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Neurturin Protects Against 6-Hydroxydopamine-Induced Reductions in Evoked Dopamine Overflow in Rat Striatum

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Abstract

Neurturin (NTN), a member of the glial cell line-derived neurotrophic factor (GDNF) family, has substantial effects on normal and lesioned nigrostriatal dopamine systems. However, its ability to protect against toxin-induced loss of striatal dopamine release has not been previously reported. The goal of the present study was to determine if NTN could protect against 6-hydroxydopamine (6-OHDA)-induced reductions in striatal dopamine overflow and tissue levels of dopamine, and to compare the effects of NTN with those of GDNF. Male Fischer-344 rats were given a single injection of vehicle, or 5 µg NTN or GDNF, into the right striatum. The following day the animals were given a single injection of 12 µg 6-OHDA into the striatum at the same site where the trophic factor was injected. Microdialysis experiments conducted three weeks later indicated that the 6-OHDA decreased basal levels of dopamine and metabolites in the lesioned striatum compared to the contralateral striatum, and NTN was able to partially protect against the 6-OHDA-induced reductions. Injection of NTN one day prior to 6-OHDA also led to significant protection against loss of both potassium and amphetamine evoked overflow of dopamine. The NTN treatments partially protected against 6-OHDA-induced reductions in striatal tissue levels of dopamine, and completely protected against loss of nigral dopamine content. The protective effects of NTN were similar in magnitude to those of GDNF. These results support that within the experimental parameters used in this study, NTN is as effective as GDNF in protecting against the dopamine-depleting effects of intrastriatal 6-OHDA.

Keywords

Neurturin; Striatum; Substantia Nigra; Dopamine; GDNF; 6-OHDA; Microdialysis

Numerous in vitro and in vivo studies have demonstrated that glial cell line-derived neurotrophic factor (GDNF) has robust effects on midbrain dopaminergic neurons (for example: Lin et al., 1993; Hudson et al., 1995; Hebert et al., 1996; Martin et al., 1996; Kobayashi et al., 1998; Bourque and Trudeau, 2000; Rosenblad et al., 2003; Salvatore et al., 2004 and 2009a). Because of its striking effects on nigrostriatal dopamine (DA) neurons in normal animals, GDNF has also been extensively evaluated in animal models as a potential therapeutic agent for Parkinson's disease. It has demonstrated both neuroprotective and neurorestorative effects in these models (Cass et al., 1998; Gash et al., 1998; Bjorklund et al.,

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2000; Kordower et al., 2000; Grondin et al., 2002), and there has been some success in two open-label clinical trials (Gill et al., 2003; Slevin et al., 2005). However, results from a larger double-blind controlled trial were disappointing due to a lack of substantial clinical benefits (Lang et al., 2006).

Neurturin (NTN), another member of the GDNF family of trophic factors (Kotzbauer et al., 1996), also has potent effects on the nigrostriatal DA system of normal, intact animals (Horger et al., 1998; Hoane et al., 1999; Hamilton et al., 2001; Cass and Peters, 2010). In addition, NTN has demonstrated protective and restorative effects for DA neurons in animal models of Parkinson's disease and aging (Tseng et al., 1998; Rosenblad et al., 1999; Oiwa et al., 2002; Kordower et al., 2006; Gasmi et al., 2007a and b; Herzog et al., 2007; Grondin et al., 2008). While in some studies the neuroprotective effects of NTN and GDNF are analogous, this is not the case in all studies. For instance, Horger et al. (1998) and Gasmi et al., (2007b) found that NTN and GDNF were equally effective in protecting against 6-hydroxydopamine (6-OHDA) lesions; Tseng et al. (1997 and 1998) found that both trophic factors protect DA neurons following medial forebrain bundle transection, but that only GDNF reduced rotation behavior; and Rosenblad et al. (1999) report that GDNF is more effective than NTN at protecting against 6-OHDA-induced nigral cell death. Thus, the literature indicates that there are both similarities and differences with regard to the protective effects of NTN and GDNF.

Several studies have demonstrated that NTN can partially protect against lesion-induced loss of nigral DA neurons (Horger et al., 1998; Rosenblad et al., 1999; Tseng et al., 1998; Akerud et al., 1999; Oiwa et al., 2002; Kordower et al., 2006; Gasmi et al., 2007a and b). However, the ability of NTN to protect against toxin-induced loss of striatal DA release has not been previously reported. The purpose of the present study was to determine if NTN could protect against 6-OHDA-induced reductions in striatal DA overflow and tissue levels of DA, and to compare the effects of NTN with those of GDNF. In vivo microdialysis was used to evaluate basal extracellular levels of DA and its primary metabolites, and stimulus-evoked overflow of DA, from the striatum of rats treated with NTN or GDNF one day before receiving a unilateral 6-OHDA lesion. Following the microdialysis experiments the protective effects of the trophic factors were further evaluated by analysis of postmortem tissue levels of DA in the striatum and substantia nigra.

1. Experimental Procedures

1.1. Animals

Young adult male Fischer-344 rats were obtained from Harlan Laboratories (Indianapolis, IN). The animals were 4–5 months old at the start of the experiments and 7–8 animals were included in each treatment group. The animals were housed in pairs under a 12-hr light-dark cycle with food and water freely available. All animal use procedures were approved by the Animal Care and Use Committee at the University of Kentucky and were in strict accordance with National Institutes of Health guidelines. All efforts were made to minimize the number of animals used and to minimize their pain and discomfort.

1.2. Trophic factor and 6-OHDA injections

All surgeries were performed using sterile procedures. The animals were anesthetized with isoflurane (2.0–2.5% as needed) and positioned in a stereotaxic apparatus. The skull was exposed and a small hole drilled in the skull over the right striatum (1.0 mm anterior to bregma, 2.8 mm lateral from midline). NTN (5 μ g in 5 μ l vehicle), GDNF (5 μ g in 5 μ l vehicle), or 5 μ l vehicle (10 mM citrate buffer with 150 mM NaCl, pH 5.0) was injected into the striatum (5.0 mm below the surface of the cortex) using a microliter syringe with a 26 gauge blunt tapered needle. The rate of injection was 0.5 μ l/min and the needle was left in place for an

additional 5 minutes following the injection. The NTN and GDNF were purchased from PeproTech Inc. (Rocky Hill, NJ). Both trophic factors were recombinant human proteins (from *E. coli*) with a purity greater than 98% (SDS-PAGE and HPLC, PeproTech product information sheets). The incision was closed with wound clips and the animals allowed to recover.

Twenty-four hours after trophic factor injection the animals were again anesthetized with isoflurane and given a single injection of 12 μ g 6-OHDA (Sigma-Aldrich, St. Louis, MO) in 4 μ l 0.9 % saline with 0.1 % ascorbic acid (pH 5.5) into the right striatum at the same site where the trophic factor was injected. The twenty-four hour interval between injections was chosen as it was effective in previous studies where GDNF was found to protect against dopaminergic toxins (Tomac et al., 1995; Cass 1996; Aoi et al., 2000; Cass et al., 2004). The 6-OHDA solution was injected over 5 minutes and the needle was left in place for an additional 5 minutes before being withdrawn. Gelfoam was placed in the burr hole and the incision closed with wound clips.

1.3. In Vivo Microdialysis

Microdialysis studies were conducted three weeks after the 6-OHDA injections. This interval was chosen as it is a time point when a significant amount of neurodegeneration has occurred (Sauer and Oertel, 1994; Kirik et al., 1998), and when significant changes in DA release and content have been demonstrated in rats following GDNF injection (Hebert et al., 1996). The animals were anesthetized with urethane (1.25–1.50 g/kg, i.p.) and positioned in a stereotaxic frame. Body temperature was maintained at 37°C with a heating pad coupled to a rectal thermometer. Microdialysis probes (CMA/11 probes, 3.0 mm length of dialysis membrane; CMA/Microdialysis, Acton, MA) were slowly lowered into both the left and right striata (0.0 mm anterior to bregma, 3.0 mm lateral from midline, tip of probe 6.3 mm below the surface of the brain). The probes were perfused at a rate of 1.2 μ l/min with artificial cerebrospinal fluid containing 145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, 1.0 mM MgCl₂, 0.2 mM ascorbic acid, and 2.0 mM NaH₂PO₄ (pH 7.4). Dialysate fractions were collected at 20-min intervals. Following a two-hour equilibration period and the collection of 3 baseline fractions, DA overflow was stimulated by increasing the potassium concentration in the perfusate to 100 mM (NaCl reduced to 47.7 mM) for a single 20-min fraction, and then two hours later by adding 100 μ M d-amphetamine to the perfusate for a single 20-min fraction. Five final fractions with normal artificial cerebrospinal fluid were collected following the d-amphetamine stimulation. Dialysate samples were immediately frozen on dry ice and stored at –80°C until assayed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).

1.4. Tissue collection and HPLC analysis

After collecting the dialysate fractions the urethane-anesthetized animals were killed by decapitation and their brains rapidly harvested and chilled in ice-cold saline. A coronal slice of brain 2 mm thick at the level of the dialysis probes was made with the aid of an ice-chilled brain mold (Rodent Brain Matrix, ASI Instruments, Warren, MI). The location of all dialysis probes was confirmed to be centered in the dorsal striatum at the level of, or just rostral to, the crossing of the anterior commissure. The site of the intrastriatal injections was also visible and was confirmed to be located in the dorsal striatum. The striatum was then dissected from each half of the slice. The substantia nigra was dissected from both sides of a 2 mm thick coronal slice through the midbrain. The tissue pieces were placed in preweighed vials, weighed, and frozen on dry ice. Samples were stored at –80°C until assayed for DA by high performance liquid chromatography (HPLC) as previously described (Cass et al., 2003). For dialysis samples, 20 μ l of the dialysate was injected directly onto the column.

1.5. Data analysis

All dialysis probes were calibrated in vitro prior to use to determine acceptable probes (recovery of DA at least 12%). However, values were not corrected for in vitro recoveries as uncorrected values may be better correlated to true values (Glick et al., 1994). Basal levels of DA and metabolites were defined as the average value in the three fractions preceding stimulation by excess potassium. Dialysis data were expressed as nM concentration of DA or metabolite in the dialysate and, for evoked overflow, as the total amount of DA in the dialysate above baseline following stimulation with potassium or amphetamine. Tissue levels of DA were expressed as ng/g wet weight of tissue. Results were analyzed statistically using mixed analyses of variance (ANOVA). When the ANOVA results indicated significant effects, Newman-Keuls tests were used for post hoc comparisons.

2. Results

2.1. Dialysate levels of DA and metabolites

The 6-OHDA treatments had significant effects on basal dialysate levels of DA, DOPAC, and HVA (Table 1). In the Vehicle + 6-OHDA treated animals there was a significant decrease in basal levels of DA of 36% ($p < 0.05$) on the lesioned side of the brain compared to the contralateral side. The decreases observed in basal DA levels on the lesioned side of the NTN (-16%) and GDNF (-23%) treated groups did not reach statistical significance. DOPAC levels were decreased in the lesioned striatum, compared to the non-lesioned contralateral striatum, by 82%, 42%, and 50% respectively in the vehicle, NTN, and GDNF treated animals ($p < 0.001$ for all 3 groups). In addition, basal levels of DOPAC on the lesioned side of the NTN and GDNF treated animals were greater than those from the vehicle treated animals ($p < 0.01$ for both). The effects of trophic factor and 6-OHDA on basal HVA levels were similar to the effects on DOPAC. Compared to the non-lesioned contralateral striatum, HVA levels were decreased in the lesioned striatum of the vehicle, NTN, and GDNF treated animals by 67%, 28%, and 40%, respectively ($p < 0.01$ for all 3 groups). Basal levels of HVA on the lesioned side of the NTN and GDNF treated animals were greater than those from the vehicle treated animals ($p < 0.05$ for both).

Time courses for DA levels from the in vivo microdialysis experiments are shown in Fig. 1. Within each treatment group, overall DA levels on the lesioned side were lower than those from contralateral side ($p < 0.001$ for all groups); and on the lesioned side, DA levels from the NTN and GDNF groups were greater than those from the vehicle group ($p < 0.05$ for both). In order to facilitate comparisons of the evoked overflow of DA between the groups and sides of the brain the data were expressed as total amount of DA in the dialysate fractions above basal levels following stimulation by excess potassium or d-amphetamine (Fig. 2). Potassium-evoked DA overflow was reduced on the lesioned side of the brain by 88% in the vehicle treated rats, by 42% in the NTN treated rats, and by 58% in the GDNF treated rats ($p < 0.001$ for all 3 groups). Amphetamine-evoked DA overflow was reduced on the lesioned side of the brain by 77% in the vehicle treated rats ($p < 0.001$), by 21% in the NTN treated rats ($p < 0.05$), and by 41% in the GDNF treated rats ($p < 0.001$). In addition, for both potassium and amphetamine evoked overflow, the 6-OHDA-induced reduction in DA overflow was significantly less in the NTN and GDNF treated animals compared to the vehicle treated animals ($p < 0.05$).

The time course data from the in vivo microdialysis experiments for DOPAC and HVA are shown in Figs. 3 and 4, respectively. As expected, the 6-OHDA treatments led to reduced levels of both metabolites on the lesioned side of the brain compared to the contralateral side for all treatment groups ($p < 0.01$ for all groups). However, on the lesioned side, metabolite levels were significantly greater in the NTN and GDNF treated animals compared to the vehicle treated controls for both DOPAC ($p < 0.01$) and HVA ($p < 0.05$).

2.2. Tissue levels of DA

The intrastriatal injection of trophic factor and 6-OHDA affected postmortem levels of DA in both the striatum and substantia nigra (Fig. 5). Compared to the contralateral side, striatal DA levels on the lesioned side were decreased by 80% in the vehicle + 6-OHDA treated animals, by 52% in the NTN + 6-OHDA treated animals, and by 51% in the GDNF + 6-OHDA treated animals ($p < 0.001$ for all 3 groups). The decrease in DA levels in the NTN and GDNF treated animals was significantly less than the decrease in the vehicle treated animals ($p < 0.05$ for both). In the substantia nigra DA content was reduced by 40% on the injected side of the vehicle + 6-OHDA treated rats ($p < 0.001$). However, there was no reduction in DA levels on the treated side of the NTN and GDNF treated rats. In addition, DA content in the substantia nigra on the lesioned side of the vehicle treated rats was significantly lower than that in the NTN and GDNF treated rats ($p < 0.01$ for both).

3. Discussion

The present study found that injection of NTN attenuated 6-OHDA-induced decreases in basal extracellular levels of striatal DA and metabolites, in potassium- and amphetamine-evoked overflow of DA from the striatum, and in striatal tissue levels of DA. In addition NTN completely prevented 6-OHDA-induced reductions in nigral tissue levels of DA. These results indicate that a single intrastriatal injection of NTN significantly protects against the effects of an intrastriatal 6-OHDA lesion on striatal DA release and nigrostriatal DA content. Furthermore, the protective effects of NTN were similar in magnitude to those of GDNF and suggest that, within the experimental parameters used in this study, NTN is as effective as GDNF in protecting against 6-OHDA.

This is the first study to document the ability of NTN to protect against neurotoxin-induced decreases in DA release in the striatum. However, several previous studies have reported on similar protective effects of GDNF. Direct intracerebral injection of GDNF (Opacka-Juffry et al., 1995; Cass and Manning, 1999), and delivery of adenoviral vector encoding for GDNF (Gerin, 2002; Smith et al., 2005), have provided partial protection against 6-OHDA-induced reductions in basal and stimulus-evoked overflow of striatal DA. In the current study both NTN and GDNF partially protected against the effects of 6-OHDA on both calcium-dependent overflow of DA (K^+ stimulation) and calcium-independent overflow of DA (amphetamine stimulation), suggesting that the protective effects encompass both vesicular exocytotic release processes and cytosolic stores of DA. Another factor that could play a role in the increased DA overflow in the trophic factor treated rats is that the NTN and GDNF may be upregulating synthesis, storage and release of DA in remaining terminals that were not as severely damaged by exposure to the 6-OHDA. It has been demonstrated that both NTN and GDNF can alter synthesis and release of DA. For example, NTN and GDNF have been shown to increase DA turnover (DOPAC/DA and HVA/DA ratios) following injection into the striatum (Horger et al., 1998; Hadaczek et al., 2010) and GDNF has been shown to increase the amount of DA released per synaptic vesicle from cultured midbrain neurons (Pothos et al., 1998). Thus, it is possible that some of the protective effects observed in this study may be due to upregulation of dopaminergic processes in remaining terminals. Still another change that could lead to increases in extracellular levels of DA would be inhibition of DA transporter (DAT) activity in the striatum. However, studies arguing against this last mechanism as contributing substantially to the present results are a recent paper demonstrating that high (100 μ g), but not lower (30 μ g), doses of GDNF reduce DAT protein levels in the striatum (Salvatore et al., 2009a), and an earlier report showing that neuroprotective injections of GDNF lead to increases in DA clearance in the striatum (Cass and Manning, 1999), which suggests an increase in DAT activity.

Both NTN and GDNF led to partial protection against 6-OHDA-induced loss of striatal DA levels, but to complete protection against loss of nigral DA content. While a role for the striatum in locomotor activity has been well established, nigral DA may also play an important role in modulating motor activity (Robertson and Robertson, 1989; Trevitt et al., 2001; Gerhardt et al., 2002; Andersson et al., 2006; Salvatore et al., 2009b), and GDNF can increase somatodendritic release of DA in the substantia nigra (Hoffman et al., 1997). Thus, protection of nigral DA levels following intrastriatal NTN or GDNF administration, in addition to protection of striatal DA release and content, is an important component of the overall protective effects of these trophic factors that may have behavioral relevance.

Other studies, using a variety of models and endpoints, have reported that NTN can protect to various degrees against lesions of the nigrostriatal system. In rodents the effects of NTN include protection against 6-OHDA-induced, and medial forebrain bundle axotomy-induced, reductions in tyrosine hydroxylase (TH) positive nigral neurons (Horger et al., 1998; Rosenblad et al., 1999; Tseng et al., 1998; Akerud et al., 1999; Oiwa et al., 2002; Gasmi et al., 2007a and b), protection against loss of striatal DA levels (Oiwa et al., 2002) and reductions in rotation behavior (Oiwa et al., 2002). In rhesus monkeys treated with the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, NTN treatment improved motor function, preserved TH+ nigral neurons, and preserved striatal TH immunoreactivity (Kordower et al., 2006); and in aged rhesus monkeys NTN treatment increased TH+ fiber staining in the striatum and cell number in the nigra (Herzog et al., 2007). However, it should be pointed out that the degree of protection observed in these studies depends on numerous experimental variables. For example, multiple intrastriatal injections of NTN starting one day after a striatal 6-OHDA lesion, a time point at which significant degenerative changes are already occurring in the striatum (Jenkins et al., 1993; Mladenovic et al., 2004; Smith and Cass, 2007), partially protected nigral TH+ cell bodies but did not preserve striatal TH+ fiber density or reduce amphetamine-induced rotations (Rosenblad et al., 1999); while a single injection of NTN into the striatum 3 days before 6-OHDA partially protected nigral TH+ cell bodies, partially preserved striatal DA levels, and decreased methamphetamine-induced rotations (Oiwa et al., 2002). In the current study the intrastriatal injection of NTN one day prior to intrastriatal 6-OHDA led to partial protection against 6-OHDA-induced decreases in evoked DA release and tissue DA content in the striatum, and to complete protection for nigral DA levels. Thus, many factors, such as timing, duration, and site of NTN administration, appear to play major roles in the overall protective effects of NTN.

Previous neuroprotection studies that examined the effect of direct injections of NTN into the striatum or nigra used doses of NTN in the low microgram range (1, 5, or 10 µg) (Horger et al., 1998; Rosenblad et al., 1999; Oiwa et al., 2002). For the current study we chose a midrange dose (5 µg) that has been shown to promote nigral TH+ cell survival following either single (Oiwa et al., 2002) or multiple (Rosenblad et al., 1999) NTN injections. It should also be noted that, although we injected equal weights of NTN and GDNF, the molar amount of NTN injected was approximately 28% higher than that of GDNF as the molecular weight of NTN (~23.6 kDa) is less than that of GDNF (~30.4 kDa). Furthermore, we compared a single dose of NTN and GDNF. Thus, it is possible that a complete dose response for both NTN and GDNF could identify doses of one trophic factor that provide greater protection than the other factor.

NTN and GDNF signal through a multi-component receptor complex that includes a cell surface bound receptor that belong to the GDNF family receptor (GFR) system, and the RET tyrosine kinase signaling protein. NTN preferentially interacts with GFR α -2 receptors, but it can also activate GFR α -1 receptors; while GDNF preferentially binds to GFR α -1 receptors but can also interact with GFR α -2 receptors (Baloh et al., 2000; Airaksinen and Saarma, 2002). Both GFR α -1 and GFR α -2 receptor expression have been demonstrated in the substantia nigra, and GFR α -1 expression has been documented in the striatum; however, there does not appear

to be significant expression of GFR α -2 in the striatum (Widenfalk et al., 1997; Golden et al., 1998; Burazin and Gundlach, 1999; Kozłowski et al., 2004). At the dose examined, we found similar protective effects of NTN and GDNF when injected into the striatum. Thus, it is possible that both NTN and GDNF are producing their effects via GFR α -1 receptors. Other investigators that have found similar results following NTN and GDNF treatments have also suggested that both trophic factors are working through similar receptor components (Horger et al., 1998; Akerud et al., 1999).

There are several other in vivo studies that have compared the neuroprotective effects of NTN and GDNF. Rosenblad and colleagues used an intrastriatal 6-OHDA lesion model and treated the animals every third day for three weeks after the lesion with intrastriatal or intraventricular injections of NTN or GDNF (Rosenblad et al., 1999). They found that multiple injections of GDNF at either site were more effective than similar injections of NTN at protecting against 6-OHDA-induced loss of TH+ nigral neurons, and point out that potential differences in solubility and diffusion between NTN and GDNF may help in explaining the lower effectiveness of NTN. Akerud et al. (1999) injected fibroblasts genetically engineered to deliver GDNF or NTN into the substantia nigra one day before a 6-OHDA lesion of the medial forebrain bundle. They found that both NTN and GDNF partially protected against 6-OHDA-induced loss of nigral TH+ neurons, but that only GDNF increased the intensity of TH staining and induced sprouting of dopaminergic neurons around the graft. Tseng and coworkers implanted polymer encapsulated cells genetically engineered to release NTN or GDNF near the substantia nigra 7 days before transection of the medial forebrain bundle (Tseng et al., 1997 & 1998). They reported that both trophic factors protected against loss of TH+ nigral neurons, but that only GDNF reduced amphetamine-induced rotation behavior. Fjord-Larsen et al. (2005), using lentiviral gene transfer, found that a modified NTN construct, but not wild type NTN, was as protective against 6-OHDA as GDNF. Horger et al. (1998) injected NTN or GDNF into the substantia nigra seven days after an intrastriatal injection of 6-OHDA and found that NTN was as effective as GDNF at promoting survival of DA neurons. Gasmi et al. (2007a and b) reported similar protection against 6-OHDA-induced loss of nigral TH+ neurons when either NTN or GDNF was delivered into the striatum using adeno-associated virus based vectors two weeks before the toxin. In the present study we found that NTN was as effective as GDNF in protecting against 6-OHDA-induced reductions in striatal DA overflow and reductions in tissue levels of DA. Thus, although some differences have been noted, previous studies and the current study document many similarities between the neuroprotective effects of NTN and GDNF. Taken together, these results suggest that NTN, like GDNF, may have substantial therapeutic potential for the treatment of disorders involving dopaminergic dysfunction.

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Figure 1.

Dialysate levels of DA from the left and right striata of animals injected in the right striatum with vehicle, NTN or GDNF one day before an injection of 6-OHDA into the right striatum. Microdialysis experiments were performed three weeks after the 6-OHDA injections. Excess potassium (100 mM) was included in the perfusate for 20-min starting at 0 min (horizontal bar above K^+), and 100 μ M amphetamine was included in the perfusate for 20-min starting at 120 min (horizontal bar above Amphetamine). Values shown are mean $\pm$ SEM from 7–8 animals per group. * $p < 0.001$ vs. left striatum, # $p < 0.05$ vs. right striatum of Vehicle + 6-OHDA group (mixed ANOVA with side of brain and time of dialysis sample collection as within factors, and treatment group as a between factor; followed by Newman-Keuls post-hoc comparisons).

Figure 2.

Potassium-evoked (top panel) and amphetamine-evoked (bottom panel) overflow of DA from the striatum of animals injected in the right striatum with vehicle, NTN or GDNF one day before an injection of 6-OHDA into the right striatum. Microdialysis experiments were performed three weeks after the injections. Values shown are mean $\pm$ SEM from 7–8 animals per group. * $p < 0.05$ vs. left side of same group, # $p < 0.05$ vs. right side of Vehicle + 6-OHDA group (mixed ANOVA with side of brain as a within factor and treatment as a between factor; followed by Newman-Keuls post-hoc comparisons).

Figure 3.

Dialysate levels of DOPAC from the left and right striata of animals injected in the right striatum with vehicle, NTN or GDNF one day before an injection of 6-OHDA into the right striatum. Microdialysis experiments were performed three weeks after the 6-OHDA injections. Excess potassium (100 mM) was included in the perfusate for 20-min starting at 0 min (horizontal bar above K^+), and 100 μ M amphetamine was included in the perfusate for 20-min starting at 120 min (horizontal bar above Amphetamine). Values shown are mean $\pm$ SEM from 7–8 animals per group. * $p < 0.001$ vs. left striatum, # $p < 0.01$ vs. right striatum of Vehicle + 6-OHDA group (mixed ANOVA with side of brain and time of dialysis sample collection as

within factors, and treatment group as a between factor; followed by Newman-Keuls post-hoc comparisons).

Figure 4.

Dialysate levels of HVA from the left and right striata of animals injected in the right striatum with vehicle, NTN or GDNF one day before an injection of 6-OHDA into the right striatum. Microdialysis experiments were performed three weeks after the 6-OHDA injections. Excess potassium (100 mM) was included in the perfusate for 20-min starting at 0 min (horizontal bar above K⁺), and 100 μ M amphetamine was included in the perfusate for 20-min starting at 120 min (horizontal bar above Amphetamine). Values shown are mean $\pm$ SEM from 7–8 animals per group. * $p < 0.01$ vs. left striatum, # $p < 0.05$ vs. right striatum of Vehicle + 6-OHDA group (mixed ANOVA with side of brain and time of dialysis sample collection as within factors, and treatment group as a between factor; followed by Newman-Keuls post-hoc comparisons).

Figure 5.

Postmortem tissue levels of DA from the striatum and substantia nigra of animals injected in the right striatum with vehicle, NTN or GDNF one day before an injection of 6-OHDA into the right striatum. Tissue was harvested three weeks after the injections. Values shown are mean $\pm$ SEM from 7–8 animals per group. * $p < 0.001$ vs. left side of same group, # $p < 0.05$ vs. right side of Vehicle + 6-OHDA group (mixed ANOVA with side of brain as a within factor and treatment group as a between factor; followed by Newman-Keuls post-hoc comparisons).

Table 1

Basal dialysate levels of DA, DOPAC and HVA from the striatum of animals injected in the right striatum with vehicle, NTN or GDNF one day before an injection of 6-OHDA into the right striatum. Dialysis experiments were performed three weeks after the 6-OHDA treatments.

Group	Dialysate level (nM)		
	DA	DOPAC	HVA
<i>Vehicle</i>			
Left striatum	5.10 ± 0.35	1810 ± 66	1095 ± 38
Right striatum	3.26 ± 0.46 ^a	325 ± 103 ^a	365 ± 89 ^a
<i>NTN</i>			
Left striatum	4.52 ± 0.42	1588 ± 55	945 ± 36
Right striatum	3.79 ± 0.57	922 ± 208 ^{a,b}	684 ± 118 ^{a,b}
<i>GDNF</i>			
Left striatum	4.87 ± 0.37	1686 ± 31	1005 ± 16
Right striatum	3.74 ± 0.25	851 ± 137 ^{a,b}	599 ± 53 ^{a,b}

Values are mean ± SEM from 7–8 animals per group.

^a p < 0.05 vs. left striatum,

^b p < 0.05 vs. right striatum of vehicle group (mixed ANOVA with treatment group as a between factor and side of brain as a within factor; followed by Newman-Keuls post hoc comparisons).