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| Original Article |

Induction of Striatal Regeneration Delays Motor Deterioration in a Mouse Model of Huntington's Disease

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Abstract : Intraventricular administration of brain-derived neurotrophic factor (BDNF) can induce striatal neurogenesis. Epidermal growth factor (EGF), by expanding the mitotic pool of neural stem/progenitor cells in the subventricular zone (SVZ) responsive to neuronal instruction by BDNF, can potentiate this process. The objective of this study was to investigate the induction of striatal regeneration and consequent functional benefits after chronic infusion of BDNF and EGF in a R6/2 transgenic mouse model of Huntington's disease (HD). At 6 weeks of age, the mice were randomly assigned to groups receiving a continuous 2-week infusion of one of the following treatments into the ventricle: combination of BDNF and EGF (B/E), BDNF, EGF, or phosphate buffered saline (PBS). Two weeks after treatment, the B/E-treated mice revealed a significant increase of new neurons co-stained with BrdU and βIII-tubulin in the ventricular side of neostriata (VZ~300 μm), compared with PBS controls. The newly generated cells were also expressed as migrating neuroblasts co-labeled with doublecortin or PSA-NCAM in the SVZ. The survival rates of the new neurons were in the range of 30~50% at 6 weeks after treatment. For behavioral assessments, the B/E combination therapy group showed a significant delay in motor deterioration relative to PBS controls in both constant and accelerating rotarod as well as locomotor activity test 6 weeks after treatment. However, administration of BDNF alone did not exhibit significant delays in motor deterioration in most of behavioral assessments. Neither did motor performance improve in R6/2 mice treated only with EGF. In conclusion, induction of striatal regeneration by the intraventricular administration of BDNF and EGF delayed disease progression in HD. Therefore, this treatment may offer a promising strategy for restoration of motor function in HD.

Key words: *brain-derived neurotrophic factor, epidermal growth factor, neurogenesis, Huntington's disease*

1. Introduction

It has been reported that stem cells and progenitor cells are present not only in the embryonic state but also in the subventricular zone (SVZ) and hippocampus of the adult brain.[1] Although the capacity of these cells themselves for repairing damaged tissue is largely limited, adult neurogenesis may be enhanced by brain-derived neurotrophic factor (BDNF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), and other growth factors.[2-5] Namely, the newly generated neurons can migrate from SVZ cells towards the olfactory bulb, and the recruitment of neurons into the striatum may be increased by administration of BDNF.[6-9] The fact that stem/progenitor cells are present in the adult brain and the production of new neurons occurs in specific sites suggests the possibility for the treatment of incurable neurological diseases in the future. Nevertheless, it has been reported that the new neurons are not sufficient to exhibit functional recovery, or that they could not survive for a long time even in the presence of BDNF.[10,11] Therefore, in this study, it was attempted to maximally increase BDNF-responsive subventricular stem/progenitor cells, and to accelerate the induction of striatal neurogenesis by combined administration of EGF to the ventricle.

The degenerative loss of medium spiny neurons in the striatum

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is the primary finding observed in Huntington's disease (HD).[12] HD is an autosomal dominant disease that expanded CAG repeats cause the production of mutant huntingtin protein which can be neurotoxic.[13] Specifically, it is a neurodegenerative disorder that striatal neurons are gradually lost, resulting in involuntary movements such as chorea and the problem of motor coordination.[12] For the treatment of HD, administration of creatine,[14-16] cystamine,[17,18] coenzyme Q10,[19] pyruvate,[20] lithium,[21] minocycline[22,23] and histone deacetylase inhibitors,[24,25] and stem cell transplant[26] have been attempted. However, an effective treatment has been still not available. Nevertheless, the theory that wild-type huntingtin plays a certain role in corticostriatal transport of BDNF was recently proposed.[27,28] The BDNF also regulates neurogenesis in addition to promoting neuroprotection.[29] Thus, administration of exogenous BDNF or other growth factors in combination may be a good therapeutic approach to prevent the progression of HD.[30]

The newly generated cells in SVZ can migrate into the affected striatum and differentiate to medium spiny neurons in HD.[31] In response to the degeneration that occurs in the neostriatum in HD, the SVZ increases production of progenitor cells that migrate towards the site of the damage, where they can differentiate into mature neurons.[32] To enhance the process, among multiple approaches to exogenous trophic factor delivery, experimental studies have employed continuous infusion via an osmotic mini-pump.[33-35] As mentioned above, if intraventricular administration of BDNF and/or EGF induces striatal neurogenesis, it is considered that the newly generated neurons can delay the symptoms of HD. Therefore, we investigated that BDNF and/or EGF administered alone or in combination has a therapeutic effect on the functional outcomes of HD.

2. Materials and Methods

2.1 Animal

As a transgenic mouse model of HD, the R6/2 model (B6CBA-Tg(HDexon1)62Gpb/1J) that approximately 145 copies of CAG repeats were inserted exon 1 of the human HD gene was used. The breeding pairs of the R6/2 model were purchased from the Jackson Laboratory (Bar Harbor, ME), and the subjects were produced by breeding ovarian transplant hemizygote females with B6CBAF1/J males. The animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) under the Animal Protection Regulation with the 12-hour light/dark cycle.

2.2 Genotyping

The confirmation of HD was determined by polymerase chain reaction (PCR) of tail-tip DNA. In other words, approximately 1-2 mm tail tip of mice was cut at 4 weeks after birth, and DNA extraction was performed in proteinase K mixture solution at 55°C overnight. PCR reactions were performed in the presence of forward primer CCGCTCAGGTTCTGCTTTTA and reverse primer GGCTGAGGA AGCTGAGGAG at 94°C for 3 minutes, 35 cycles × (94°C for 30 seconds, 58°C for 1 minute, and 72°C for 1 minute), 72°C for 2 minutes, and 4°C afterward. R6/2 transgenic mice were confirmed by performing electrophoresis on 1.2 % agarose gel and detecting 611 bp bands (Fig 1A).

2.3 Experimental Design

For behavioral testing, at 6 weeks of age, 76 HD mice were randomly assigned to receive a continuous 2-week intraventricular

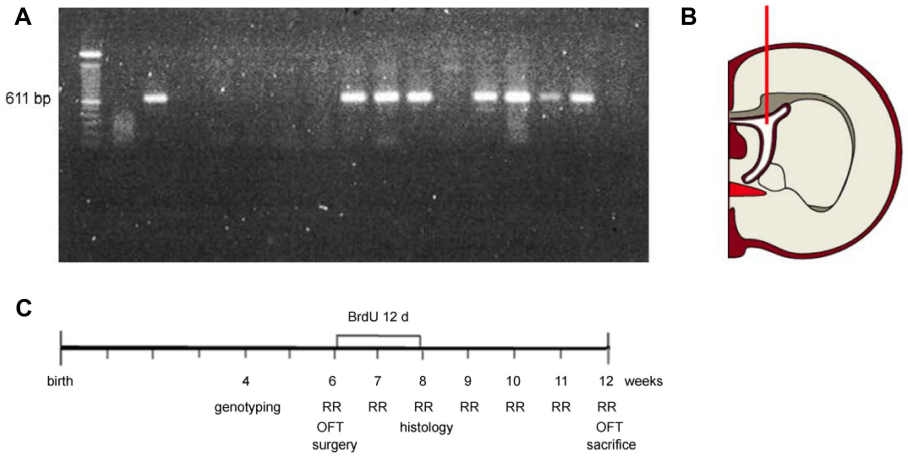

Figure 1. Genotyping and experimental design. Huntington's disease was confirmed by detecting 611 bp bands (A) At 6 weeks of age, intraventricular administration of BDNF and/or EGF was performed as shown in the above schematic figure (B) Experimental schedule was described from birth to 12 weeks of age (C) BrdU: 5-bromo-2-deoxyuridine, RR: rotarod, OFT: open-field test.

infusion of one of the following four treatments: a combination of BDNF and EGF (B/E), BDNF, EGF, or phosphate buffered saline (PBS) (n = 19 each). BDNF and EGF were each infused at a concentration of 1 μg/ml using an Alzet micro-osmotic pump (model 1002; 0.25 μl/hr infusion rate, 100 μl volume; Durect). The infusion cannula (Brain Infusion Kit 3) was inserted using stereotaxic coordinates (AP -0.5 mm from Bregma; ML -0.7 mm from Bregma; DV -2.0 mm from dura) to the lateral ventricle (Fig 1B), and the osmotic pump connected to this was inserted into the dorsal subcutaneous tissue. In the other set of immunohistochemistry, 2 weeks after surgery, newborn neurons were evaluated in the neostriatum of HD and WT subjects (n = 5/group). The schematic timeline of this experiment from birth to 12 weeks of age is provided in Fig 1C.

2.4 Behavioral Assessment

All subjects were assessed at 6 weeks of age as pre-operative evaluation. To evaluate motor coordination, rotarod test was performed at one week interval from 6 weeks to 12 weeks of age using a rotarod treadmill (Cat No. 47600, UGO Basile, VA, Italy). The latency when animals fall from the rod was measured at the constant speed of 12 rpm and at the accelerating speed from 4 rpm to 40 rpm for 5 min. In each trial, it was performed 3 times, and their mean latency was analyzed. For open-field test, locomotor activity was also assessed at 2-week intervals using a 41×41×33 cm activity cage (Cat No. 7420, UGO Basile, VA, Italy). The activity was automatically measured by the number of the beams a mouse crossed in the activity cage consisting of 2 pairs of arrays emitting and sensing 16 infrared beams.

2.5 Immunohistochemistry

Mice were given an intraperitoneal injection of 5-bromo-2-deoxyuridine (BrdU; 50 mg/kg, Sigma-Aldrich) once per day for 12 days, beginning 1 day after stereotaxic surgery. Two weeks after chronic infusion, new neurons were evaluated in the neostriatum of HD and WT mice (n = 5/group). Briefly, the animals were sacrificed, given an intracardiac perfusion of 4% paraformaldehyde, and the brain tissues were harvested. They were frozen and cryosectioned at 16-μm intervals, and immunostaining was performed on six sections over a range of 256 μm. Sections were stained with the cell proliferation marker BrdU and the neuronal marker βIII-tubulin (1:400, Covance, NJ), and the double-labeled cells of $BrdU^+/\beta III\text{-}tubulin^+$ were assessed by confocal imaging. The number of newly generated neurons was evaluated at intervals of 300 μm from the ventricular zone (VZ) to evaluate the distribution of new neurons in the neostriatum. The area of the neostriatum were obtained using the MetaMorph Imaging System (Molecular Device, Sunnyvale, CA), converted to the volume (area×16 μm), and quantified as the density (/mm^3). They were also immunostained with BrdU and doublecortin (DCx; 1:400, Chemicon), or PSA-NCAM (1:400, Sigma-Aldrich) to identify migrating neuroblasts. In addition, the survival rates of the newly generated cells were evaluated at 6 weeks after infusion in another cohort of HD and WT mice (n = 5/group).

2.6 Statistical Analysis

The effect of BDNF and/or EGF on the generation of new neurons and the functional outcomes after the intraventricular infusion was evaluated for each group. Namely, the numbers of $BrdU^+$ or $BrdU^+/\beta III\text{-}tubulin^+$ cells (/mm^3) in the neostriatum were analyzed as a function of distribution from the VZ by one-way ANOVA followed by post hoc Bonferroni comparison using SPSS. In addition, the findings of the delay in functional deterioration by striatal regeneration or neuroprotective effect of BDNF itself were examined. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Rotarod Performance

The mice consist of the four groups as a function of administrating the BDNF and/or EGF at 6 weeks of age; B/E, BDNF, EGF, and PBS. The rotarod performance prior to surgery of each group was not significantly different. However, when the rotarod test was performed weekly, the B/E combination therapy group started to show a significant delay in motor deterioration in comparison with the PBS group 6 weeks after surgery (12 weeks of age) at both 1 min constant rorarod (Fig 2a) and at 5 min constant rotarod (Fig 2B) ($p < 0.05$ by one-way ANOVA with posthoc Bonferroni test). The mice treated with BDNF alone also showed a significantly longer latency at 12 weeks of age, compared with the PBS group at 1 min constant rotarod ($p < 0.05$) (Fig 2A). At the accelerating rotarod, significant treatment effects were shown in the B/E combination therapy group in comparison with the EGF group post-treatment 6 weeks (12 weeks of age) as well as the PBS group post-treatment 5-6 weeks (11-12 weeks of age) ($p < 0.05$) (Fig 2C).

3.2 Locomotor Activity

When open-field locomotor activity was evaluated for 10 min or 60 min, the activity of each group was not significantly different before treatment. In addition, when evaluation was limited to the initial 10 min, statistical differences were not shown among the R6/2 HD subjects, although the B/E-treated mice exhibited a higher tendency of beam crossing incidences

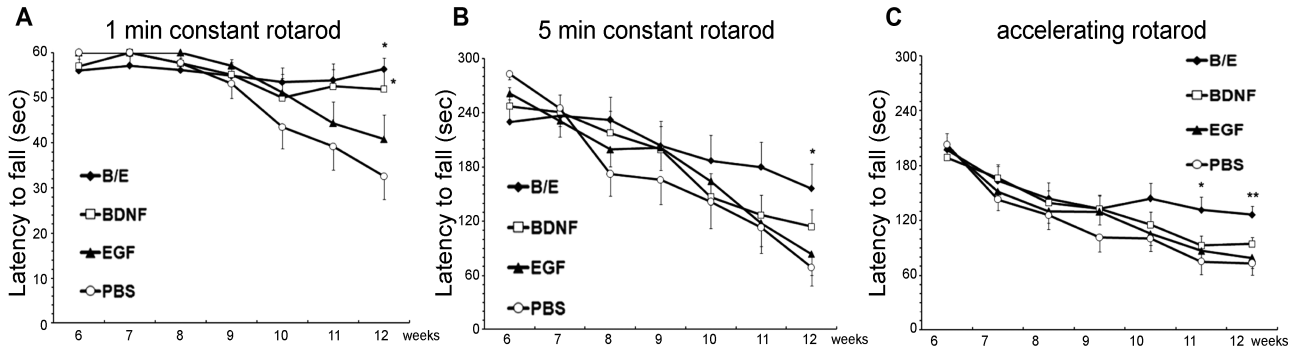

Figure 2. Rotarod performance. At 1-min constant rotarod (A), the B/E and BDNF group significantly delayed motor deterioration compared with PBS controls post-treatment 6 weeks (12 weeks of age). At 5-min constant rotarod (B), mean rotarod latency was significantly sustained post-treatment 6 weeks after intraventricular infusion of BDNF and EGF. At accelerating rotarod (C), the B/E-treated mice showed a significant delay in motor deterioration 5-6 weeks after treatment. *$p < 0.05$ compared with PBS controls. **$p < 0.05$ compared with EGF and PBS group. B/E: combination of BDNF and EGF, BDNF: brain-derived neurotrophic factor, EGF: epidermal growth factor, PBS: phosphate-buffered saline.

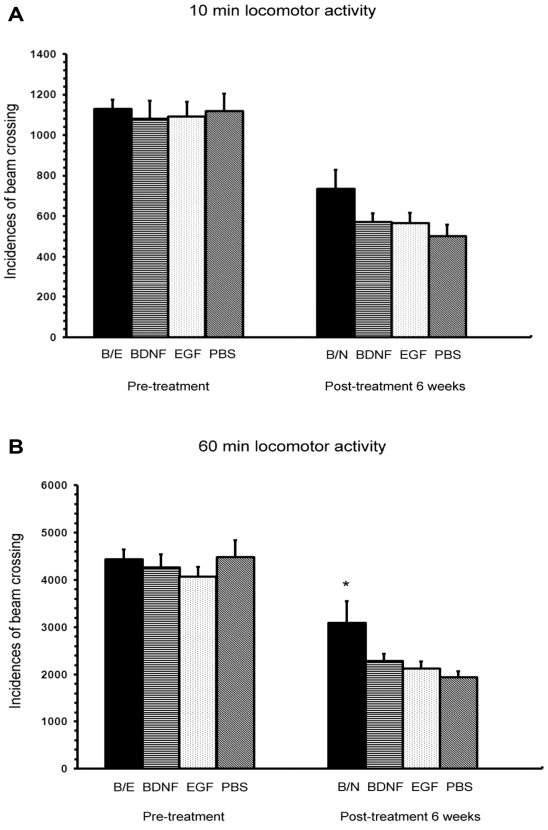

Figure 3. Open field activity test in the 10-min locomotor activity test (A), statistical differences were not shown among the subjects. in the 60-min locomotor activity test (B), the B/E combination therapy group significantly delayed activity deterioration compared with PBS controls post-treatment 6 weeks (12 weeks of age). *$p < 0.05$ compared with PBS controls. B/E: combination of BDNF and EGF, BDNF: brain-derived neurotrophic factor, EGF: epidermal growth factor, PBS: phosphate-buffered saline.

6 weeks after surgery (12 weeks of age) (Fig 3A). However, in the locomotor activity test for total 60 min, the B/E combination therapy group showed a significant delay in activity deterioration in comparison with the PBS controls at 12 weeks of age ($p < 0.05$ by one-way ANOVA with posthoc Bonferroni test) (Fig 3B).

3.3 Immunohistochemistry

The ability of BDNF combined with EGF to induce striatal neurogenesis from endogenous neural stem cells in the SVZ was assessed immunohistologically by counting the number of newly generated neurons co-labeled with BrdU and βIII-tubulin in WT and HD mice after 2 weeks of chronic infusion (Fig 4A,B). As described previously,[24] EGF could potentiate striatal neurogenesis by expanding the mitotic pool of SVZ cells responsive to neuronal instruction by BDNF. In addition, we found that the newly generated cells were expressed as migrating neuroblasts labeled with DCx or PSA-NCAM in the SVZ and the ventricular side of neostriatum (Fig 4C,D). When the new neurons were evaluated at 6 weeks after infusion, their survival rates were in the range of 30~50%, suggesting that more than a half of the newly generated neurons might be prone to die (Table 1). Particularly, both WT and R6/2 transgenic HD mice treated with B/E showed a significant induction of striatal neurogenesis in the ventricular side of neostriatum (VZ~300 μm) compared with PBS controls ($p < 0.05$ by one-way ANOVA with posthoc Bonferroni test) (Fig 4E). In other words, approximately 50~80% of the new neurons were distributed in the area within 300 μm from VZ (Table 2). However, striatal neurogenesis did not show a statistical difference among the groups in the other area of neostriatum

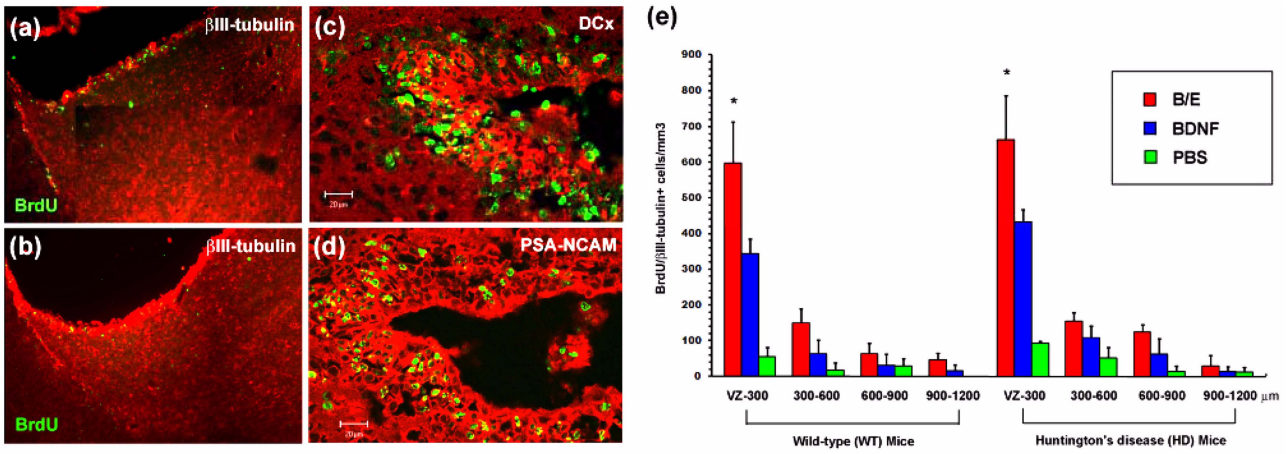

Figure 4. Induction of striatal neurogenesis. HD mice treated with B/E (A) showed more striatal recruitment of new neurons stained with $BrdU^+$/βIII-$tubulin^+$ than PBS controls (B). The newly generated neurons were expressed as migrating neuroblasts labeled with DCx^+ (C) or $PSA\text{-}NCAM^+$ (D) in the SVZ. Both WT and R6/2 transgenic mice treated with B/E exhibited a significant induction of striatal neurogenesis in the ventricular side of neostriatum (VZ~300 μm) compared with PBS controls (E). *$p < 0.05$ compared with PBS controls. BrdU: 5-bromo-2-deoxyuridine, DCx: doublecortin, B/E: combination of BDNF and EGF, BDNF: brain-derived neurotrophic factor, PBS: phosphate-buffered saline. Scale bars: 20 μm (C, D).

Table 1. Newly generated striatal neurons and survival rates as a function of disease and treatment

	Treatment	2 weeks after infusion	6 weeks after infusion	Survival rate
Volume ($\times 10^{-3} mm^3$)				
WT mice	B/E	50.7±0.7	46.6±4.1	-
	BDNF	53.8±1.8	51.1±2.6	-
	PBS	49.6±1.3	52.2±2.4	-
HD mice	B/E	42.5±1.9	36.2±2.0	-
	BDNF	47.0±4.6	38.5±2.4	-
	PBS	44.3±3.9	33.9±5.7	-
$BrdU^+$ cells/mm^3				
WT mice	B/E	2062±148*	1581±114*	75.9%
	BDNF	1571±229	1097±116*	69.8%
	PBS	965±126	497±82	51.3%
HD mice	B/E	2184±197*	1909±271*	78.3%
	BDNF	1735±69	1350±52*	77.3%
	PBS	1389±47	770±89	55.4%
$BrdU^+$/βIII-$tubulin^+$cells/mm^3				
WT mice	B/E	211±40*	90±23*	42.3%
	BDNF	114±25	44±8	38.1%
	PBS	25±10	7±5	30.1%
HD mice	B/E	260±64*	123±24*	49.9%
	BDNF	156±14	63±11	40.7%
	PBS	43±8	14±6	33.7%

Values are mean±S.E. *$p < 0.05$ compared with PBS controls.
VZ: ventricular zone, WT: wild-type, HD: Huntington's disease, B/E: combination of BDNF and EGF, BDNF: brain-derived neurotrophic factor, PBS: phosphate-buffered saline, BrdU: 5-bromo-2-deoxyuridine

Table 2. Distribution of newly generated striatal neurons according to the distance from ventricular zone

	From VZ	~ 300 μm	300~600 μm	600~900 μm	900~1200 μm
Volume ($\times 10^{-3}mm^3$)					
WT mice	B/E	12.5±0.4 (24.7%)	13.0±0.3 (25.5%)	12.6±0.4 (24.8%)	12.7±0.3 (25.0%)
	BDNF	13.8±1.0 (25.6%)	13.4±1.0 (24.9%)	12.8±0.3 (23.8%)	13.9±1.0 (25.8%)
	PBS	11.6±0.4 (23.3%)	12.7±0.5 (25.5%)	12.5±0.8 (25.1%)	12.9±0.2 (26.1%)
HD mice	B/E	10.0±0.8 (23.5%)	10.3±0.5 (24.3%)	11.2±0.5 (26.3%)	11.0±0.9 (26.0%)
	BDNF	11.9±1.1 (25.4%)	12.4±1.2 (26.4%)	10.4±0.9 (22.2%)	12.2±1.2 (26.0%)
	PBS	11.0±0.8 (24.8%)	11.0±0.6 (24.7%)	11.3±0.7 (25.5%)	11.0±1.5 (24.9%)
$BrdU^+$ cells/mm^3					
WT mice	B/E	4579±640* (54.8%)	1633±308 (19.5%)	1185±264 (14.2%)	966±255 (11.6%)
	BDNF	3697±389 (59.4%)	996±304 (16.0%)	851±219 (13.7%)	680±198 (10.9%)
	PBS	2537±479 (63.1%)	637±72 (15.9%)	506±112 (12.6%)	339±43 (8.4%)
HD mice	B/E	4786±330* (55.6%)	1714±140 (19.9%)	1143±212 (13.3%)	969±68 (11.3%)
	BDNF	4263±365 (61.8%)	1239±302 (17.9%)	713±115 (10.3%)	687±137 (10.0%)
	PBS	3403±103 (60.9%)	972±110 (17.4%)	577±107 (10.3%)	631±102 (11.3%)
$BrdU^+$/βIII-tubulin$^+$cells/mm^3					
WT mice	B/E	598±114* (69.7%)	150±39 (17.5%)	64±28 (7.4%)	46±19 (5.4%)
	BDNF	343±42 (75.7%)	63±39 (13.9%)	31±31 (6.8%)	16±16 (3.6%)
	PBS	55±22 (53.7%)	18±18 (17.6%)	29±18 (28.7%)	0±0 (0%)
HD mice	B/E	663±124* (68.4%)	155±23 (15.9%)	123±21 (12.7%)	29±29 (3.0%)
	BDNF	432±36 (70.2%)	108±33 (17.5%)	62±43 (10.0%)	14±14 (2.3%)
	PBS	92±5 (54.2%)	52±21 (30.4%)	14±14 (8.4%)	12±12 (7.0%)

Values are mean±S.E. *$p<0.05$ compared with PBS controls.
VZ: ventricular zone, WT: wild-type, HD: Huntington's disease, B/E: combination of BDNF and EGF, BDNF: brain-derived neurotrophic factor, PBS: phosphate-buffered saline, BrdU: 5-bromo-2-deoxyuridine

(300~1200 μm) (Fig 4E, Table 2).

4. Discussion

Recently, experimental studies engaging various neurotrophic factors alone or in combination with stem cell therapy or induced pluripotent stem cell technology have been actively investigated for various diseases.[26,36,37] Nonetheless, its treatment effectiveness on HD is not yet clear, and the standardized methods for the evaluation are not available. Particularly, a consensus on partial effects has not been reached. In our study, after the intraventricular administration of BDNF and/or EGF, functional effects derived from striatal neurogenesis, in other words, the delay of motor deterioration with time, were assessed by the evaluation of rotarod and locomotor activity. For this, as an animal model of HD, R6/2 transgenic mice prepared to contain approximately 145 CAG repeats in the first exon of human HD gene were used.[38,39] The R6/2 model has been widely investigated, and their neuropathological findings as well as clinical symptoms are similar to juvenile HD patients. They usually began to show the degeneration of motor function at 6 weeks after birth. From 8-9 weeks of age, they exhibit involuntary motor symptoms such as tremor as well as chorea, and overt behavior symptoms such as gait disturbance. From approximately 12-13 weeks of age, severe reduction of the locomotor activity is shown, and afterward, they die in most cases.[40,41] Therefore, the mice were treated with BDNF and/or EGF at 6 weeks of age when the animals began to exhibit behavioral dysfunction, and sacrificed at 12 weeks of age before death.

Our study demonstrated that intraventricular administration of BDNF and EGF could delay the motor deterioration 6 weeks after treatment. The histological results also showed that combination therapy significantly induced striatal neurogenesis after 2 weeks of chronic infusion. In particular, the new neurons were significantly recruited into the ventricular side of neostriatum, suggesting that striatal regeneration might be derived from neural stem/progenitor cells in the SVZ. Moreover, the newly generated cells, expressed as migrating neuroblasts immunostained with DCx or PSA-NCAM in the

SVZ, migrated and differentiated to striatal neurons with proof of neuronal marker βIII-tubulin.

Administration of BDNF and EGF promoted functional benefits consistently in various motor functions. However, mice treated with BDNF alone did not show any statistical increment in 5 min constant rotarod, accelerating rotarod, and locomotor activity test, although they exhibited an improved rotarod latency only in the 1 min constant rotarod test. In other words, the finding that significant differences of rotarod latency and locomotor activity in the B/E group were shown compared with PBS controls and the finding that administration of BDNF alone did not show a significant delay in motor deterioration in most of the behavioral assessments could be considered as a treatment effect mediated by newly generated neurons induced by combination therapy rather than the neuroprotective effect of BDNF itself. Furthermore, any behavioral performance in the mice treated with EGF alone was not different from that in PBS controls. Rather than a direct benefit, EGF seems to expand the mitotic pool of SVZ cells responsive to neuronal instruction by BDNF, and thereafter to potentiate striatal neurogenesis and functional benefits.[9]

In this study, the rotarod tests were performed with various methods to evaluate motor coordination and balance sensitively and reliably.[8,42] Namely, both constant speed and accelerating speed method were used, and the mean latency of the constant rotarod was analyzed by terminating at 1 min or 5 min. In addition, general locomotor activity was investigated by dividing the evaluation time to 10 min or 60 min. In such manners, the most sensitive and reliable evaluation methods were assessed by varying the test time. As a result, the 1 min constant rotarod test detected the differences between the mice treated with BDNF alone and PBS controls, whereas the BDNF group did not exhibit a significant delay of motor deterioration in 5 min constant and accelerating rotarod tests. Based on the observation that constant rotarod performance was terminated as a maximal latency of 1 min, it suggests that 1 min rotarod test might detect partial neuroprotective effect of BDNF more sensitively. Therefore, it was found that appropriate evaluation duration should be required to compare treatment groups showing partial effects. Considering our results, it is thought that appropriate test methods should be selected depending on the subject groups to be compared. Similarly, when locomotor activity was evaluated by dividing the test results to 10 min and 60 min in a R6/2 HD mouse model, the difference between B/E combination therapy group and PBS controls was shown only in the 60 min locomotor activity test. Thus, it is thought that previous result of the evaluation for 10 min activity may not be sufficient to obtain reliability.[26]

We confirmed that administration of BDNF and EGF induced striatal neurogenesis and migration from SVZ cells into the neostriatum in HD. The striatal regeneration could exert treatment effects of delaying motor deterioration and disease progression, implying a causal association of functional outcomes with histological results. In other words, the functional outcomes may be derived from the induction of striatal neurogenesis rather than from the neuroprotective effects of BDNF itself in HD. This result is consistent with our previous study in an animal model of hypoxic-ischemic brain injury.[9] It is also considered that various behavioral assessments should be performed by appropriate methods to evaluate treatment effects more sensitively and reliably.

As a limitation of this study, R6/2 transgenic mice may not be a sufficient animal model of HD to evaluate striatal regeneration and functional effects of our treatment. It thus is thought if N171-82Q or YAC transgenic mouse model with neurological symptoms similar to HD patients were used, the direct link between motor function and histological outcome could be determined simultaneously at the appropriate time. In addition, we could not define the role of the newly generated cells, because this study did not show functional synapses and neural pathway regenerated from the survived new neurons. Through the further studies, we should be able to elucidate the rapeutic mechanism of functional improvement such as synaptic formation and the connection of striatopallidal pathway.

5. Conclusion

The induction of striatal regeneration by intraventricular administration of BDNF and EGF for 2 weeks delayed motor deterioration and disease progression in HD. Therefore, this treatment may offer a promising strategy for the restoration of motor function in incurable neurodegenerative diseases such as HD.

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