Intravenous Brain-Derived Neurotrophic Factor Enhances Poststroke Sensorimotor Recovery and Stimulates Neurogenesis

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Background and Purpose—The discovery of spontaneous neuronal replacement in the adult brain has shifted experimental stroke therapies toward a combined approach of preventing neuronal cell death and inducing neuronal plasticity. Brain-derived neurotrophic factor (BDNF) was shown to induce antiapoptotic mechanisms after stroke and to reduce infarct size and secondary neuronal cell death. Moreover, in intact animals, BDNF is a potent stimulator of adult neurogenesis.

Methods—The current study analyzed the effects of BDNF on induction of neuronal progenitor cell migration and sensorimotor recovery after cortical photothrombotic stroke.

Results—Daily intravenous bolus applications of BDNF during the first 5 days after stroke resulted in significantly improved sensorimotor scores up to 6 weeks. At the structural level, BDNF significantly increased neurogenesis in the dentate gyrus and enhanced migration of subventricular zone progenitor cells to the nearby striatum of the ischemic hemisphere. BDNF treatment could not, however, further stimulate progenitor cell recruitment to the cortex.

Conclusions—These findings consolidate the role of BDNF as a modulator of neurogenesis in the brain and as an enhancer of long-term functional neurological outcome after cerebral ischemia. (Stroke. 2007;38:2165-2172.)

Key Words: animal models ■ brain recovery, experimental ■ growth factors ■ neural stem cells ■ neuroregeneration

Neurotrophic factors control intercellular and intracellular signaling pathways that sculpt neuronal circuits during brain development and fundamentally regulate plasticity as well as cell survival in the adult brain.1 One of the prominent factors and, within the central nervous system, one of the most widely distributed factors is brain-derived neurotrophic factor (BDNF), which acts specifically via a high-affinity cell surface receptor (TRKB). By activating intracellular protein kinase B (ie, Akt), mitogen-activated protein kinases, and the extracellular signal–regulated kinases, BDNF effectively prevents neuronal cell death after various traumatic events such as cerebral ischemia.2 BDNF also crucially promotes synaptic and axonal plasticity associated with learning, memory, and sensorimotor recovery.3,4

In addition to qualitatively enhancing neural structural plasticity, BDNF was shown to increase the number of newborn neurons in several brain areas. However, BDNF-induced neurogenesis in vivo was observed only in unlesioned healthy animals.5–8 After global ischemia, blockade of endogenously occurring BDNF as well as exogenous delivery of BDNF counteracted neuronal differentiation in the affected dentate gyrus.9,10 In the present study, we explored the effect of peripheral BDNF application on neurogenesis in a focal cortical ischemia model. We assessed functional sensorimotor outcome as well as the generation and migration of neuronal progenitors in the cortex, striatum, and hippocampus.

Materials and Methods

Photothrombotic Ischemia Model

All animal experiments were performed in accordance with national and international regulations and were approved by government authorities. Male Wistar rats weighing 280 to 320 g were anesthetized with ketamine hydrochloride (100 mg/kg body weight IM; Ketanest) and xylazine hydrochloride (8 mg/kg body weight IM; Rompun). A PE-50 tube was inserted into the right femoral artery for monitoring mean arterial blood pressure and blood gases. During the experiment, rectal temperature was maintained at 37°C by a thermostatically controlled heating pad (Föhr Medical Instruments). Photothrombotic ischemia was induced in the rat
parietal cortex (right side) according to the method of Watson et al. In brief, after injection of Bengal rose (0.133 mL/kg body weight IV), the skull was illuminated with a cold, white light beam (150 W) for 20 minutes at 4 mm posterior to and lateral from the bregma. Sham-operated animals received vehicle infusion and were not illuminated.

Before surgery, animals were assigned in a fully randomized and blinded fashion to the following groups: ischemia + vehicle (n = 10), sham + vehicle (n = 7), ischemia + BDNF (n = 10), or sham + BDNF (n = 7). The randomization code was broken after all data were acquired, including those from morphological analysis. Animals were treated with 20 μg BDNF IV or vehicle 1 hour after induction of ischemia. Treatment was repeated on days 2 to 5. Four hours after treatment, dividing cells were labeled with bromodeoxyuridine (BrdU; 50 mg · kg⁻¹ · d⁻¹ IP). Animals were perfused 37 days after the last injection, and 6 animals per group were randomly chosen for immunohistochemistry.

**Sensorimotor Measurements**

In all animals, sensorimotor tests were performed during the light cycle 1 to 3 days before ischemia after a training period of 3 days and at 2, 3, 4, 5, and 6 weeks after ischemia by an investigator blinded to the experimental groups. Tests such as the Rotorod, adhesive tape removal, and beam balance were done as described in detail. By using the previously described Neurological Severity Score (NSS), neurological function was graded on a scale of 0 to 16 (normal score = 0, maximal deficit score = 16). The NSS is a composite of motor, sensory, and reflex tests, including beam balance.

**Neurogenesis Detection In Vivo**

Neuronal progenitor cells and new neurons were visualized by immunofluorescence against double cortin and by double labeling of BrdU with the mature neuronal marker NeuN. Antibodies used were as follows: rat anti-BrdU (1:500, Accurate); mouse anti-NeuN (1:500, Chemicon); goat anti–double cortin (DCX) C-18 (1:500, Santa Cruz); and anti-rat fluorescein isothiocyanate, anti-goat rhodamine X, and anti-mouse CY5 (Jackson ImmunoResearch). Free-floating sections were treated with 0.6% H2O2 in Tris-buffered saline (TBS = 0.15 mol/L NaCl, 0.1 mol/L Tris-HCl; pH 7.5) for 30 minutes. BrdU-labeled nuclei were detected by DNA denaturation steps after a 2-hour incubation in 50% formamide/2× standard saline citrate (2× standard saline citrate is 0.3 mol/L NaCl and 0.03 mol/L sodium citrate) at 65°C, a 5-minute rinse in 2× standard saline citrate, a 30-minute incubation in 2 mol/L HCl at 37°C, and a 10-minute rinse in 0.1 mol/L boric acid, pH 8.5. Incubation in TBS/0.1% Triton X-100/3% normal donkey serum for 30 minutes was followed by incubation with primary antibodies (48 hours, 4°C). After being washed in TBS/0.1% Triton X-100/5% normal donkey serum, sections were incubated with secondary antibodies for 2 hours, washed in TBS, and mounted on glass slides. Fluorescence was detected by confocal scanning laser microscopy (Leica).

**Histological Quantification**

A systematic random-counting procedure was used as previously described. The dentate gyrus was analyzed on sections adjacent to the ventricle wall for the striatum.

**Data and Statistical Analysis**

Values are presented as mean ± SEM. Thionin/basic fuchsin-stained slices (400-μm interval) were obtained from the infarcted region. The slice with the largest length, diameter, and height was used for infarct volume calculation (see supplemental Figure I, available online at http://stroke.ahajournals.org). Infarct volumes were calculated from the equation for ellipsoids: 4/3πr1r2r3, where r1 is 1/2 the length, r2 is 1/2 the diameter, and r3 is 1/2 the height. A 2-tailed t test was used to determine significant differences in infarct volumes. Sensorimotor measurements were analyzed with a 1-way ANOVA or Kruskal-Wallis test for each time point. The area under the curve (AUC) was analyzed by 1-way ANOVA and a post hoc Duncan’s test. One-way ANOVA and post hoc Tukey’s test were used to determine the statistical significance of differences in physiological parameters and neurogenesis. Statistical calculations and analyses were done with NCSS statistical software and Prism 4 (Graphpad Inc). A probability value <0.05 was considered significant.

**Results**

**Peripheral Application of BDNF Improves Functional Outcome After Cerebral Ischemia**

Systemic BDNF treatment (20 μg IV for 5 postischemic days) in the present study was tailored to enhance poststroke recovery without affecting infarct size, and indeed, infarct volumes between BDNF-treated animals and controls were not statistically different (27.7 ± 9.5 versus 37.8 ± 9.4 mm³; see supplemental Figure I). Physiological parameters (rectal temperature, pH, PCO2, PO2, and mean arterial pressure during surgery; see supplemental Table I, available online at http://stroke.ahajournals.org) as well as body weight and mortality (no animals died during the experiment) were not different between the groups.

Sensorimotor deficits were statistically significant in vehicle-treated ischemic animals (lesion + vehicle) compared with sham-operated rats ( sham + vehicle) in the Rotorod test, adhesive tape removal test, and NSS for up to 6 weeks after the insult (Figure 1, a–d; compare yellow versus blue). BDNF-treated ischemic rats ( lesion + BDNF) performed significantly better than did vehicle-treated animals in the overall NSS as well as in the adhesive tape removal test by comparing group means per time point (Figure 1, b–d, green versus yellow curves; P < 0.05 by 1-way ANOVA, Kruskal-Wallis test). By analyzing the even more appropriate time series measurements in subjects (comparison of individual AUCs over time; see Figure 1, a–d, bar graphs), the data demonstrated a robust improvement with BDNF treatment versus vehicle in all test paradigms, including the Rotorod test (Figure 1, a–d, green versus yellow bars; P < 0.01, AUC and Duncan’s test). Interestingly, the treatment effect was most pronounced in the adhesive tape removal paradigm on the affected, ie, paretic, left forepaw, consisting of a substantial sensory component in contrast to the motor function–focused NSS (compare Figures 1c versus 1b; P < 0.0001, AUC and Duncan’s test). No difference was noted between sham + vehicle and sham + BDNF treatment (data not shown).
Cortical Ischemia Stimulates Recruitment of Neuronal Progenitor Cells From the SVZ Toward the Lesion Site, but BDNF Fails to Increase SVZ Progenitor Cell Recruitment to the Cortical Lesion Site

In intact animals, migration of neuronal progenitor cells originating in the SVZ was restricted to chain migration toward the olfactory bulb (Figure 2a), with few cells oriented toward the cortex. In the corpus callosum of sham vehicle animals, we very rarely detected DCX-positive cells (Figure 2b) and no BrdU/NeuN double-positive cells. We observed a trend toward more DCX-positive cells passing through the corpus callosum in lesion+vehicle and lesion+BDNF animals, not significant due to the high intragroup variability (Figure 2b; ANOVA F(3,20)=0.87, P=0.47). In ischemic animals, the cortical area between the lesion site and the

Figure 1. BDNF treatment improves long-term functional outcome after cortical ischemia. a, BDNF-treated ischemic animals had better running function compared with controls (green bar vs yellow bar). b, BDNF-treated ischemic animals (green bar and line) displayed better motor performance (NSS including beam balance) compared with controls (yellow bar and line). c and d, BDNF-treated ischemic animals (green bar and line) displayed better sensorimotor function (adhesive tape removal test) compared with controls (yellow bar and line). Line graphs are group comparisons over time (1-way ANOVA, post hoc Kruskal-Wallis test, *P<0.05). Bar graphs are comparison for each rat over time per group (AUC, post hoc Duncan’s test, *P<0.01). Yellow indicates ischemia+vehicle; green, ischemia+BDNF; and blue, sham-lesion+vehicle.

Figure 2. Cortical ischemia stimulates recruitment of neuronal progenitor cells from the SVZ toward the lesion. a, DCX-positive cells leave the rostral migratory stream (rms) to penetrate the corpus callosum (cc) toward the overlying neocortex. Green indicates DCX; red , NeuN. Scale bar in a=25 μm. b, Quantification of DCX-positive cells. Cell counts represent 1000 cells/μm²; mean±SEM.
ventricle wall displayed a substantial amount of DCX-positive progenitors (Figure 3a), whereas no DCX-positive cells were present in the corresponding cortical region of sham-vehicle or sham-BDNF animals (data not shown). The area and intensity of DCX immunoreactivity in lesion/vehicle animals were comparable to those in lesion/BDNF animals (Figures 3c and 3d; t test $t=0.4135$, $df=10$, $P=0.68$ for area and $t=0.3566$, $df=10$, $P=0.73$ for intensity). Using confocal microscopy and 3-dimensional analysis, we found no incorporation of BrdU into NeuN-expressing cells. Instead, BrdU-positive nuclei were frequently detected in very close proximity but were distinctly different from neuronal cell bodies, indicative of newly formed satellite cells (Figure 4d). As an indicator of beginning neuronal differentiation of DCX-positive cells, we frequently detected the coexpression of DCX and NeuN in cells surrounding the lesion site and in the striatum (Figure 4, a–e).

**BDNF Increases SVZ Progenitor Cell Migration Into the Ipsilateral Striatum After Cortical Stroke**

A significant increase in the density of BrdU-positive cells in the striatum of lesion+vehicle and lesion+BDNF animals compared with sham-lesioned animals (Figure 5c; ANOVA $P<0.001$, $F_{3,20}=8.5$, Tukey’s post hoc test: sham+vehicle versus lesion+vehicle $q=5.8$, $P<0.01$; sham+vehicle versus lesion+BDNF $q=4.0$, $P<0.05$) was observed, but no difference between lesion+vehicle and lesion+BDNF was found. More important, however, the number of DCX-positive neuronal progenitor cells in the striatum was increased only in lesion+BDNF animals (Figure 5d, ANOVA $P<0.001$, $F_{3,20}=8.5$, Tukey’s post hoc test: sham+vehicle versus lesion+BDNF $q=6.6$, $P<0.001$; sham+BDNF versus lesion+BDNF $q=5.7$, $P<0.01$; lesion+vehicle versus lesion+BDNF $q=7.2$, $P<0.001$; all other comparisons $P=NS$). Similar to the situation in the cortex, we observed colabeling of DCX with NeuN in the striatum, indicating that neuronal progenitor cells began to express mature neuronal markers at 6 weeks after lesion induction (Figure 4e). Although present, BrdU/NeuN double-positive cells were so infrequently detected that quantification of this observation was not possible because of low counts (total of 8 BrdU/NeuN cells in all animals).

**Hippocampal Neurogenesis Is Increased in BDNF-Treated Animals After Cortical Stroke**

First we analyzed the total number of BrdU-positive cells in the hippocampus, which represents all new cells including neurons, astrocytes, oligodendrocytes, microglia, and macrophages. Animals subjected to cortical photothrombosis had an
increased number of BrdU-positive cells in the hippocampus of the infarcted side (Figure 6d; ANOVA \( P<0.001, F_{3,20}=9.7 \), Tukey’s post hoc test: sham + vehicle versus lesion + vehicle \( q=5.8, P<0.01 \)). However, there was no statistically significant increase in the number of new cells due to BDNF treatment under control or lesion conditions (Figure 6d; ANOVA \( P<0.001, F_{3,20}=9.7 \), Tukey’s post hoc test: sham + vehicle versus sham + BDNF \( q=2.38, P=\text{NS} \) and lesion + vehicle versus lesion + BDNF \( q=0.83, P=\text{NS} \)).

We determined the neuronal fraction among the new cells (BrdU/NeuN double-positive) by confocal micros-
copy. Compared with all other groups, only the lesion+vehicle animals had a significantly smaller fraction of neurons (38% for lesion+vehicle versus 78% for sham+vehicle, 67% for sham+BDNF, and 64% for lesion+BDNF; ANOVA $F_{(3,20)}=11.9$, Tukey’s post hoc test: sham+vehicle versus lesion+vehicle $q=7.5$, $P<0.001$; sham+BDNF versus lesion+vehicle $q=7.0$, $P<0.01$; lesion+vehicle versus lesion+BDNF $q=5.2$, $P<0.01$; all other comparisons $P=NS$).

To compare whether the total amount of hippocampal neurogenesis was altered, the number of BrdU-positive cells was multiplied by the percentage of NeuN/BrdU double-positive cells and expressed as the total number of new neurons per dentate gyrus (Figure 6e). BDNF treatment after lesion induction significantly increased hippocampal neurogenesis compared with all other groups (Figure 6e; ANOVA $F_{(3,20)}=6.98$, $P=0.002$; Tukey’s post hoc sham+vehicle versus lesion+BDNF $q=6.3$, $P<0.01$; sham+BDNF versus lesion+BDNF $q=4.3$, $P<0.05$; lesion+vehicle versus lesion+BDNF $q=4.1$, $P<0.05$; all other comparisons $P=NS$). However, ischemia alone or BDNF alone was insufficient to increase the level of neurogenesis above control levels (Figure 6e).

**Discussion**

The treatment paradigm and dose of BDNF in the present study were chosen on the basis of previous evidence, with the idea of allowing brain penetration by the growth factor after systemic application, leading to enhanced poststroke recovery without changing stroke size. Therefore, BDNF was administered as a low-dose intravenous infusion (20-µg repetitive bolus on 5 consecutive days) that should exert no protective effects. This procedure indeed left infarct volumes of BDNF-treated animals and controls unchanged, whereas poststroke sensorimotor function was enhanced. Sensorimotor function gradually improved from weeks 3 to 6 in the Rotorod test (running function), the NSS (walking and balancing function, reflexes) as well as in the adhesive tape removal test (sensory awareness of the hand, grip function). These results clearly suggest a true recovery-enhancing effect for BDNF.

Blood-brain barrier (BBB) penetration by BDNF was beyond the scope of the present study, because passage of BDNF through the intact BBB is a well-characterized phenomenon. A much smaller dose of intravenously injected $^{125}$I-BDNF (2 ng compared with 20 µg as single bolus in our study) had an early and rapid influx into the brain. Most of this BDNF was later (10 minutes) associated with the brain parenchyma of the cortex, demonstrating complete passage across the BBB. BDNF influx into the brain is likely even further augmented by the photothermotic stroke model, which leads to rapid disruption of the BBB that persists for days.

When analyzing the distribution of DCX-expressing progenitor cells in the ventricle wall and the overlying corpus callosum and cortex, we found robust recruitment of progenitor cells into the peri-ischemic area of the neocortex. This observation is in accordance with other studies describing the migration of neuronal progenitor cells from the lateral ventricle wall in response to a cortical lesion. BDNF treatment, however, failed to further increase the recruitment of DCX-positive cells into the ischemic cortex. Although a large number of DCX-positive cells were detected in the immediate periphery of the ischemic lesion, the colabeling of BrdU with

![Figure 6. BDNF treatment increases neurogenesis in the dentate gyrus of the hippocampus.](image)
a marker for mature neurons (NeuN) analyzed by confocal microscopy did not indicate differentiation of progenitor cells into cortical neurons. Induction of neurogenesis in the lesioned cortex was reported only by Magavi and colleagues, who used a very selective apoptotic elimination model of individual cortical neurons. Among the possible reasons for our inability to detect cortical neurogenesis are (1) the lesion model, because Arvidsson and colleagues, using the middle cerebral artery occlusion stroke model, were also unable to detect cortical neurogenesis, and (2) the possibility that the ventricle wall produces new neurons for the cortex not during the first 5 days after lesion but rather at a later time. However, we frequently observed that DCX-positive cells were labeled with NeuN (Figure 4), a characteristic that indicates that these cells progressed from a migrating to a differentiating progenitor cell stage. Whether these cells fully differentiate into neurons requires additional experiments with a delayed BrdU labeling paradigm.

In contrast to the neocortex, neurogenesis in the striatum is a well-described though relatively rare phenomenon. As shown here, peripheral delivery of BDNF increased SVZ progenitor cell migration to the ipsilateral striatum after cortical stroke, which corresponds to previous findings that intrathecal infusion or viral vector–mediated delivery of BDNF increased the number of striatal progenitor cells. Our lesion paradigm produced no BrdU/NeuN-positive cells in the striatum, although we observed DCX/NeuN double labeling.

A prominent effect of BDNF on neurogenesis was seen in the dentate gyrus. Though distant from the site of stroke, this region typically responds to ischemia with induction of neurogenesis through glutamatergic stimulation. This excitation-neurogenesis coupling hypothesis suggests that adult hippocampal neural stem cells/progenitor cells possess their activity-sensing capability by acting in part via N-methyl-D-aspartate receptors to adapt the adult neural network both to physiological demands and to pathological insults. In our study, BDNF, which is endogenously expressed by granule cells of the hippocampus, significantly increased the number of newly generated granule cells in ischemic brains. Both ischemia alone and combined ischemia/BDNF treatment increased the number of newly generated cells (Figure 6d); however, under ischemic conditions, a significant reduction in the neuronal portion of the newly generated cells was observed, leading to only slightly elevated hippocampal neurogenesis levels after cortical stroke (Figure 6e). BDNF, on the other hand, reversed this lesion effect on neurogenesis by increasing the neuronal differentiation of new cells (Figure 6f). Direct intrahippocampal delivery of high doses of BDNF (12 or 36 \( \mu \)g/d), however, has been reported to stimulate the formation of newly generated cells in the unlesioned dentate gyrus. This finding is supported by similar results in BDNF-deficient mice. In the present study, BDNF failed to increase neurogenesis in the intact dentate gyrus of sham-lesioned and nonischemic animals, possibly due to the relatively low (20 \( \mu \)g/d) and systemically (intravenous) administered dose of BDNF.

The question remains how BDNF-induced neurogenesis in the dentate gyrus corresponds to improved sensorimotor behavior after cortical ischemia. It is well known that BDNF interacts significantly with acute stroke pathophysiology and has particularly potent antiapoptotic functions. For example, a reduction in BDNF’s endogenous biological activity by replacement of the coding sequence in a single allele of the BDNF gene resulted in markedly enlarged infarctions. After hypoxia and/or ischemia, BDNF was shown to induce the extracellular signal–regulated kinase signaling pathway, to counteract proapoptotic caspase 3 and bax, and to upregulate the antiapoptotic bcl-2. BDNF may, however, not unani-

ously exert neuroprotective or antiapoptotic effects in the hippocyn and/or ischemic brain as observed in a cardiac arrest study in rats. Although it cannot be excluded that BDNF-induced prevention of apoptosis may have improved poststroke recovery, similar infarct sizes in BDNF-treated animals and controls makes this primary mechanism quite unlikely. More important in this context are BDNF’s well-known stimulatory effects on neuroplasticity at several levels, including axonal growth, dendritic branching, and synaptic transmission. BDNF treatment after stroke, for instance, enhances MAP1B expression as a marker of axonal growth in the ischemic border zone and synaptophysin expression as a marker of synaptogenesis in the contralateral cortex. Thus, BDNF’s strong trophic activity after stroke likely contributed to enhancement of sensorimotor recovery, independent of the possible involvement of hippocampal neurogenesis. Nevertheless, there are also data supporting a possible role for hippocampal structures in functional recovery from motor deficits. The hippocampus produces a rhythmic slow-wave activity, which is involved in sensorimotor integration and movement initiation that can be disrupted by cortical lesions. Preservation of sensorimotor integration after cortical stroke as measured here by the adhesive tape removal test could therefore be related to hippocampal function. Indeed, BDNF’s treatment effects were particularly pronounced in this test paradigm (see Figures 1c and 1d). A possible connection between the motor system and hippocampal neurogenesis is further supported by the finding that voluntary exercise is a strong activator of neurogenesis and hippocampal upregulation of BDNF in mice. However, the causal relation would be reversed: ie, improved motor function leads to more neurogenesis and not vice versa.

Previous reports as well as our current study are limited by the fact that enhancement of poststroke neurogenesis is so far just correlative to an improvement in sensorimotor and cognitive recovery. Currently unavailable experiments are needed to specifically impair hippocampal neurogenesis to dissociate neurogenesis effects from other structural plasticity effects during poststroke functional recovery.

Summary
We have demonstrated that systemic application of BDNF during the first 5 days after cortical stroke induces hippocampal neurogenesis and improves functional recovery during a 6-week period in several sensorimotor tasks. These findings consolidate the role of BDNF as a modulator of neurogenesis...
in the brain and as an enhancer of long-term functional neurological outcome after cerebral ischemia. Our study could influence poststroke recovery trials and calls for further studies that specifically target trophic factor–enhanced poststroke physical therapy.

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Disclosures
None.

References
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