was quantified using a computer-associated image analyzer software (WinRoof version 5). Sham; sham-operated. 1 w; 1 week after ischaemia. 2 w; 2 weeks after ischaemia. 6 w; 6 weeks after ischaemia. 20 w; 20 weeks after ischaemia. All values were expressed as means ± SD. *P < 0.01 compared with sham-operated group (Fisher’s PLSD multiple comparison test). n = 4–5.
in the number of TH immunoreactive neurones was found in the ipsilateral substantia nigra 6 weeks after focal cerebral ischaemia. Twenty weeks after focal ischaemia, however, no significant change in the number of TH immunoreactive neurones was observed in the ipsilateral substantia nigra, as shown in Figure 3 (b). On the other hand, a significant decrease in optical densities of TH immunoreactivity was observed in areas of the ipsilateral substantia nigra 6 weeks after focal ischaemia. However, no significant change in optical densities of TH immunoreactivity was observed in areas of the ipsilateral substantia nigra 20 weeks after focal ischaemia, as shown in Figure 3 (c).

Long-term changes of areas in the ipsilateral substantia nigra pars compacta after focal cerebral ischaemia

The area of the substantia nigra pars compacta with TH immunoreactivity was decreased progressively from 1 up to 20 weeks after focal cerebral ischaemia, as shown in Figure 4.

Figure 3 (a) Representative microphotographs of TH immunostaining in the rat ipsilateral substantia nigra after transient focal cerebral ischaemia. (A) sham-operated; (B) 1 week after ischaemia; (C) 2 weeks after ischaemia; (D) 6 weeks after ischaemia; (E) 20 weeks after ischaemia. Bar (A–E) = 100 μm. (b) TH immunopositive cell number in the ipsilateral substantia nigra of rats after transient focal cerebral ischaemia. Total number of TH immunopositive cells was quantified using a computer-associated image analyzer software. (c) Densities of TH immunoreactivity in the ipsilateral substantia nigra of rats after transient focal cerebral ischaemia. Densities of TH immunoreactivity were quantified using a computer-associated image analyzer software. Sham; sham-operated. 1 w; 1 week after ischaemia. 2 w; 2 weeks after ischaemia. 6 w; 6 weeks after ischaemia. 20 w; 20 weeks after ischaemia. All values were expressed as means ± SD. *P < 0.01 compared with sham-operated group (Fisher’s PLSD multiple comparison test). #P < 0.01 compared with 6 weeks group (Student’s t-test). n = 4–5.

Figure 4 Areas (mm²) in the ipsilateral substantia nigra pars compacta with TH immunoreactivity after focal cerebral ischaemia. Changes of areas of TH immunoreactivity were quantified using a computer-associated image analyzer software. Sham; sham-operated. 1 w; 1 week after ischaemia. 2 w; 2 weeks after ischaemia. 6 w; 6 weeks after ischaemia. 20 w; 20 weeks after ischaemia. All values were expressed as means ± SD. *P < 0.05, **P < 0.01 compared with sham-operated group (Fisher’s PLSD multiple comparison test). n = 4–5.
NeuN immunostaining in the ipsilateral substantia nigra after focal cerebral ischaemia

Representative microphotographs of NeuN immunostaining in the substantia nigra are shown in Figure 5 (a). In sham-operated rats, NeuN immunoreactivity was clearly observed in neurones of the substantia nigra. In contrast, a significant decrease in the number of NeuN-immunopositive neurones was observed in the ipsilateral substantia nigra from 6 up to 20 weeks after focal cerebral ischaemia, as shown in Figure 5 (b). On the other hand, a significant decrease in optical densities of NeuN immunoreactivity was observed in areas of the ipsilateral substantia nigra from 2 up to 20 weeks after focal ischaemia. However, the decrease in optical densities of NeuN immunoreactivity in areas of the ipsilateral substantia nigra 6 weeks after focal cerebral ischaemia was more pronounced than that in the ipsilateral substantia nigra 20 weeks after focal ischaemia, as shown in Figure 5 (c).

Iba-1 immunostaining in the ipsilateral substantia nigra after focal cerebral ischaemia

Representative microphotographs of Iba-1 immunostaining in the substantia nigra are shown in Figure 6 (a). In sham-operated rats, Iba-1 immunoreactivity was slightly observed in glial cells of the substantia nigra. In contrast, a significant increase in the number of Iba-1-immunopositive microglia was observed in the ipsilateral substantia nigra from 1 up to 6 weeks after focal cerebral ischaemia. However, the increase in the number of Iba-1-immunopositive microglia showed a tendency to decrease gradually in the ipsilateral substantia nigra from 1 up to 6 weeks after focal cerebral ischaemia. Thereafter, no significant change in the number of Iba-1-immunopositive microglia was observed in the ipsilateral substantia nigra 20 weeks after focal cerebral ischaemia, as shown in Figure 6 (b).
GFAP immunostaining in the ipsilateral substantia nigra after focal cerebral ischaemia

Representative microphotographs of GFAP immunostaining in the substantia nigra are shown in Figure 7 (a). In sham-operated rats, GFAP immunoreactivity was slightly observed in glial cells of the substantia nigra. In contrast, a significant increase in number of GFAP immunopositive astrocytes was observed gradually in the ipsilateral substantia nigra from 1 up to 20 weeks after focal cerebral ischaemia, as shown in Figure 7 (b).

BDNF immunostaining in the ipsilateral substantia nigra after focal cerebral ischaemia

Representative microphotographs of BDNF immunostaining in the substantia nigra are shown in Figure 8. In sham-operated rats, BDNF immunoreactivity was slightly observed in glial cells and neurones of the substantia nigra. In contrast, a significant decrease in number of BDNF immunopositive glial cells was observed in the ipsilateral substantia nigra 6 weeks after focal cerebral ischaemia. Thereafter, a significant increase in number of BDNF immunopositive glial cells was observed in the ipsilateral substantia nigra 20 weeks after focal cerebral ischaemia. In contrast, a significant increase in number of BDNF immunopositive neurones was found in the ipsilateral substantia nigra 1 and 20 weeks after focal cerebral ischaemia.

Double-labelled immunostainings in the ipsilateral substantia nigra after focal cerebral ischaemia

Representative double-labelled immunostainings with anti-GFAP and anti-BDNF antibodies in the ipsilateral...
substantia nigra after focal ischaemia. 

**Figure 7** (a) Representative microphotographs of GFAP immunostaining in the rat ipsilateral substantia nigra after transient focal cerebral ischaemia. (A) sham-operated; (B) 1 week after ischaemia; (C) 2 weeks after ischaemia; (D) 6 weeks after ischaemia; (E) 20 weeks after ischaemia; (F) sham-operated (high magnification); (G) 1 week after ischaemia (high magnification); (H) 6 weeks after ischaemia (high magnification); (I) 20 weeks after ischaemia (high magnification). Bar (A–E) = 100 μm. Bar (F–I) = 10 μm. (b) GFAP immunopositive cell number in the ipsilateral substantia nigra of rats after transient focal cerebral ischaemia. Total number of GFAP immunopositive cells was quantified using a computer-associated image analyzer software. Sham; sham-operated. 1 w; 1 week after ischaemia. 2 w; 2 weeks after ischaemia. 6 w; 6 weeks after ischaemia. 20 w; 20 weeks after ischaemia. The dotted lines indicate the regions of substantia nigra compacta. All values were expressed as means ± SD. *P < 0.05, **P < 0.01 compared with sham-operated group (Fisher’s PLSD multiple comparison test).

**Figure 8** (a) Representative microphotographs of BDNF immunostaining in the rat ipsilateral substantia nigra after transient focal cerebral ischaemia. (A) sham-operated; (B) 1 week after ischaemia; (C) 2 weeks after ischaemia; (D) 6 weeks after ischaemia; (E) 20 weeks after ischaemia. Bar (A–E) = 100 μm. (b) BDNF immunopositive glial cell number in the ipsilateral substantia nigra of rats after transient focal cerebral ischaemia. Total number of BDNF immunopositive glial cells was quantified using a computer-associated image analyzer software. (c) BDNF immunopositive neuronal cell number in the ipsilateral substantia nigra of rats after transient focal cerebral ischaemia. Total number of BDNF immunopositive neurons was quantified using a computer-associated image analyzer software. Densities of BDNF immunoreactivity were quantified using a computer-associated image analyzer software. Sham; sham-operated. 1 w; 1 week after ischaemia. 2 w; 2 weeks after ischaemia. 6 w; 6 weeks after ischaemia. 20 w; 20 weeks after ischaemia. The dotted lines indicate the regions of substantia nigra pars compacta. All values were expressed as means ± SD. *P < 0.05, **P < 0.01 compared with sham-operated group (Fisher’s PLSD multiple comparison test).
nigra of rats 20 weeks after focal cerebral ischaemia as compared to rats 1 week postischaemia, as shown in Figure 9 (b) and (c).

**TH immunostaining in the ipsilateral striatum after focal cerebral ischaemia**

Representative microphotographs of TH immunostaining in the striatum are shown in Figure 10 (a). In sham-operated rats, TH immunoreactivity was clearly observed in striatum. In contrast, a significant decrease in densities of TH immunoreactivity was observed in the ipsilateral striatum 1 week after focal cerebral ischaemia. Thereafter, a significant increase in densities of TH immunoreactivity was found in the ipsilateral striatum 20 weeks after focal cerebral ischaemia, as shown in Figure 10 (b).

**Discussion**

The substantia nigra is situated in the brain stem and displays a large distance to the striatum and the globus pallidus. Perforating branches of the posterior cerebral artery supply the substantia nigra. The substantia nigra has strong connections preferentially to the striatum. The nigrostriatal projections originate within the pars compacta of the substantia nigra are dopaminergic. In contrast, the striatalnigral projections are GABA (γ-aminobutyric acid)ergic and project mainly to the pars reticulata of the substantia nigra.

It is well known that focal cerebral ischaemia leads to secondary neuronal death within the substantia nigra (Nagashawa & Kogure 1990; Tamura *et al.* 1990; Dihne et al. 2001). However, little is known about long-term changes in the

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**Figure 9** (a) Representative microphotographs of double-labelled immunostaining with anti-BDNF and anti-GFAP antibodies in the rat ipsilateral substantia nigra after transient focal cerebral ischaemia. (A) 1 week after ischaemia; (B) 20 weeks after ischaemia; (C) 20 weeks after ischaemia (high magnification). Bar (A, B) = 20 μm. Bar (C) = 10 μm. (b) GFAP and BDNF immunopositive glial cell number in the ipsilateral substantia nigra of rats 1 and 20 weeks after transient focal cerebral ischaemia. Total number of GFAP + BDNF immunopositive glial cells was quantified using a computer-associated image analyzer software. (c) BDNF immunopositive neuronal cell number in the ipsilateral substantia nigra of rats 1 and 20 weeks after transient focal cerebral ischaemia. Total number of BDNF immunopositive neurones was quantified using a computer-associated image analyzer software. 1 w; 1 week after ischaemia. 20 w; 20 weeks after ischaemia. All values were expressed as means ± SD. *P < 0.05, **P < 0.01 compared with 1 weeks group (Student’s t-test). n = 4.
ipsilateral substantia nigra after transient focal cerebral ischaemia in rats.

In the present study, severe damage of NeuN immunopositive neurones was observed in the ipsilateral substantia nigra from 1 up to 20 weeks after focal cerebral ischaemia. A significant decrease in optical densities of NeuN immunoreactivity was observed in areas of the ipsilateral substantia nigra from 2 up to 20 weeks after focal cerebral ischaemia. However, the decrease in optical densities of NeuN immunoreactivity in areas of the ipsilateral substantia nigra 20 weeks after focal cerebral ischaemia was more pronounced than that in the ipsilateral substantia nigra 20 weeks after focal cerebral ischaemia. These results demonstrate that the atrophy of the ipsilateral substantia nigra was not static but progressive after focal cerebral ischaemia. Our present findings also suggest that the neuronal cell loss was observed in the ipsilateral substantia nigra from 2 up to 20 weeks after focal cerebral ischaemia. A previous interesting study reported that the mechanism of the atrophy in the ipsilateral substantia nigra, which is remote from the site of infarction, might be explained by transsynaptic, GABA-mediated disinhibition as a result of infarction of the striatum after focal cerebral ischaemia (Tamura et al. 1990). Furthermore, a disinhibition of nigral neurones due to abolition of striatal GABAergic input has been proposed to be involved in this neuronal damage (Saji & Reis 1987). Therefore, our present findings suggest that the atrophy in the ipsilateral substantia nigra may be explained by the disinhibition of the striatal GABAergic input as a result of infarction of the striatum after focal cerebral ischaemia. However, a significant increase in the number of TH immunopositive neurones and in optical densities of TH immunoreactivity was observed in areas of the ipsilateral substantia nigra in rats 20 weeks after focal cerebral ischaemia, as compared to rats 6 weeks postischaemia. Similarly, a significant increase in optical densities of NeuN immunoreactivity was observed in areas of the ipsilateral substantia nigra in rats 20 weeks after focal cerebral ischaemia, as compared to rats 6 weeks postischaemia. The reason for this phenomenon is presently unclear. A previous study reported that transient focal cerebral ischaemia caused a significant decrease of TH immunopositive neurones in the ipsilateral substantia nigra at 7 and 14 days after ischaemia (Soriano et al. 1997). They also demonstrated that the decrease of TH immunoreactivity recovered at 30 days and recovered completely at 60 days after ischaemia. Furthermore, TH immunopositive neurones in the ipsilateral substantia nigra were transiently reduced in number and recovered to the level in the contralateral side at 60 days.

Figure 10 (a) Representative microphotographs of TH immunostaining in the rat ipsilateral striatum after transient focal cerebral ischaemia. (A) sham-operated; (B) 1 week after ischaemia; (C) 20 weeks after ischaemia. Bar (A–C) = 50 μm. (b) Densities of TH immunoreactivity in the ipsilateral striatum of rats after transient focal cerebral ischaemia. The densities of TH immunoreactivity were quantified using a computer-associated image analyzer software. All values were expressed as means ± SD. *P < 0.01 compared with sham-operated group (Fisher’s PLSD multiple comparison test). n = 4–5.
after focal cerebral ischaemia (Huh et al. 2003). However, the mechanisms by which recovery of TH immunoreactivity in the ipsilateral substantia nigra is induced were not well understood.

In the present study, a significant increase in the number of Iba-1 immunopositive microglia was observed in the ipsilateral substantia nigra from 1 up to 6 weeks after focal cerebral ischaemia. However, the increase in the number of Iba-1 immunopositive microglia showed a tendency to decrease gradually in the ipsilateral substantia nigra from 1 up to 6 weeks after focal cerebral ischaemia. Thereafter, no significant change in the number of Iba-1 immunopositive microglia was observed in the ipsilateral substantia nigra 20 weeks after focal cerebral ischaemia. In contrast, a significant increase in number of GFAP immunopositive astrocytes was observed gradually in the ipsilateral substantia nigra from 1 up to 20 weeks after focal cerebral ischaemia. Therefore, we speculate that GFAP immunopositive astrocytes may play a key role in the survival of nigral dopaminergic neurones in the ipsilateral substantia nigra 20 weeks after focal cerebral ischaemia. In addition, our immunohistochemical study showed no significant change in the contralateral substantia nigra from 1 up to 20 weeks after focal ischaemia (data not shown).

BDNF is one of a family of neurotrophins. It is known that BDNF is involved in neuronal survival, synaptic plasticity, learning and memory, and neuronal plasticity (Miller & Kaplan 2001; Ploughman et al. 2009). Numerous studies have identified BDNF as playing a key role in various forms of neuronal plasticity in both the intact and damaged brain (Kleim et al. 2003; Ploughman et al. 2007; Bekinschtein et al. 2008). Furthermore, it is known that administrating BDNF in the acute postischaemic period decreases neuronal cell death and infarct volume (Wu & Partridge 1999; Zhang & Partridge 2006). Based on these observations, it is conceivable that BDNF may play an important role in the survival of dopaminergic neurones in the ipsilateral substantia nigra 20 weeks after focal cerebral ischaemia.

Our double-labelled immunohistochmical study showed that BDNF immunoreactivity was observed in both GFAP-positive astrocytes and neurones of the ipsilateral substantia nigra from 1 to 20 weeks after focal cerebral ischaemia. Furthermore, the present study demonstrates that the BDNF immunoreactivity was expressed significantly in the ipsilateral striatum and substantia nigra pars compacta 20 weeks after focal ischaemia, compared to rats 1 week postischaemia. These results demonstrate that BDNF released by GFAP positive astrocytes may play a key role in the survival of dopaminergic neurones in the ipsilateral substantia nigra at chronic stage after focal cerebral ischaemia, although the areas of the ipsilateral substantia nigra were decreased progressively after ischaemia. However, previous studies reported that BDNF-mutant mice exhibit impaired long-term potentiation (LTP) and are unable to learn (Patterson et al. 1996; Linanarsson et al. 1997). Therefore, we cannot exclude the possibility that other neurotrophic factors may play an important role in the survival of dopaminergic neurones in the ipsilateral substantia nigra after focal cerebral ischaemia.

In addition, we did not observe the presence and/or a possible upregulation of specific receptors (TrkB) on surviving dopaminergic neurones in the ipsilateral substantia nigra after focal cerebral ischaemia. Also, we did not examine fully the functional role on surviving dopaminergic neurones in the ipsilateral substantia nigra after focal cerebral ischaemia. Furthermore, we did not study the effect of motor function in rats subjected to focal cerebral ischaemia. Further studies are required to clarify our findings.

In conclusion, the present study shows that transient focal cerebral ischaemia in rats can cause a severe and prolonged neuronal damage in the ipsilateral striatum. Furthermore, our results indicate that the atrophy of the ipsilateral substantia nigra was not static but progressive after focal cerebral ischaemia. These results demonstrate that BDNF released by GFAP-positive astrocytes may play a key role in the survival of dopaminergic neurones in the ipsilateral substantia nigra at chronic stage after focal cerebral ischaemia, although the areas of the ipsilateral substantia nigra were decreased progressively after ischaemia. Thus our study provides further valuable information for the pathogenesis after transient focal cerebral ischaemia.

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