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Effects of intrastriatal GDNF on the response of dopamine neurons to 6-hydroxydopamine: Time course of protection and neurorestoration

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Abstract

Glial cell line-derived neurotrophic factor (GDNF) protects dopamine (DA) neurons from 6-hydroxydopamine (6-OHDA) toxicity. We have now explored this protection over 8 weeks following toxin administration. Infusion of Fluoro-Gold (FG) into striatum was followed 1 week later by GDNF (9 μg) or its vehicle. Six hours later, animals received 6-OHDA (4 μg) into the same site. 6-OHDA caused a loss of cells in the substantia nigra that expressed both FG and tyrosine hydroxylase (TH) and striatal terminals expressing TH, the high affinity dopamine transporter (DAT), and the vesicular monoamine transporter 2 (VMAT2) as assessed 2-8 weeks later. Loss of FG^+ cells, and striatal DA was completely blocked by GDNF by 2 weeks. In contrast, GDNF only slightly attenuated the loss of TH, DAT, or VMAT2 in striatum at 2 wks, but had restored these markers by 4-8 weeks. Thus, GDNF prevents DA cell death and loss of striatal DA content, but several weeks are required to fully restore the dopaminergic phenotype. These results provide insight into the mechanism of GDNF protection of DA neurons, and may help avoid incorrect interpretations of temporary phenotypic changes.

Keywords

Neuroprotection; oxidative stress; Parkinson's disease; striatum; substantia nigra; glial cell line derived neurotrophic factor

1. Introduction

Among the cells lost in Parkinson's disease (PD) are the dopamine (DA) neurons projecting from the substantia nigra (SN) to the striatum. The loss of these neurons is believed to be responsible for many of the motor deficits associated with the disease. Current pharmacotherapy for PD can alleviate many symptoms of the disorder but does not appear to significantly attenuate the neurodegenerative process. However, neurotrophic factors are a promising avenue for neuroprotective therapies. Much of the evidence for this comes from

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studies of glial cell line-derived neurotrophic factor (GDNF), a member of the TGFβ family member that is highly expressed in the striatum (Stromberg et al., 1993) as well as other regions of the brain. First, GDNF is a potent survival factor for cultured dopaminergic cells (Lin et al., 1993; Kramer et al, 1999; Gong et al., 1999; Schatz et al., 1999; Ugarte et al., 2003; Ding et al., 2004), and GDNF or a viral vector containing the GDNF gene can protect animals from the behavioral and neuropathological effects of 6-OHDA (Hoffer et al., 1994; Bowenkamp et al., 1995; 1996; Kearns & Gash, 1995; Choi-Lundberg et al., 1998; Garbayo et al., 2009). Second, injury to the brain can increase GDNF (Naveilhan et al., 1997; Liberatore et al., 1997; Sakurai, et al., 1999; Wei et al., 2000; Smith et al., 2003; Cheng et al., 2008). Third, age-related loss of tyrosine hydroxylase (TH) expression in the SN is accelerated in a heterozygous mouse model containing only one copy of the GDNF gene (Boger et al., 2006). Fourth, the loss of DA neurons in patients with PD is accompanied by a reduction of GDNF as compared to age-matched controls (Siegel and Chauhan 2000), suggesting that reduced trophic support may be a causal factor in the genesis of the disease (Appel 1981).

Studies have been somewhat equivocal regarding the efficacy of exogenous GDNF in the treatment of PD. Some groups have reported improvements in clinical symptoms and neuropathology (Gill et al., 2003; Patel et al., 2005; Slevin et al., 2006), whereas others have shown no clinical improvement (Nutt et al., 2003; Lang et al., 2006) (see Sherer et al., 2006 for review of the issues). Despite this controversy, we believe GDNF and its family members to be prime candidates as a therapeutic treatment against degeneration of the nigrostriatal DA system in PD, and that a full understanding of the neuroprotective effects of GDNF will be useful in the development of additional therapies for the disease. Moreover, a better understanding of the changes produced by GDNF on DA neurons should also shed light on the best ligands to use in quantifying the impact of treatment via imaging approaches such as SPECT and PET.

In this report we explore the effects of GDNF in a 6-OHDA rat model of the DA deficiency in PD. We examine several phenotypic markers of the nigrostriatal system over an 8-wk period to gain further insight into the nature of GDNF-induced protection of DA neurons against oxidative stress.

2. Results

2.1 Distribution of exogenous GDNF after intrastriatal administration

No GDNF immunoreactivity was observed in animals treated with 6-OHDA alone or with the 6-OHDA vehicle at any time point examined. Two weeks after infusion of GDNF alone or with 6-OHDA, a large spread of GDNF immunoreactivity beyond the needle track was observed in striatum. However, by 4 and 8 wks, GDNF was largely confined to the needle track (Fig. 1). No GDNF was observed in the SN of any animals at any time point (data not shown).

2.2 Effect of GDNF on the 6-OHDA-induced loss of TH, DAT, and VMAT2 immunoreactivity in the striatum

Treatment with vehicle or GDNF alone had no effect on TH, DAT, or VMAT2 immunoreactivity in striatum at any time point examined. In contrast, a one-way ANOVA revealed that animals treated with 6-OHDA alone (n = 25) displayed a significant loss of these phenotypic markers at each time point examined ($p < 0.001$ vs. vehicle and GDNF alone; Fig 2) [Overall group effect: TH: $F_{(6,55)} = 27.96$; $p < 0.0001$; Fig 2a; DAT: $F_{(6,55)} = 22.29$; $p < 0.0001$; Fig 2b; VMAT2: $F_{(6,55)} = 38.65$; $p < 0.0001$; Fig 2c]. A significant reduction in lesion size was observed with TH, DAT, and VMAT2 at 4 and 8 wks in animals

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that received GDNF prior to 6-OHDA compared to animals treated with 6-OHDA alone, showing an 84, 80, and 76% reduction in the loss of immunoreactivity ($p < 0.01$) at 8 wks post-infusion, respectively.

2.3 Effect of GDNF on the 6-OHDA-induced loss of TH positive neurons

6-OHDA caused a significant loss in TH^+ cells in the SN at 2 wks post-6-OHDA infusion that persisted for at least 8 wks ($p < 0.001$) [Overall group effect: $F_{(7,60)} = 15.14$; $p < 0.001$]. GDNF alone had no significant effect on the number of TH^+ cells at any time point ($p > 0.05$), and GDNF infusion prior to 6-OHDA did not prevent the loss of TH^+ cells in the SN at 2 wks. However, by 8 wks there was no longer any significant difference between the number of TH^+ cells in the SN of animals treated with GDNF plus 6-OHDA and vehicle-treated animals sacrificed at 2, 4 and 8 wks depicted as time 0 ($p < 0.001$; Fig 3 and supplemental figure).

Since we had injected FG into the striatum 1 wk prior to administering 6-OHDA into the same region, we also were able to determine the number of FG^+ cells in the SN, the great majority of which were co-labeled with TH in control animals (Fig 3 and supplemental figure). A 57% loss of FG^+ cells was observed at 2 wks in animals given 6-OHDA alone ($p < 0.001$), which was even greater at 4 wks (78%; $p < 0.05$) and 8 wks (83%; $p < 0.001$), indicating that 6-OHDA caused a gradual but profound loss of the DA cells in the SN. In contrast, GDNF given 6 hr prior to 6-OHDA completely blocked the loss of FG^+ cells at each time point examined ($p < 0.001$, Fig 3 and supplemental figure) [Overall group effect: FG: ($F_{(7,60)} = 18.74$; $p < 0.001$) Co-labeled: ($F_{(7,60)} = 41.81$; $p < 0.001$)]. GDNF pretreatment failed to block the loss of co-labeling by FG and TH at 2 wks ($p < 0.001$). However, by 8 wk there was no longer any significant loss of co-labeled cells in the GDNF pretreated animals (Fig 3 and supplemental figure). GDNF alone had no effect on the number of co-labeled cells. In vehicle treated animals, ~56% of TH+ SN cells were co-labeled for TH and FG (data not shown).

2.4 Effect of GDNF on the 6-OHDA-induced loss of DA content in the striatum

Since TH immunoreactivity was only gradually restored by GDNF pretreatment at early timepoints, we surmised that dopaminergic function also would return gradually. To begin to assess the functional impact of GDNF on the response to 6-OHDA, we measured striatal DA content. Contrary to our prediction, whereas 6-OHDA infused into the striatum produced a significant 60% depletion of DA content in the striatum 2-4 wks post-6-OHDA infusion ($p < 0.01$) [Overall group effect: ($F_{(4,24)} = 13.89$; $p < 0.0001$], prior treatment with GDNF completely blocked this loss at these same time points (Fig 4).

3. Discussion

3.1 Protection of DA cells versus restoration of the DA phenotype

Our study confirms previous observations that GDNF can protect DA neurons from 6-OHDA in the adult rat (see Introduction), and extends those findings to indicate that protection occurs by several steps, including stabilization of SN cells and maintenance of DA stores, followed by restoration of phenotypic markers of DA neurons. MPTP and 6-OHDA have been reported to cause loss of the TH phenotype without loss of DA neurons in mice and rats (Sauer and Oertel, 1994; Jackson-Lewis et al., 1995; Bowenkamp et al., 1996; Ara et al., 1998; Rosenblad et al., 2003), and infusion of GDNF into the SN has been shown to block loss of SN cells produced by intra-nigral 6-OHDA or axotomy without affecting the initial loss of TH (Bowenkamp et al., 1996; Lu and Hagg, 1997). Thus, loss of the dopaminergic phenotype does not necessarily reflect the loss of the cell. In order to distinguish between these two events, we infused FG into the striatum prior to infusion of 6-

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OHDA, thereby retrogradely labeling SN neurons that would later be exposed to the toxin. After 6-OHDA treatment, a significant loss of FG^+ cells was observed within 2 wks, and cell loss progressed over the next 6 wks as previously reported (Sauer and Oertel, 1994). 6-OHDA also caused a loss of TH^+ cells in SN. However, the loss of TH was maximal at 2 wks, the earliest time studied. Similar results were observed when we examined cells co-labeled for TH and FG.

Collectively, these results and those of others (e.g., Sauer and Oertel, 1994; Bowenkamp et al., 1996; Lu and Hagg, 1997) indicate that some of the initial loss of TH immunoreactivity results from downregulation of TH gene expression rather than cell loss. This is an important distinction since it indicates that a window of opportunity is likely to exist during which effects of oxidative stress might be reversible. Indeed, GDNF-induced protection of DA neurons has been observed with intervals as long as 4-5 wks between treatments with neurotoxins and GDNF (Hoffer et al., 1994; Bowenkamp et al., 1996; Aoi et al., 2001; Kirik et al., 2001; Yang et al., 2009).

We administered GDNF 6 hrs prior to 6-OHDA. This treatment ultimately protected against the toxin, but full protection took several weeks. Whereas GDNF blocked the 6-OHDA-induced loss of FG^+ cells at all times examined, the trophic factor failed to block the loss of TH^+ cells in SN at 2 wks. Instead, TH immunoreactivity in SN – as well as phenotypic markers for DA terminals in striatum – was gradually restored by GDNF over an 8 wk period.

The inability of GDNF to protect cells from the initial 6-OHDA-induced decrease in phenotypic markers indicates that the trophic factor does not initially block the full impact of oxidative stress caused by 6-OHDA. Instead, GDNF may cause a temporary shift in DA neurons from normal maintenance to a mode in which the cell has prioritized repair or regenerative processes. Perhaps only after neuronal survival has been assured, can the full DA phenotype be restored. Studies of gene expression will be required to test this hypothesis.

Our observations contrast with the report that GDNF downregulates TH protein in the striatum and SN many months after a large increase in striatal GDNF produced by the intrastriatal delivery of the GDNF gene using a viral vector and GDNF protein injection in the rat (Rosenblad et al., 2003, Salvatore et al., 2004) since we were unable to detect any significant effects of intrastriatal GDNF given alone on total TH protein or striatal DA content. This discrepancy may reflect differences in the times of measurement and/or the levels of GDNF that were achieved in the nigrostriatal pathway.

3.2 Impact of GDNF on the 6-OHDA-induced loss of DA stores

In order to begin to determine the functional effects of GDNF, we examined striatal DA content. 6-OHDA produced a persistent loss of the neurotransmitter as would be expected from neuronal degeneration. To our surprise, however, no loss of DA was seen at either time point in animals pretreated with GDNF. We had previously observed differential regulation of TH activity and total TH protein in noradrenergic neurons compromised by 6-OHDA (Acheson et al., 1980; Acheson and Zigmond, 1981; see also Reis et al., 1975). In those studies, the ratio of TH activity to norepinephrine was increased while total TH protein was reduced. We and others have also reported an increase in the ratio of DOPAC to DA after the 6-OHDA-induced loss of striatal DA (e.g., Zigmond et al., 1984, Hefti et al., 1985), as well as the maintenance of extracellular DA despite apparent DA neurons loss (e.g., Abercrombie et al.,1990; Robinson et al., 1994). Such phenomenon suggest that mechanisms exist that allow catecholamine neurons to compensate for partial damage. The maintenance of DA content in the face of reduced total TH protein in our study may be an

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example of such a compensatory mechanism, and could be mediated by the activation of pre-existing TH molecules. Support for this hypothesis comes from several reports that GDNF can phosphorylate TH and increase its activity both in vitro (Kobori et al. 2004) and in vivo (Salvatore et al. 2004). A GDNF-induced increase in DA content (Hudson et al., 1995; Beck et al., 1996; Martin et al., 1996, Rosenblad et al., 2003) and in the stimulus-evoked release of DA (Herbert et al., 1996; Herber & Gerhardt, 1997; Hoffman et al., 1997) has also been reported.

3.3 Duration of action of exogenous GDNF

We observed that GDNF immunoreactivity was detectable for at least 8 wks following administration. GDNF staining was maximal at 2 wks post-infusion, the earliest time point examined, and was largely confined to the needle track at 4 and 8 wks. Of course, we do not know if the GDNF detected by immunohistochemistry at 2-8 wks was biologically active. Thus, in addition to its initial effects in protecting DA neurons against 6-OHDA toxicity, the protein may play a long-term role in the dynamic changes in TH, DAT, and VMAT2, or simply set in motion pro-survival intracellular signaling cascades whose effects are manifest over the subsequent several weeks. We also cannot be certain that all of the GDNF we detected was exogenous, since we and others have shown that endogenous GDNF can be increased in response to injury caused by 6-OHDA (e.g., Smith et al., 2003). However, it is unlikely that such injury-induced increases in endogenous GDNF played a role in our studies, since no such effects were observed in our 6-OHDA-treated animals examined 2-8 wks later (see also Stromberg et al., 1993).

As in our study, Lu and Hagg (1997) demonstrated an initial downregulation of TH after GDNF and 6-OHDA that gradually recovered, as well as the protection of DA cells as measured by a retrograde tracer. However, the protection they observed disappeared if GDNF infusion was terminated, which contrasts with our findings. This might reflect the difference in our experimental paradigms. Lu and Hagg (1997) infused 6-OHDA into the medial forebrain bundle, producing a much larger, more rapid loss of nigrostriatal DA neurons than in our striatal infusion model. In addition, the previous investigators infused GDNF chronically and directly into the SN, whereas in our studies a single bolus of GDNF was infused in the striatum. These may be crucial differences as it has been suggested that GDNF infused in the SN is ineffective at protecting axons and axon terminals from 6-OHDA (Kirik et al., 2004). On the other hand, Bowenkamp and coworkers (Bowenkamp et al., 1996), who delivered 6-OHDA into the medial forebrain bundle followed by a single injection of GDNF into the SN 2 wks later, observed long-lasting restoration of DA neurons, as did we.

3.4 Mechanism of GDNF-induced neuroprotection

Our current hypothesis is that GDNF protects from the loss of DA neurons, but that there is an initial downregulation of the DA phenotype, which is only gradually restored. It remains possible that recovery of striatal markers at later timepoints is a result of sprouting. Indeed, GDNF has been shown to produce axonal sprouting in the striatum after a 6-OHDA lesion (Rosenblad et al., 1998; 1999). However, in a preliminary experiment we found no change of growth associated protein-43, a well established marker of sprouting, in either the striatum or SN of animals treated similarly with GDNF and 6-OHDA. Assuming, then, that GDNF acts to rescue DA neurons from an initial impairment induced by 6-OHDA, a critical question for future research will be the mechanism of that rescue. One factor likely to be involved is GDNF-induced activation of several survival cascades, including those involving Ras/ERK and PI3K/Akt, both of which have been shown by several labs including our own to be associated with GDNF-induced protection (Ugarte et al., 2003; Vallegas et al., 2006; Du et al., 2008; Lindgren et al., 2008). This, in turn may protect mitochondria and/or

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enhance antioxidant capacity. For example, GDNF has been shown to increase several antioxidant defense enzymes, including copper zinc superoxide dismutase, glutathione peroxidase and catalase, as well as glutathione (Chao and Lee, 1999) and to decrease 6-OHDA induced oxidative stress (Smith and Cass, 2007).

In conclusion, these data indicate that GDNF protected against the 6-OHDA-induced loss of DA neurons. This appeared to occur in several steps. Initially, there was an immediate protection of DA cell as assessed by FG but a loss of several phenotypic markers – TH, VMAT2, and DAT. At the same time there was maintenance of striatal DA stores at normal levels. Protection of DA neurons and striatal DA stores was followed by the gradual restoration of the phenotypic markers over 4-8 weeks. These results confirm previous reports that GDNF can protect DA neurons against oxidative stress, but that this process is more complex than might have previously been realized. Finally, they suggest that studies of GDNF-induced protection in both experimental models and in patients must take into consideration the specific markers used and the interval between trophic factor delivery and analysis of its effects.

4. Experimental Procedures

4.1 Animals

Male Sprague Dawley rats (Hilltop Lab Animals, Scottdale, PA) weighing 250-350 grams were used in these experiments. All animals were housed two per cage and maintained on 12 h light/dark cycle with food (Purina Lab Chow; Purina Labs, St. Louis, MO) 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 Committee at the University of Pittsburgh.

4.2 Surgical Procedures

Seven days prior to GDNF and/or 6-OHDA infusion, animals were anesthetized with isoflurane (1-2% in 100% O_2; Halocarbon, River Edge, NJ) and Fluoro-gold (FG) (Fluorochrome, Denver, CO; 2% in sterile saline; 0.2 μl at 0.05 μl/min) was infused into the right striatum (+0.7 mm anterior, −3.1 mm lateral of bregma, and 6.0 mm ventral to dura) according to the atlas of Paxinos and Watson (1982). One week after FG infusion, animals were again deeply anesthetized with isoflurane and 9 μg/3 μl GDNF (Amgen, Thousand Oaks, CA) or 3 μl of its vehicle (citrate buffer, pH 7.2) was infused into the right striatum at 0.5 μl/min at the same coordinates as FG. Six hours following GDNF administration animals were again deeply anesthetized with isoflurane and 6-OHDA (4 μg/0.75 μl at 0.5 μl/min; Regis, Morton Grove, IL) or vehicle (0.02% ascorbate in 0.9% sterile saline; Sigma, St. Louis, MO) was infused into the right striatum at the same coordinates as above.

4.3 Histological Analysis

At 2, 4, or 8 wks after 6-OHDA administration, animals were deeply anesthetized with Equithesin and sacrificed via transcardial perfusion using ice cold saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.6. Following 48 hr cryoprotection in 30% sucrose, brains were sectioned at 60 μm on a cryostat at −20°C. Coronal slices were collected in a one-in-six series. Every sixth section cut from the SN was labeled for TH and every sixth section from the striatum was labeled for TH, vesicular monoamine transporter 2 (VMAT2), DA transporter (DAT), and GDNF.

Immunohistochemistry—All sections were rinsed 3 times in 10 mM phosphate buffered saline (PBS), pH 7.6, prior to and between each incubation. PBS with 0.3% triton-X 100 was used as the diluent for all treatments unless specified otherwise. Sections were

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pretreated for 15 min with 1% H_2O_2 in PBS followed by blocking for 1 hr with 10% normal donkey serum. Primary antibody incubations occurred on a rotator overnight at 4°C using a 1:1000 dilution of mouse anti-TH antibody (Chemicon Inc, Temecula, CA, Cat. #MAB318); a 1:250 dilution of goat anti-VMAT2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, Cat.#SC7721); a 1:500 dilution of rat anti-DAT antibody (Chemicon Inc, Temecula, CA, Cat. #MAB369); or a 1:1000 dilution of a goat anti-GDNF antibody (R&D systems, Minneapolis, MN, Cat. #AF-212-NA) with 1% of the appropriate normal serum. This was followed by incubation for 1 hr at room temperature in a 1:200 dilution of the appropriate biotinylated secondary antibody (Jackson ImmunoResearch, West Grove, PA). Tissue was then treated with avidin biotin peroxidase complex (ABC-Elite, Vector Laboratories, Burlingame, CA) and subsequently with 0.02% 3,3-diaminobenzadine as the chromogen. Sections were mounted on gelatin coated slides and dehydrated in ascending concentrations of ethanol before being rinsed in xylenes and coverslipped with Permount mounting medium (Fisher Scientific, Pittsburgh, PA).

Double immunofluorescent labeling in SN—To visualize FG and TH in the same neurons, immunofluorescent labeling was utilized. Sections were treated with 10% normal goat serum for 1 hr and then incubated overnight at 4°C in a 1:1000 dilution of mouse anti-TH antibody. Sections were subsequently labeled with Alexafluor 488 (goat anti-mouse; Molecular Probes, Invitrogen, CA) at a concentration of 1:500 in PBS for 2 hrs at room temperature, washed, mounted, coverslipped in DPX mounting medium (Fisher Scientific, Pittsburgh, PA), and viewed under epifluorescent illumination on a Nikon Inverted Eclipse TE microscope (Nikon Inc., Melville, NY)

4.4 Image Analysis

Lesion area in striatum—Quantification of lesion area was performed in the striatum using MetaMorph software (Molecular Devices Corp., Downington, PA). For quantification of the area devoid of immunoreactivity, the entire coronal section was visualized using a Nikon Supercool scanner (Nikon Inc., Melville, NY). Images were then pseudocolored in MetaMorph, the lesion area on the three sections exhibiting the largest loss was circumscribed in blind fashion, and the area within this region calculated as mm^2 by MetaMorph software. The average of these three sections was used as a representation of lesion area for a given animal.

Cell counts in SN—For cell counts in the SN, one of every six sections from each animal was randomly selected by Microsoft Excel for staining. We then counted all FG^+, TH^+, and co-labeled cells in three sections of the SN at 360 μm intervals.

4.5 HPLC Analysis

Animals were sacrificed via decapitation and a 2 mm section of striatum was dissected centered on the needle track made by prior injections. Dissected striata were assayed using minor modifications of previous method (Smith et al. 2003). Striatal tissue was suspended in 0.1 N $HClO_2$, homogenized and centrifuged at 16,000 × g for 20 min at 4°C, and the supernatant was removed. Tissue samples were assayed for DA by injecting a 10 μl aliquot of the sample onto a reverse phase column (2.0 × 150 mm, ESA Inc., Chelmsford, MA). The mobile phase consisted of 50 mM H_2NaPO_4, 0.72 mM sodium octyl sulfate, 0.075 mM Na_2EDTA and 16% methanol (v/v), pH 2.7. The mobile phase was pumped through the system at 0.3 ml/min using an ESA 580 pump (ESA Inc., Chelmsford, MA). Analyses were performed using an ESA Coulochem Model 4100A detector, an ESA Model 5010 conditioning cell, and an ESA Model 5014B microdialysis cell (ESA, Inc., Chelmsford, MA). The settings for detection were E_1=−75mV, E_2=+220mV, and guard cell=+350mV. The limits of detection for DA were in the low femtomole range.

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4.6 Statistical Analysis

Data were analyzed with a two-way ANOVA, using Bonferonni-corrected multiple comparisons for post hoc analysis. There was no significant difference between the lesion area in striatum of animals treated only with 6-OHDA at the 2 (n = 9), 4 (n = 8), and 8 wk time points (n = 6); therefore these groups were pooled for further analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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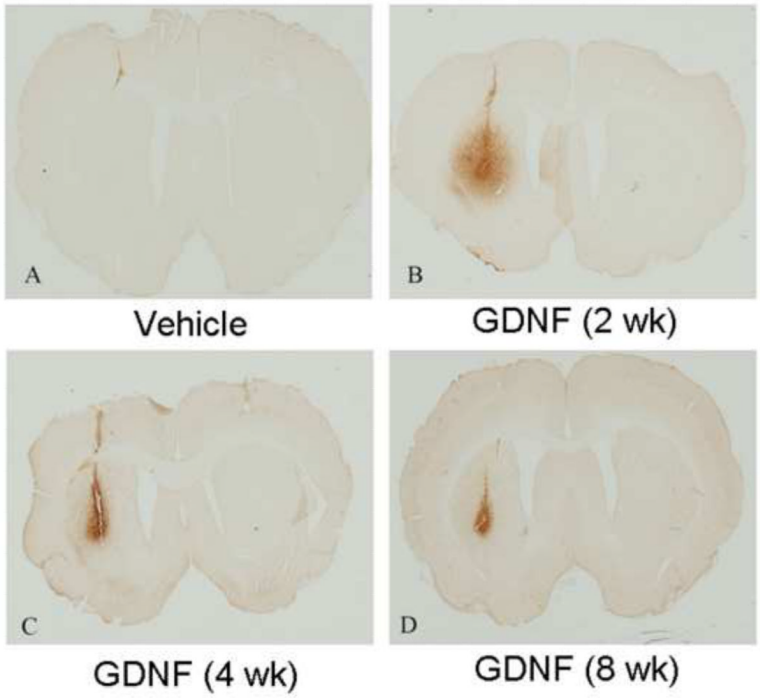

Figure 1.
Photomicrographs of GDNF-immunoreactivity in the striatum. Vehicle animals (a) showed no GDNF immunoreactivity in the striatum, while GDNF immunoreactivity was present in the striatum of GDNF (2 wk) (b), GDNF (4 wk) (c), and GDNF (8 wk) (d) treated animals.

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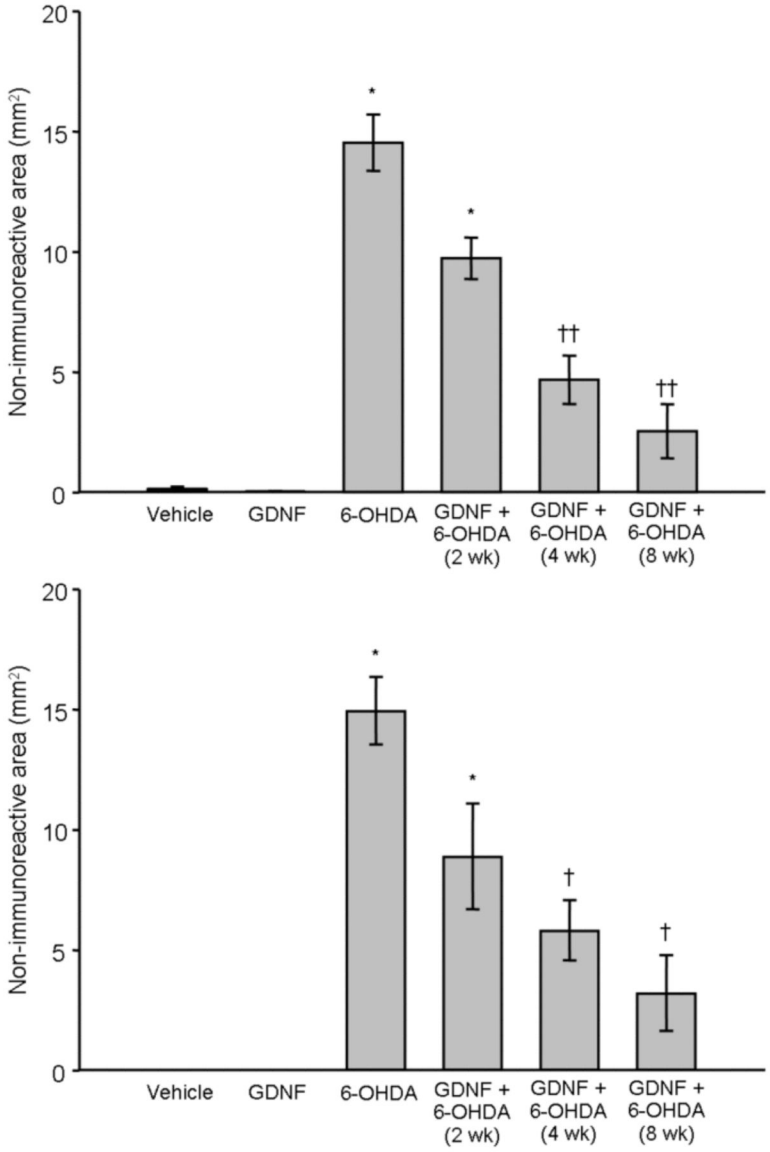

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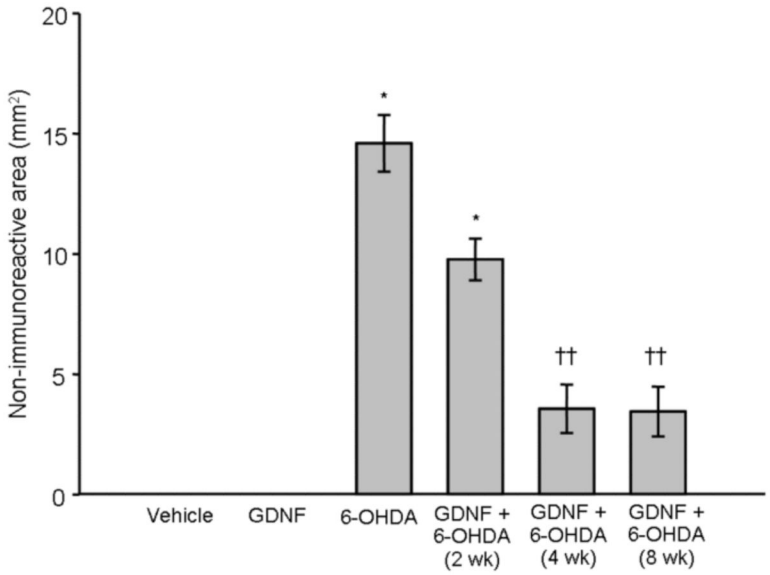

Figure 2.

Effects of GDNF on phenotypic markers of DA terminals in striatum. Significant loss of TH (panel A), DAT (panel B), and VMAT2 (panel C), was observed in the striatum after 6-OHDA (* $p < 0.01$ vs. vehicle and GDNF). Animals given 6-OHDA with GDNF displayed significant loss of these markers after 2 wks when compared to animals that just received vehicle or GDNF (* $p < 0.01$). However, this 6-OHDA-induced loss was greatly attenuated by GDNF at 4 and 8 wks (†$p < 0.01$; ††$p < 0.001$ vs. 6-OHDA alone) and was not statistically different from animals treated with just vehicle or GDNF. All values are expressed as average lesion area in mm^2 ± SEM.

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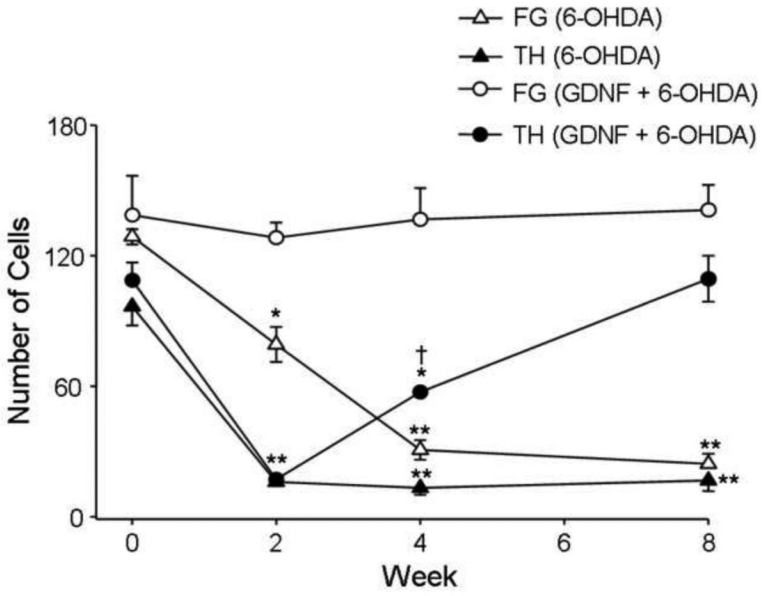

Figure 3.

Effects of GDNF on loss of TH^+ and FG^+ cells in the SN after 6-OHDA. Since no significant effect of vehicle treatment was observed at any time, time 0 represents the mean values for all vehicle treated animals sacrificed at 2, 4, and 8 wks. There was a significant loss of TH^+ cells in the SN at 2, 4 and 8 wks post infusion in animals treated with only 6-OHDA when compared to vehicle treated animals depicted as time 0 (**$p < 0.001$). Infusion of GDNF did not prevent a 6-OHDA-induced loss of TH^+ cells when assessed at 2 wks compared to vehicle treated animals (**$p < 0.001$). However, by 4 wks the number of TH^+ cells in the animals given both GDNF and 6-OHDA was significantly higher than that at 2 wks (†$p < 0.05$), although it was still significantly lower than vehicle (*$p < 0.05$). By 8 wk, no significant loss of TH^+ cells was observed in 6-OHDA-treated animals given GDNF when compared to vehicle treated animals. No significant loss of FG^+ cells in the SN was observed at any time in animals given 6-OHDA and GDNF. All values are expressed average number of cells ± S.E.M.

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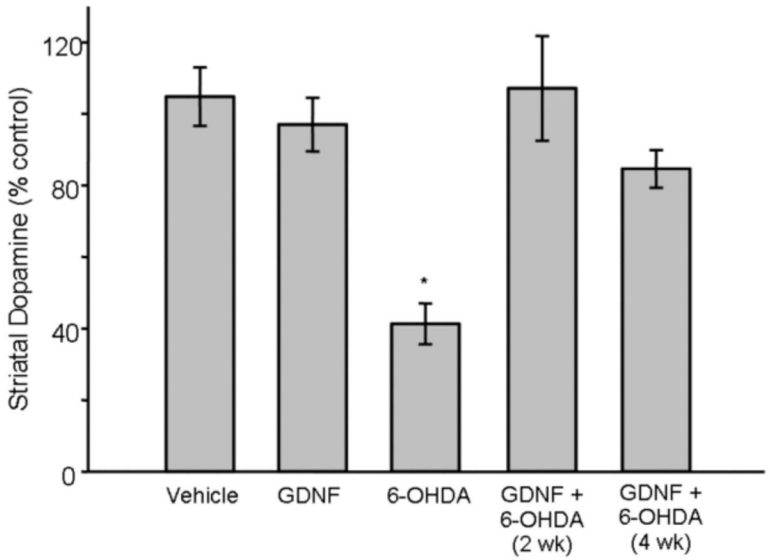

Figure 4.

Effects of GDNF on DA content in the striatum after 6-OHDA. 6-OHDA-treated animals displayed a significant decrease in DA content (* $p < 0.01$). An injection of GDNF 6 hr prior to 6-OHDA infusion into the same location prevented this loss of DA content as assessed 2 and 4 wks later. Vehicle and GDNF animals showed no significant loss of DA content in the striatum. All values are expressed as a percent of loss of DA content compared to the contralateral side ± SEM.