

Neuroprotective effects of prior limb use in 6-hydroxydopamine-treated rats: possible role of GDNF

Ann D. Cohen,^{*,†} Jennifer L. Tillerson,[†] Amanda D. Smith,^{*} Timothy Schallert^{†,‡} and Michael J. Zigmond^{*}

^{*}Department of Neurology and Center for Neuroscience, University of Pittsburgh, Philadelphia, USA

[†]Institute for Neuroscience, University of Texas at Austin, Texas, USA

[‡]Department of Neurosurgery, University of Michigan, Michigan, USA

Abstract

Unilateral administration of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) causes a loss of dopamine (DA) in the ipsilateral striatum and contralateral motor deficits. However, if a cast is placed on the ipsilateral limb during the first 7 days following 6-OHDA infusion, forcing the animal to use its contralateral limb, both the behavioral and neurochemical deficits are reduced. Here, we examine the effect of forced reliance on a forelimb during the 7 days prior to ipsilateral infusion of 6-OHDA on the deficits characteristic of this lesion model. Casted animals displayed no behavioral asymmetries as measured 14–28 days postlesion and a marked attenuation in the loss of striatal DA and its

metabolites at 30 days. In addition, animals receiving a unilateral cast alone had an increase in glial cell-line derived neurotrophic factor (GDNF) protein in the striatum corresponding to the overused limb. GDNF increased within 1 day after the onset of casting, peaked at 3 days, and returned to baseline within 7 days. These results suggest that preinjury forced limb-use can prevent the behavioral and neurochemical deficits to the subsequent administration of 6-OHDA and that this may be due in part to neuroprotective effects of GDNF.

Keywords: behavior, exercise, GDNF, neurotrophic factor, Parkinson's disease, striatum.

J. Neurochem. (2003) **85**, 299–305.

Parkinson's disease (PD) is a neurological disorder characterized by the degeneration of dopamine (DA) cells in the substantia nigra. The loss of dopaminergic control over striatal output neurons leads to a variety of neurological deficits, including akinesia. Most current treatments for PD are pharmacological, temporarily restoring dopaminergic tone in the striatum, and therefore focus on alleviating symptoms of the disorder. However, when the degree of DA cell loss becomes too extensive, the efficacy of drug treatment diminishes, and motor and psychiatric side-effects become more problematic. Thus, it is essential to develop strategies for slowing or preventing ongoing cell death in this disorder.

Animal models of PD have been produced using the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) (Ungerstedt 1971; Zigmond and Keefe 1997). When delivered intracerebrally to rats, 6-OHDA causes selective loss of DA neurons via oxidative stress, a proposed mechanism in the pathogenesis of PD (Halliwell 2001), and a marked impairment in limb use for movement initiation or skilled

motor functions (e.g. Marshall *et al.* 1974; Spirduso *et al.* 1985; Schallert *et al.* 1992; Miklyaeva and Whishaw 1996). However, like patients with PD, 6-OHDA-lesioned rats can show paradoxical kinesia. For example, they show transient improvements in motor performance when placed in an ice bath or exposed to a cat (Marshall *et al.* 1976), returned to their home cage after exposure to an unfamiliar environment (Schallert 1989), or placed in a deep pool of water (Marshall *et al.* 1976; Keefe *et al.* 1989, 1990).

Received November 26, 2002; accepted December 12, 2002.

Address correspondence and reprint requests to Dr Michael J. Zigmond, Department of Neurology, S-510 Biomedical Science Tower, Pittsburgh, PA 15213, USA. E-mail: zigmond@pitt.edu

Abbreviations used: BDNF, brain-derived neurotrophic factor; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; FGF-2, fibroblast growth factor-2; GDNF, glial cell-line derived neurotrophic factor; IGF-1, insulin-like growth factor 1; MFB, medial forebrain bundle; NGF, nerve growth factor; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease.

Increases in physical activity levels throughout life have been associated with a lower propensity to develop PD (Sasco *et al.* 1992; Tsai *et al.* 2002). Additionally, constraining the non-impaired upper extremity in stroke patients, thereby forcing use of the affected limb, improves the motor function and increases the use of the affected limb (Taub *et al.* 1999; Taub and Morris 2001; Sterr *et al.* 2002). There is also limited evidence for efficacy of physical therapy regimens in the treatment of PD (Toole *et al.* 1999).

We have recently shown that forcing animals to exercise their impaired limb for 7 days beginning 0–3 days after the unilateral administration of 6-OHDA can dramatically attenuate both the behavioral and neurobiological effects of the lesion (Tillerson *et al.* 2001, 2002). Although the mechanism by which forced use ameliorates behavioral and biochemical deficits is unknown, one hypothesis that can be drawn from the literature is that forced use of the impaired forelimb initiates a cascade of events that involves an increase in the availability of key neurotrophic factors in the brain. One such factor is glial cell line-derived neurotrophic factor (GDNF).

GDNF has been shown to be a potent survival factor for DA neurons (Lin *et al.* 1993). Moreover, administration of exogenous GDNF or a viral vector containing the GDNF gene is known to protect DA neurons from the neurotoxic effects of 6-OHDA *in vitro* (Gong *et al.* 1999; Kramer *et al.* 1999; Schatz *et al.* 1999) and the behavioral and neurotoxic effects *in vivo* (Hoffer *et al.* 1994; Kearns and Gash 1995; Choi-Lundberg *et al.* 1998; Akerud *et al.* 1999). Although levels of GDNF protein decrease markedly after development, there is evidence that GDNF can increase after experience, such as an enriched environment (Young *et al.* 1999). We therefore reasoned that forced-use might increase the expression of endogenous GDNF and attenuate the effects of 6-OHDA.

Materials and methods

Animals

Male rats weighing 350–450 g were used throughout these experiments. All animals were housed two per cage and maintained on 12 h light/dark cycle with food and water available *ad libitum*. All procedures were in strict accordance with the guidelines for the NIH Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at the University of Texas and the University of Pittsburgh. The studies reported here of the effects of casting on the behavioral and neurochemical effects of 6-OHDA were carried out in hooded Long-Evans rats (Charles River Laboratories, Wilmington, MA, USA), whereas studies of GDNF were carried out with Sprague–Dawley rats (Hilltop Laboratory Animals, Scottsdale, PA, USA). A small pilot study indicated that the neuroprotective effects of casting seen with the Long–Evans strain (see Figs 1–3) were comparable to those seen with Sprague–Dawley strain (see legend to Fig. 4). In addition,

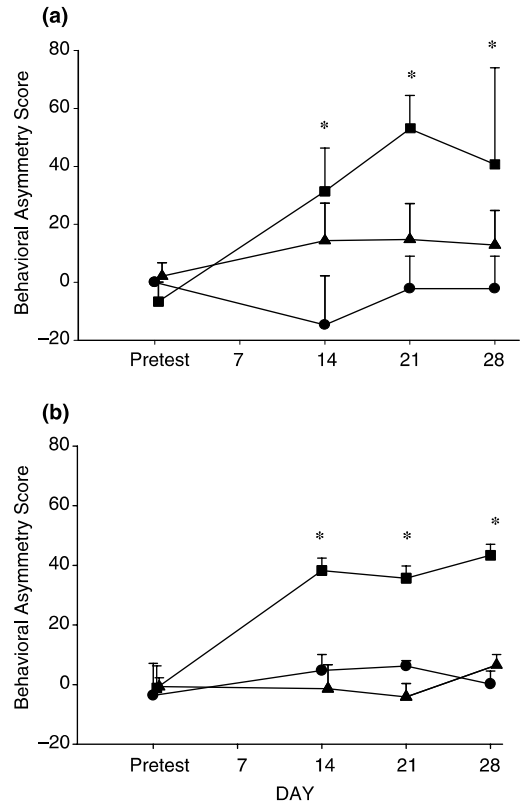


Fig. 1 (a) Effects of prior forced limb use on forelimb use asymmetry after 6-OHDA. Unilateral casting of a forelimb prior to ipsilateral infusion of 6-OHDA into the MFB prevented the limb use asymmetry associated with unilateral 6-OHDA lesion. Sham-lesioned animals (●) showed no asymmetry of limb use whereas animals receiving 6-OHDA into the MFB without a cast (■) showed a significant asymmetry of limb use ($*p < 0.01$ versus sham-lesioned animals). Animals that received a cast prior to 6-OHDA infusion (▲) displayed a significant decrease in limb use asymmetry ($*p < 0.01$ versus lesioned animals). All values are expressed as mean asymmetry score \pm SEM. (b) Effects of prior forced limb use on forelimb akinesia after 6-OHDA. Forelimb akinesia was prevented by unilateral casting prior to ipsilateral 6-OHDA infusion into the MFB. Sham-lesioned animals showed no significant akinetic behavior. 6-OHDA-lesioned animals that were not fitted with a cast displayed significant akinetic behavior when compared with sham-lesioned animals ($*p < 0.01$). This akinetic behavior was prevented in casted/lesioned animals ($*p < 0.01$, lesioned versus casted/lesioned) and comparable to behavior observed in sham-lesioned animals. All values are expressed as mean asymmetry score \pm SEM.

previous investigators also have found that forced exercise has the same effect on 6-OHDA lesioned rats from the Long–Evans and Sprague–Dawley strains (Moroz *et al.* 2002; Tillerson *et al.* 2003).

Preoperative and surgical procedures

Seven days prior to surgery animals were randomly assigned to one of four groups. Sham-lesioned/casted animals ($n = 3$) received a

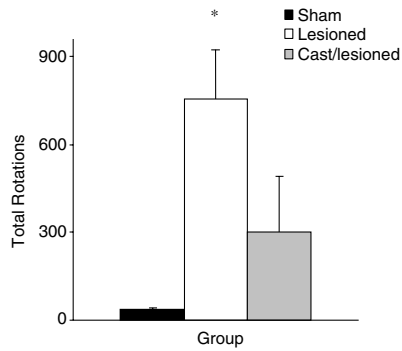


Fig. 2 Effects of prior forced limb use on apomorphine-induced rotational behavior after 6-OHDA. Pre-6-OHDA forced limb use attenuated apomorphine-induced rotations. Sham-lesioned animals did not exhibit contralateral rotational behavior in response to apomorphine. 6-OHDA infusion increased the number of contralateral rotations in lesioned-animals ($*p < 0.05$, lesioned versus sham-lesioned). Forcing reliance on a forelimb prior to contralateral infusion of 6-OHDA greatly attenuated apomorphine-induced rotational behavior ($*p < 0.05$, lesioned versus casted/lesioned animals). All values are expressed as mean rotations \pm SEM.

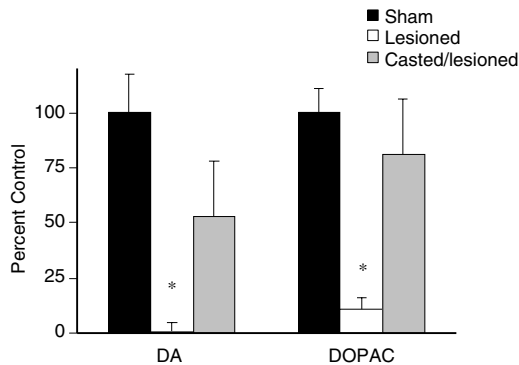


Fig. 3 Effects of prior forced limb use on striatal DA and its metabolites after 6-OHDA. Animals in the lesioned group showed a significant loss of DA and DOPAC when compared with sham-lesioned (DA, $0.84 \pm 3.6\%$; DOPAC, $11 \pm 5\%$, lesioned values; $*p < 0.01$) and casted/lesioned (DA, $47 \pm 25\%$; DOPAC, $81 \pm 21\%$; $*p < 0.05$) animals. This loss was attenuated in casted/lesioned animals such that no significant difference was detected between sham-lesioned and casted/lesioned animals. All results are expressed as mean percent of control \pm SEM, which were 2.7 ± 0.34 ng/20 μ L for DA, 1.2 ± 0.16 ng/20 μ L for DOPAC.

cast for the 7 days prior to the sham lesion; sham-lesioned animals ($n = 2$) received a sham lesion alone; lesioned animals ($n = 9$) received unilateral 6-OHDA alone, whereas casted/lesioned animals ($n = 7$) received a cast on one limb for 7 days prior to being given an ipsilateral infusion of 6-OHDA into the medial forebrain bundle (MFB).

Animals in the casted groups were fitted with plaster of Paris casts to immobilize the forelimb in a naturally retracted position against the sternum (Jones and Schallert 1994). At the end of the

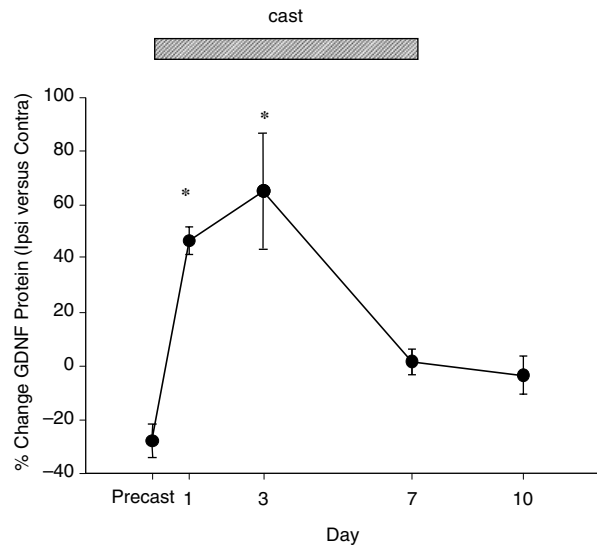


Fig. 4 Effects of forced limb use on striatal GDNF levels. GDNF protein levels increased in a unilateral and time-dependent manner during unilateral casting. This increase was significant ($*p < 0.05$) at 24 and 72 h after the cast was placed on the animal. The increase in GDNF was only observed in the striatum corresponding to the non-casted, overused limb. All results are expressed as a mean percent of contralateral side \pm SEM which was 0.31 ± 0.04 ng/mg wet tissue weight for GDNF. These studies were carried out with a Sprague-Dawley strain of rats, whereas the experiments reported in Figs 1–3 used a Long-Evans strain. However, in a small pilot study ($n = 2$ –3/group), we observed that the altered strain showed comparable neuroprotective effects of casting, with respect to apomorphine-induced rotational behavior (sham, 0.06 ± 0.07 rotations/min; lesioned 3.27 ± 0.27 ; casted/lesioned, -0.07 ± 0.17) and neurochemical changes (sham, 94.20% of control $\pm 23.87\%$; lesioned, $8.86\% \pm 0.57\%$; casted/lesioned, $62.29\% \pm 9.16$). This strain-independence of exercise-induced protection against 6-OHDA-induced toxicity is consistent with findings from other labs (see Materials and methods).

7-day casting period, all animals were anesthetized with Equithesin (25 mg/kg pentobarbital and 150 mg/kg chloral hydrate, intraperitoneally), the casts were removed (if present), and the animals were prepared for surgery. Animals began to use the previously casted forelimb immediately upon awakening from surgery. Thirty minutes prior to surgery, animals were given desipramine (15 mg/kg, intraperitoneally), an inhibitor of norepinephrine uptake that serves to block the entry of 6-OHDA into noradrenergic neurons. 6-OHDA (10 μ g in 4 μ L of 0.9% NaCl, 0.02% ascorbic acid) was infused (0.5 μ L/min) into either the right or left MFB (the side ipsilateral to the cast; 3.3 mm posterior, ± 1.7 mm lateral of bregma, and 9 mm ventral to dura) (Paxinos and Watson 1982). Sham-lesioned animals received all surgical procedures up to but not including lowering of infusion cannulae.

Behavioral assessment

One week before and 2–4 week after lesioning, animals were assessed for forelimb asymmetry and akinesia. The extent of asymmetry in the forelimbs during exploratory movements was

determined by videotaping rats in a clear Plexiglass cylinder (20 cm diameter; 30 cm high) for 3 min and later analyzing a slow motion version of the tape (Schallert and Tillerson 2000; Tillerson *et al.* 2001, 2002; Cenci *et al.* 2002). We assessed the amount of time animals made independent use of the impaired forelimb, the independent use of the non-impaired forelimb, and the simultaneous use of both limbs (including alternating steps) for support and weight shifting movements along the walls. For a single score, these values were converted to percentages and the percent independent use of the non-impaired forelimb was subtracted from the percent independent use of the impaired forelimb. To assess forelimb akinesia, animals were held with their hindquarters suspended and allowed to initiate stepping movements in a 10-min period. Stepping movements were assessed for both limbs and an asymmetry score was computed: [(ipsilateral steps/ipsilateral plus contralateral steps) – (contralateral steps/ipsilateral plus contralateral steps)] (Schallert and Tillerson 2000; Schallert *et al.* 1992; Cenci *et al.* 2002). Three-week postlesion animals received apomorphine (0.5 mg/kg, subcutaneously), were placed in a 37-cm diameter plastic bowl and rotations were counted over a 90-min period to estimate the extent of denervation induced up-regulation of DA receptors (Ungerstedt 1971).

Neurochemical assessment

Dopamine assay

Thirty days postlesion, animals from sham-lesioned, sham-lesioned/casted, lesioned, and casted/lesioned groups were killed, brains were removed, and a section of the striatum from 1 to 2 mm anterior of bregma was dissected from both the ipsilateral and contralateral hemispheres. Dissected striata were assayed using minor modifications of previous methods (Smith *et al.* 2002). Striatal tissue was suspended in 0.1 M HClO₂ containing 347 μM NaHSO₃ and 134 μM Na₂EDTA, homogenized and centrifuged at 16 000 g for 20 min at 4°C, and the supernatant was removed. Tissue samples were assayed for DA and 3,4-dihydroxyphenylacetic acid (DOPAC) by injecting a 20-μL aliquot of the sample onto a Symmetry C18 column (3.9 × 140 mm, Waters Corporation, Milford, MA, USA). The mobile phase consisted of 50 mM H₂NaPO₄, 0.72 mM sodium octyl sulfate, 0.075 mM Na₂EDTA and 16% methanol (v/v), pH 2.7. The mobile phase was pumped through the system at 0.7 mL/min using a Shimadzu LC-10AD pump (Shimadzu Corp., Columbia, MD, USA). Analyses were detected coulometrically using an ESA Coulochem Model 4100 A detector, an ESA Model 5010 conditioning cell, and an ESA Model 5014B microdialysis cell (ESA, Inc., Chelmsford, MA, USA). The settings for detection were E1 = + 0.26 V, E2 = + 0.28 V, and guard cell = + 0.4 V. The limits of detection for DA and DOPAC were in the femtomole range.

GDNF assay

Thirty-three male Sprague–Dawley rats were casted and decapitated 1–10 days after placement of the cast, and the striata were removed, homogenized in lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (10 μg/mL), and 0.5 mM sodium vanadate. Homogenate was then centrifuged at 12 000 g for 20 min at 4°C, supernatant was removed, acid-treated with 1 M HCl (1 μL/10 μL of sample), and then neutralized with 1 M NaOH (1 μL/10 μL of sample), respectively, to increase the sensitivity of the assay (Okragly and Haak-

Friendscho 1997). Samples were assayed for GDNF using an ELISA kit (Promega Corporation, Madison, WI, USA) according to the protocol provided. GDNF values were compared with those observed in uncasted animals.

Statistical analysis

Neurochemical, trophic factor, and rotational behavior data were analyzed using a one-way ANOVA, and *post-hoc* analyses were carried out using Bonferonni-corrected multiple comparison tests. A two-way repeated measures ANOVA was used to analyze all other behavioral data. *Post-hoc* analyses were performed using Bonferonni-corrected multiple comparison tests.

Results

Effect of casting prior to injury on effects of unilateral 6-OHDA on forelimb rearing and akinesia

Animals casted prior to 6-OHDA infusion showed reductions in forelimb asymmetry (Fig. 1a) and akinesia (Fig. 1b) normally associated with unilateral 6-OHDA lesions. The casted sham group and non-casted sham group showed no significant difference in asymmetric behaviors, therefore these groups were pooled for further analysis ($F_{1,11} = 2.336$; $p > 0.05$). Analysis of variance indicated an overall group effect for forelimb asymmetry ($F_{2,47} = 8.74$; $p < 0.01$) and forelimb akinesia ($F_{2,39} = 48.33$; $p < 0.01$). *Post-hoc* analysis of these behavioral measures indicated significant attenuation of asymmetric behavior in animals casted prior to infusion of 6-OHDA when compared with animals that received 6-OHDA alone ($p < 0.01$).

Effect of casting on apomorphine-induced turning normally associated with unilateral 6-OHDA

Casting-induced attenuation of the effects of 6-OHDA-induced forelimb rearing and akinesia might conceivably be caused by compensatory changes in pathways that parallel the nigrostriatal DA projection. Thus, we also used a third behavioral test, apomorphine-induced turning. This phenomenon depends on a large (>90%) reduction in the availability of DA at DA receptors (Ungerstedt 1971). We observed that contralateral turning in response to apomorphine was markedly attenuated by casting prior to injury when compared with animals receiving a 6-OHDA alone ($F_{2,18} = 19.32$; $p < 0.01$; Fig. 2).

Effect of casting prior to injury on 6-OHDA-induced changes in DA and DOPAC in the striatum

There was no significant difference in striatal DA and DOPAC content between sham-lesioned animals therefore data from these groups were pooled for further analysis ($F_{2,2} = 1.276$; $p > 0.05$). A one-way ANOVA revealed a significant group effect for both DA and DOPAC content [DA: ($F_{2,18} = 10.03$; $p < 0.01$); DOPAC: ($F_{2,18} = 11.16$;

$p < 0.01$]. 6-OHDA alone resulted in an almost complete loss of both striatal DA and DOPAC content compared with levels observed in sham-lesioned animals (Fig. 3; $p < 0.01$, shams versus 6-OHDA alone). *Post hoc* analysis revealed a significant attenuation of the loss of striatal DA and DOPAC content in animals casted prior to lesion when compared with animals receiving lesion alone ($p < 0.05$). No significant changes in striatal DA or DOPAC content were observed between groups in the non-lesioned hemisphere relative.

Effect of casting on striatal GDNF

Finally, we examined the profile of striatal GDNF protein levels during and after forced limb-use in Sprague–Dawley animals that were not subjected to 6-OHDA. A one-way ANOVA revealed GDNF levels significantly increased in the striatum corresponding to the overused limb compared with non-casted control animals ($F_{2,28} = 5.934$; $p < 0.01$), whereas GDNF levels in the opposite hemisphere were unchanged. *Post hoc* analysis indicated this increase was time dependent, increasing significantly above control 24 h and 3 days after placement of the cast ($p < 0.05$) and returned to near baseline levels on day 7 (Fig. 4).

Discussion

Unilateral infusion of 6-OHDA along the MFB causes degeneration of DA neurons in the ipsilateral substantia nigra and loss of striatal DA and DOPAC content. This loss of striatal DA produces a number of motor deficits that include a tendency to limit the use of the contralateral limb during rearing, contralateral akinesia, and contraversive turning in response to the systemic administration of apomorphine (Ungerstedt 1971; Marshall and Ungerstedt 1977; Schallert and Tillerson 2000). In this study we report three observations. First, placing a cast on an animal for 7 days prior to the ipsilateral infusion of 6-OHDA prevented the development of behavioral deficits characteristic of this lesion model. Second, casting prior to injury greatly attenuated the loss of striatal DA. Third, casting prior to injury caused a significant but transient increase in striatal GDNF. These data extend our previous findings (Tillerson *et al.* 2001, 2002), indicating that casting the unimpaired forelimb for 7 days *prior* to and following 6-OHDA infusion protects against 6-OHDA toxicity.

It is our assumption that the ability of casting to protect against the behavioral effects of 6-OHDA results from the casting-induced attenuation of the loss of DA. The strongest support from this assumption comes from the ability of casting to greatly reduce the apomorphine-induced rotation normally associated with unilateral lesions of the nigrostriatal DA projections. Our working hypothesis is that the casting-induced attenuation of the loss of striatal DA was, in turn, a result of an exercise-induced increase in GDNF in the region that subsequently received 6-OHDA. The neuroprotective effects of GDNF are well known (see Introduction).

Although GDNF had declined to baseline prior to 6-OHDA infusion, this does not preclude GDNF as a possible participant in the neuroprotective effects of forced limb-use. Indeed, previous investigators have shown that exogenous GDNF can be neuroprotective if administered as much as 7 days prior to 6-OHDA (Kearns and Gash 1995; Choi-Lundberg *et al.* 1998), and in those experiments it is unlikely that levels of this trophic factor were still elevated at the time of toxin administration.

To be effective, GDNF would need to act on its receptor complex, thereby initiating a signaling cascade that produces cytoplasmic and/or translational changes in DA neurons. It is possible that these downstream effects of GDNF are activated after the protein has returned to baseline levels. Moreover, extracellular signal regulated kinase (ERK), a downstream effector of GDNF, has been shown to be activated after exercise, and to remain elevated for up to 1 month (Shen *et al.* 2001).

Although we have focused our initial attention on GDNF, there are many other factors that may play a role in the casting-induced protection that we observed. Exercise, primarily running, has been shown to increase brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (Neeper *et al.* 1996), fibroblast growth factor-2 (FGF-2) (Gomez-Pinilla *et al.* 1995), and insulin-like growth factor 1 (IGF-1) (Carro *et al.* 2001) in certain regions of the CNS, particularly the hippocampus. Although there are no previous reports on the effects of exercise on trophic factors in the striatum, each of these factors has potent trophic activity towards DA neurons (Hyman *et al.* 1991; Altar *et al.* 1992; Lin *et al.* 1993; Winkler *et al.* 1996) and, where examined, has been shown to protect against the neurotoxic effects of 6-OHDA (Hoffer *et al.* 1994; Kearns and Gash 1995; Levivier *et al.* 1995; Choi-Lundberg *et al.* 1998; Akerud *et al.* 1999; Gong *et al.* 1999; Kramer *et al.* 1999; Schatz *et al.* 1999; Shults *et al.* 2000; Wang *et al.* 2002). For example, rats exposed to enriched environments, exercise regimens, dietary restriction, and learning tasks all show an increase in protein and mRNA levels of trophic factors in the brain (Neeper *et al.* 1995; Gomez-Pinilla *et al.* 1998; Torasdotter *et al.* 1998; Pham *et al.* 1999; Young *et al.* 1999; Duan *et al.* 2001). Many of these environmental changes are protective against hippocampal damage incurred from insults, such as ischemia, and it has been proposed that this neuroprotection is driven by increases in trophic factors (Duan *et al.* 2001; Young *et al.* 1999). Moreover, it seems likely that more than one factor will ultimately be found to be responsible for exercise-induced protection. In this regard, we note that FGF-2, one of the trophic factors shown to be up-regulated by exercise (Gomez-Pinilla *et al.* 1995), has also been shown *in vitro* to induce expression of both GDNF and BDNF (Suter-Crazzolara and Unsicker 1996; Kwon 1997).

In conclusion, these data suggest that forced limb-use is protective against subsequent injury to the nigrostriatal DA

system and this protection is accompanied by increases in striatal GDNF. These findings provide further evidence that physical therapy may be beneficial to patients with PD, as well as the possibility that exercise throughout life may protect against development of PD. In fact, our group has recently shown that forced use is not only protective but that lack of use may exacerbate damage associated with 6-OHDA, indicating that motor weakness early in the disorder may be an exacerbating factor (Tillerson *et al.* 2002). As early detection methods are developed, physical activity may be an important factor in slowing or even halting the neurodegenerative cascade associated with PD.

Acknowledgements

This research was supported in part by USPHS grants NS19608, NS23979 and MH29670. ADC was supported as a predoctoral trainee on USPHS grant NS07433. Our thanks to Susan D. Giegel for assistance in preparing the manuscript.

References

- Akerud P., Alberch J., Eketjall S., Wagner J. and Arenas E. (1999) Differential effects of glial cell line-derived neurotrophic factor and neurturin on developing and adult substantia nigra dopaminergic neurons. *J. Neurochem.* **73**, 70–78.
- Altar C. A., Boylan C. B., Jackson C., Hershenson S., Miller J., Wiegand S. J., Lindsay R. M. and Hyman C. (1992) Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover *in vivo*. *Proc. Natl Acad. Sci. USA* **89**, 11347–11351.
- Carro E., Trejo J. L., Busiguina S. and Torres-Aleman I. (2001) Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. *J. Neurosci.* **21**, 5678–5684.
- Cenci M. A., Whishaw I. Q. and Schallert T. (2002) Animal models of neurological deficits: How relevant is the rat? *Nat. Rev. Neurosci.* **3**, 574–579.
- Choi-Lundberg D. L., Lin Q., Schallert T., Crippens D., Davidson B. L., Chang Y. N., Chiang Y. L., Qian J., Bardwaj L. and Bohn M. C. (1998) Behavioral and cellular protection of rat dopaminergic neurons by an adenoviral vector encoding glial cell line-derived neurotrophic factor. *Exp. Neurol.* **154**, 261–275.
- Duan W., Guo Z. and Mattson M. P. (2001) Brain-derived neurotrophic factor mediates an excitoprotective effect of dietary restriction in mice. *J. Neurochem.* **76**, 619–626.
- Gomez-Pinilla F., Vu L. and Cotman C. W. (1995) Regulation of astrocyte proliferation by FGF-2 and heparan sulfate *in vivo*. *J. Neurosci.* **15**, 2021–2029.
- Gomez-Pinilla F., So V. and Kessler J. P. (1998) Spatial learning and physical activity contribute to the induction of fibroblast growth factor: neural substrates for increased cognition associated with exercise. *Neuroscience* **85**, 53–61.
- Gong L., Wyatt R. J., Baker I. and Masserano J. M. (1999) Brain-derived and glial cell line-derived neurotrophic factors protect a catecholaminergic cell line from dopamine-induced cell death. *Neurosci. Lett.* **263**, 153–156.
- Halliwell B. (2001) Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging* **18**, 685–716.
- Hoffer B. J., Hoffman A., Bowenkamp K., Huettl P., Hudson J., Martin D., Lin L. F. and Gerhardt G. A. (1994) Glial cell line-derived neurotrophic factor reverses toxin-induced injury to midbrain dopaminergic neurons *in vivo*. *Neurosci. Lett.* **182**, 107–111.
- Hyman C., Hofer M., Barde Y. A., Juhasz M., Yancopoulos G. D., Squinto S. P. and Lindsay R. M. (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **350**, 230–232.
- Jones T. A. and Schallert T. (1994) Use-dependent growth of pyramidal neurons after neocortical damage. *J. Neurosci.* **14**, 2140–2152.
- Kearns C. M. and Gash D. M. (1995) GDNF protects nigral dopamine neurons against 6-hydroxydopamine *in vivo*. *Brain Res.* **672**, 104–111.
- Keefe K. A., Salamone J. D., Zigmond M. J. and Stricker E. M. (1989) Paradoxical kinesia in parkinsonism is not caused by dopamine release. Studies in an animal model. *Arch. Neurol.* **46**, 1070–1075.
- Keefe K. A., Stricker E. M., Zigmond M. J. and Abercrombie ed. (1990) Environmental stress increases extracellular dopamine in striatum of 6-hydroxydopamine-treated rats: *in vivo* microdialysis studies. *Brain Res.* **527**, 350–353.
- Kramer B. C., Goldman A. D. and Mytilineou C. (1999) Glial cell line derived neurotrophic factor promotes the recovery of dopamine neurons damaged by 6-hydroxydopamine *in vitro*. *Brain Res.* **851**, 221–227.
- Kwon Y. K. (1997) Expression of brain-derived neurotrophic factor mRNA stimulated by basic fibroblast growth factor and platelet-derived growth factor in rat hippocampal cell line. *Mol. Cells.* **7**, 320–325.
- Levivier M., Przedborski S., Bencsics C. and Kang U. J. (1995) Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. *J. Neurosci.* **15**, 7810–7820.
- Lin L. F., Doherty D. H., Lile J. D., Bektesh S. and Collins F. (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* **21**, 1130–1132.
- Marshall J. F., Richardson J. S. and Tietlebaum P. (1974) Nigrostriatal bundle damage and the lateral hypothalamic syndrome. *J. Comp. Physiol. Psychol.* **87**, 808–830.
- Marshall J. F., Levitan D. and Stricker E. M. (1976) Activation-induced restoration of sensorimotor functions in rats with dopamine-depleting brain lesions. *J. Comp. Physiol. Psychol.* **90**, 536–546.
- Marshall J. F. and Ungerstedt U. (1977) Supersensitivity to apomorphine following destruction of the ascending dopamine neurons: quantification using the rotational model. *Eur. J. Pharmacol.* **41**, 361–367.
- Miklyaeva E. I. and Whishaw I. Q. (1996) Hemiparkinson analogue rats display active support in good limbs versus passive support in bad limbs on a skilled reaching task of variable height. *Behav. Neurosci.* **110**, 117–125.
- Moroz I., Cohen A. D., Tillerson J. L., Maxwell K., Martinez E., Schallert T. and Stewart J. (2002) Effects of forced limb use on behavioral outcome and FGF-2-IR after partial unilateral 6-OHDA lesions of nigrostriatal dopamine neurons. *Soc. Neuroscience Abstr.* Program No. 885.5.
- Neeper S. A., Gomez-Pinilla F., Choi J. and Cotman C. W. (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res.* **726**, 49–56.
- Okragly A. J. and Haak-Frendscho M. (1997) An acid-treatment method for the enhanced detection of GDNF in biological samples. *Exp. Neurol.* **145**, 592–596.
- Paxinos G. and Watson C. (1982) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

- Pham T. M., Ickes B., Albeck D., Soderstrom S., Granholm A. C. and Mohammed A. H. (1999) Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. *Neuroscience* **94**, 279–286.
- Sasco A. J., Paffenbarger R. S. Jr, Gendre I. and Wing A. L. (1992) The role of physical exercise in the occurrence of Parkinson's disease. *Arch. Neurol.* **49**, 360–365.
- Schallert T. (1989) Preoperative intermittent feeding or drinking regimens enhance post-lesion sensorimotor function, in *Preoperative Events: Their Effects on Behavior Following Brain Damage* (Schulkin J., ed.), pp. 1–20. Lawrence Erlbaum Association, New Jersey.
- Schallert T. and Tillerson J. L. (2000) Intervention strategies for degeneration of dopamine neurons in Parkinsonism: optimizing behavioral assessment of outcome, in *Central Nervous System Diseases* (Emerich D. F., Dean R. L., III and Sanberg, P. R., eds), pp. 131–151. Humana, Totowa, NJ.
- Schallert T., Norton D. and Jones T. A. (1992) A clinically relevant unilateral rat model of parkinsonian akinesia. *J. Neural Transplant Plastic.* **3**, 332–333.
- Schatz D. S., Kaufmann W. A., Saria A. and Humpel C. (1999) Dopamine neurons in a simple GDNF-treated meso-striatal organotypic co-culture model. *Exp. Brain Res.* **127**, 270–278.
- Shen H., Tong L., Balazs R. and Cotman C. W. (2001) Physical activity elicits sustained activation of the cyclic AMP response element-binding protein and mitogen-activated protein kinase in the rat hippocampus. *Neuroscience* **107**, 219–229.
- Shults C. W., Ray J., Tsuboi K. and Gage F. H. (2000) Fibroblast growth factor-2-producing fibroblasts protect the nigrostriatal dopaminergic system from 6-hydroxydopamine. *Brain Res.* **883**, 192–204.
- Smith A. D., Amalric M., Koob G. F. and Zigmond M. J. (2002) Effect of bilateral 6-hydroxydopamine lesions of the medial forebrain bundle on reaction time. *Neuropsychopharmacology* **26**, 756–764.
- Spirduso W. W., Gilliam P. E., Schallert T., Upchurch M. and Vaughn D. M., Wilcox R. E. (1985) Reactive capacity: a sensitive behavioral marker of movement initiation and nigrostriatal dopamine function. *Brain Res.* **335**, 45–54.
- Sterr A., Elbert T., Berthold I., Kolbel S., Rockstroh B. and Taub E. (2002) Longer versus shorter daily constraint-induced movement therapy of chronic hemiparesis: an exploratory study. *Arch. Phys. Med. Rehabil.* **83**, 1374–1377.
- Suter-Crazzolara C. and Unsicker K. (1996) GDNF mRNA levels are induced by FGF-2 in rat C6 glioblastoma cells. *Brain Res. Mol. Brain Res.* **41**, 175–182.
- Taub E. and Morris D. M. (2001) Constraint-induced movement therapy to enhance recovery after stroke. *Curr. Atheroscler. Rep.* **3**, 279–286.
- Taub E., Uswatte G. and Pidikiti R. (1999) Constraint-induced movement therapy: a new family of techniques with broad application to physical rehabilitation – a clinical review. *J. Rehabil. Res. Dev.* **36**, 237–251.
- Tillerson J. L., Cohen A. D., Philhower J., Miller G. W., Zigmond M. J. and Schallert T. (2001) Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine. *J. Neurosci.* **21**, 4427–4435.
- Tillerson J. L., Cohen A. D., Caudle M. W., Zigmond M. J., Schallert T. and Miller G. W. (2002) Forced nonuse in unilateral parkinsonian rats exacerbates injury. *J. Neurosci.* **22**, 6790–6799.
- Tillerson J. L., Caudle W. M., Reveron M. E. and Miller G. W. (2003) Exercise induces behavioral recovery and attenuates neurochemical deficits in rats with unilateral Parkinsonism. *Neurosci.* In press.
- Toole T., Hirsch M. A., Forknok A., Lehman D. A. and Maitland C. G. (1999) The effects of balance and strength training program on equilibrium in Parkinsonism: a preliminary study. *Neurorehabilitation* **14**, 1–10.
- Torasdotter M., Metsis M., Henriksson B. G., Winblad B. and Mohammed A. H. (1998) Environmental enrichment results in higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus. *Behav. Brain Res.* **93**, 83–90.
- Tsai C. H., Lo S. K., See L. C., Chen H. Z., Chen R. S., Weng Y. H., Chang F. C. and Lu C. S. (2002) Environmental risk factors of young onset Parkinson's disease: a case-control study. *Clin. Neurol. Neurosurg.* **104**, 328–333.
- Ungerstedt U. (1971) Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand. Suppl.* **367**, 69–93.
- Wang L., Muramatsu S., Lu Y., Ikeguchi K., Fujimoto K., Okada T., Mizukami H., Hanazono Y., Kume A., Urano F., Ichinose H., Nagatsu T., Nakano I. and Ozawa K. (2002) Delayed delivery of AAV-GDNF prevents nigral neurodegeneration and promotes functional recovery in a rat model of Parkinson's disease. *Gene Ther.* **9**, 381–389.
- Winkler C., Sauer H., Lee C. S. and Bjorklund A. (1996) Short-term GDNF treatment provides long-term rescue of lesioned nigral dopaminergic neurons in a rat model of Parkinson's disease. *J. Neurosci.* **16**, 7206–7215.
- Young D., Lawlor P. A., Leone P., Draganow M. and During M. J. (1999) Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nat. Med.* **5**, 448–453.
- Zigmond M. J. and Keefe K. A. (1997) 6-Hydroxydopamine as a tool for studying catecholamines in adult animals: lessons from the neostriatum, in: *Highly Selective Neurotoxins: Basic and Clinical Applications* (Kostrezewa, R. M., ed.), pp. 75–107. Humana, Totowa, NJ.