Published in final edited form as:
Neurobiol Aging. 2012 February ; 33(2): 427.e1–427.e14. doi:10.1016/j.neurobiolaging.2010.10.013.

Enhanced Dopamine Transporter Activity in Middle-Aged Gdnf Heterozygous Mice

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Abstract

Glial cell line-derived neurotrophic factor (GDNF) supports the viability of midbrain dopamine (DA) neurons that degenerate in Parkinson's disease. Middle aged, 12-month-old, *Gdnf* heterozygous (*Gdnf*$^{+/-}$) mice have diminished spontaneous locomotor activity and enhanced synaptosomal DA uptake compared to wildtype mice. In this study, dopamine transporter (DAT) function in middle-aged, 12-month-old *Gdnf*$^{+/-}$ mice was more thoroughly investigated using *in vivo* electrochemistry. *Gdnf*$^{+/-}$ mice injected with the DAT inhibitor, nomifensine, exhibited significantly more locomotor activity than wildtype mice. *In vivo* electrochemistry with carbon fiber microelectrodes demonstrated enhanced clearance of DA in the striatum of *Gdnf*$^{+/-}$ mice, suggesting greater surface expression of DAT than in wildtype littermates. Additionally, 12 month old *Gdnf*$^{+/-}$ mice expressed greater D_2 receptor mRNA and protein in the striatum than wildtype mice. Neurochemical analyses of striatal tissue samples indicated significant reductions in DA and a faster DA metabolic rate in *Gdnf*$^{+/-}$ mice than in wildtype mice. Altogether, these data support an important role for GDNF in the regulation of uptake, synthesis, and metabolism of DA during aging.

Keywords

In vivo electrochemistry; Dopamine; neurodegeneration; Glial cell-line derived neurotrophic factor; Striatum; Movement disorders; Dopamine transporter

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Disclosures

Dr. Greg Gerhardt is the owner of the manufacturer of the FAST – 16 mk II systems, Quanteon, LLC.

1. Introduction

The decline in motor function associated with aging has been widely demonstrated in animal models of aging (Hebert and Gerhardt, 1998, Willig, et al., 1987, Yurek, et al., 1998, Zhang, et al., 2000) and parallels a similar decline in human aging (Bennett, et al., 1996, Kluger, et al., 1997, Richards, et al., 1993). Dopamine (DA) neuron dysfunction has been related to age-associated motor impairment in both humans (Volkow, et al., 1998) and animals (Gerhardt, et al., 2002, Hebert and Gerhardt, 1998, Yurek, et al., 1998). The loss of functional DA neurons in the substantia nigra pars compacta (SNpc) and consequent loss of striatal DA are a hallmark of Parkinson's disease (PD) (Marsden, 1990), with its motor symptoms of bradykinesia, rigidity, and tremor (Hornykiewicz and Kish, 1987). The presence of some of these motoric deficits observed in many aged individuals has been termed age-related parkinsonism (Bennett, et al., 1996), and likely involves changes in the functional properties of DA neurons rather than neuronal loss as demonstrated in aged rats and monkeys (Grondin, et al., 2003, Hebert and Gerhardt, 1999, Hebert, et al., 1999, Yurek, et al., 1998).

Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β superfamily (Lin, et al., 1993). It has been hypothesized that age-related decreases in neurotrophic factor levels contribute to DA neuron degeneration and/or alterations in DA neuron function (Yurek and Fletcher-Turner, 2001). *In vivo* application of exogenous GDNF to the SN is reported to enhance DA neuron function in normal young (Hebert, et al., 1996), aged (Grondin, et al., 2003, Hebert and Gerhardt, 1997), and lesioned (Hoffer, et al., 1994, Tomac, et al., 1995) animals. In addition, GDNF is neuroprotective and neurorestorative in rat DA systems subjected to neurotoxic doses of methamphetamine (Cass, et al., 2000, Cass, et al., 2006) and produces functional restoration in rhesus monkeys exhibiting 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced parkinsonism (Gash, et al., 1996, Grondin, et al., 2002). GDNF has also been shown to enhance DA neuron function as indicated by 1) increased evoked DA release, 2) augmented locomotor behavior, 3) increased DA content in nigral tissue (Hebert and Gerhardt, 1997, Hebert, et al., 1996), and 4) increased high affinity DA uptake (Lin, et al., 1993). However, the exact role (s) of GDNF in normal aging, specifically the maintenance of DA neuron system function, is not known.

To further elucidate the effects of GDNF, a knockout mouse model was created (Pichel, et al., 1996) which exhibits a partial and stable reduction of the GDNF protein in brain tissues (Boger, et al., 2006). At birth, midbrain DA systems in mice homozygous for the GDNF null mutation ($Gdnf^{-/-}$) appear unaffected (Moore, et al., 1996); however, DA neuron function after the major apoptotic waves, which occur 2 and 14 days after birth (Mahalik, et al., 1994), cannot be assessed because these mice die at birth due to kidney agenesis (Moore, et al., 1996). Therefore, postnatal *in vivo* studies have used mice with a partial deletion of the *Gdnf* gene ($Gdnf^{+/-}$) (Boger, et al., 2006). GDNF expression in the striatum appears to be critical for appropriate innervation, survival, and differentiation of midbrain DA neurons to their striatal targets during early development (Stromberg, et al., 1993). Indeed, postnatal development of these DA neurons is compromised in the absence of GDNF (Granholm, et al., 2000). Behavioral and immunohistochemical characterization of multiple age groups (4, 8, 12, 16, and 20 months of age) of $Gdnf^{+/-}$ mice compared to wildtype (WT) controls, provides evidence of a unique aging phenotype in the $Gdnf^{+/-}$ mice. A partial GDNF depletion leads to an earlier (12 months of age) loss of tyrosine hydroxylase positive (TH-positive) neurons in the SNpc as well as diminished spontaneous locomotor activity (Boger, et al., 2006). The diminished locomotor activity and decline in TH-positive neurons also show an accelerated age-associated decline from 8 to 12 months in the $Gdnf^{+/-}$ mice while both measures in WT mice of the same age groups are unchanged (Boger, et al., 2006).

Young *Gdnf*$^{+/-}$ mice (6–9 months) exhibit no effect of GDNF reduction on spontaneous or methamphetamine-induced locomotor behavior (Boger, et al., 2006, Boger, et al., 2007, Gerlai, et al., 2001). *In vitro* synaptosomal preparations from *Gdnf*$^{+/-}$ mice show increased dopamine transporter (DAT) activity, which has been suggested to predispose DA neurons to methamphetamine-induced toxicity (Boger, et al., 2007). Consistent with the age related changes in DA systems noted above, DAT activity in the striatal pathway is altered in the normal aging process in animal models (Friedemann, 1992, Friedemann and Gerhardt, 1992, Hebert and Gerhardt, 1999, Hebert, et al., 1999), and may be correlated with age-related motor deficits. Such dynamic changes in DA uptake using parameters associated with DAT activity have been characterized by *in vivo* chronoamperometry using carbon fiber microelectrodes (Cass, et al., 1993, Gerhardt, et al., 1999, Gerhardt, et al., 1986). The high spatial (microns) and temporal (seconds) resolution of these techniques allows for the rapid and sensitive quantification of DA uptake in discrete DA terminal regions.

Based on previous *in vitro* findings that DA uptake in *Gdnf*$^{+/-}$ mice was greater than in WT mice (Boger, et al., 2007), the present study investigated this age-associated deficit by using *in vivo* electrochemistry to address the effects of a partial and chronic genetic reduction of GDNF on the function of DA terminals in the striatum of middle-aged 12-month-old *Gdnf*$^{+/-}$ mice. The following questions were addressed: (1) Do *Gdnf*$^{+/-}$ mice differ in behavioral response to DAT modulation via DAT inhibition? (2) Do *Gdnf*$^{+/-}$ mice exhibit altered DAT activity *in vivo*, and if so, what is the spatial pattern of this effect within striatal subregions? (3) Do *Gdnf*$^{+/-}$ mice have altered striatal dopamine D2 receptor expression? (4) Is whole tissue neurochemical content altered in *Gdnf*$^{+/-}$ mice? Thus, these studies focus on the functional properties of DA neurons in 12-month-old *Gdnf*$^{+/-}$ mice that demonstrate accelerated age-related motor deficits and loss of TH-positive neurons as compared to WT littermate controls (Boger, et al., 2006).

2. Methods

2.1. Animals

A nonfunctional GDNF allele was generated by replacing part of exon 3 which encodes the GDNF protein with a selectable marker neomycin phosphotransferase expressing cassette. Generation and genotyping of *Gdnf*$^{+/-}$ mice is described in detail in previous work (Pichel, et al., 1996). Mice were obtained from a colony established at the Medical University of South Carolina. Mice were bred on a C57Bl/6J background consistent with NIH approved protocols. After transfer to the University of Kentucky, mice were acclimated for a minimum of 1 week before experimentation. Male *Gdnf*$^{+/-}$ mice (12 months of age) were compared with age-matched WT mice in all experiments. Mice were housed 3–4 per cage with food and water provided *ad libitum*. Mice were maintained under 12:12 h light/dark cycle at an ambient temperature of 20–22° C. Protocols for animal care were in agreement with NIH approved guidelines and compliant with local institutional protocols at the University of Kentucky Medical Center and Medical University of South Carolina. Procedures were in strict agreement with the *Guide for the Care and Use of Laboratory Animals.*

2.2. Locomotor activity

Locomotor activity (total distance traveled) was assessed with a Digiscan Animal Activity Monitor system (Omnitech Electronics Model RXYZCM (8); TAO, Columbus, OH, USA), details of which have been previously described (Halberda, et al., 1997). Animals of each genotype (N = 8) were injected with 0.9% NaCl (0.01ml/g body weight, i.p.) and placed into the activity boxes for a 1 hr period of habituation. After the habituation period, the mice

were injected with the DAT inhibitor, nomifensine (7.5 mg/kg, i.p.) and placed into the activity boxes for a 2 hr recording period.

2.3 *In vivo* electrochemistry measurements of dopamine clearance

High-speed *in vivo* chronoamperometric recordings of DA clearance were carried out using Nafion®-coated carbon fiber microelectrodes (electrode tips were ~30 μm o.d. × 150 μm length; Quanteon, LLC, Lexington, KY) as previously described (Cass and Gerhardt, 1994, Thomas, et al., 2007). The sensitivity and selectivity for DA *versus* other endogenous electroactive molecules were enhanced by the use of a high temperature Nafion® coating (5% solution, 1 coat at 200°C, Aldrich Chemical Co., Milwaukee, WI (Gerhardt and Hoffman, 2001). High-speed chronoamperometric electrochemical measurements were made using the FAST-16 system (Quanteon, LLC, Nicholasville, KY). A square wave potential (0.0 to +0.55 V, 200ms total) was applied to the carbon fiber microelectrode *versus* the Ag/AgCl reference electrode at a frequency of 1 Hz. The resulting oxidation of DA and subsequent reduction of dopamine-o-quinone was digitally integrated during the last 80 ms of each 100 ms pulse.

The microelectrodes were calibrated *in vitro* in 0.05 M phosphate buffered saline solution. Parameters quantified included: linearity of response to DA, selectivity, and limit of detection (LOD) for DA. Data are expressed as mean ± SEM. The microelectrodes showed linear responses to serial additions of DA (2–6 μM, effective beaker concentrations) with correlation coefficients (R^2) of 0.9978 to 0.9998. Additions of ascorbate (250 μM) were used to determine the selectivity of the recordings for DA that averaged 908 ± 166:1 (N = 13). The LOD for DA was 11 nM ± 3 with a signal to noise ratio ≥ 3 (N = 13). Changes in DA *in vivo* were expressed as changes from a stable baseline response using the calibration curve for a particular microelectrode. DA clearance in the mouse striatum was studied *in vivo* using microelectrodes attached to a single barrel micropipette. A micropipette (1 mm o.d., 0.58 i.d., glass, A-M Systems, Inc., Everett, WA) with an inner tip diameter of 10–15 μm was positioned 180–220 μm from the carbon fiber electrode tip with sticky wax (Kerr Corp., Orange, CA, USA). The micropipette was filled with a DA solution (100 μM DA, 200 μM ascorbate, in 0.9% NaCl). The solution was filtered (0.22 μm) and pH adjusted between 7.2 and 7.4 before *in vivo* use.

WT (N=7) or *Gdnf*$^{+/-}$ (N=5) mice were anesthetized, placed into a stereotaxic frame and rested on a re-circulating water bath connected to a heating pad (Gaymar Industries, Inc., Orchard Park, NY), which was maintained at 37° (Thomas, et al., 2007). A craniotomy was performed for access to the striatum and the overlying dura was reflected prior to insertion a carbon fiber microelectrode/micropipette assembly into the brain. A burr hole distant from the recording site(s) was created and used for the placement of a miniature Ag/AgCl reference electrode (0.008 in. Teflon coated wire; A-M Systems Inc., Carlborg, WA, USA) in contact with brain tissue (Hascup, et al., 2007). Two striatal recording tracts per hemisphere were used to characterize DA uptake in the left hemisphere. The electrode assembly was stereotaxically placed (Kopf Instruments, Tujunga, CA, USA) in contact with dura and lowered via a microdrive apparatus at 400 μm increments. Stereotaxic coordinates from bregma were (mm) Site 1 (rostral-medial): anterior-posterior +1.0, medial-lateral +1.2, dorsal-ventral −3.0 to −4.6; *Site* 2 (caudal-lateral): anterior-posterior +0.1, medial-lateral +2.2, dorsal-ventral −2.5 to −4.1 (incisor bar positioned with *lambda* level to *bregma*; see Fig. 1A; (Franklin and Paxinos, 2001). The microelectrode recordings were allowed to reach a stable baseline response in the mouse cortex for a minimum of 1 hour after initial placement into the brain. The microelectrode was then lowered to the targeted recording area in the striatum for studies of DA uptake through the local application of the DA solution. The DA solution was applied by pressure ejection (Palmer, et al., 1980) using a Picosprizter III (Parker-Hannifin, NJ) and solution volume applied was monitored using a

stereomicroscope fitted with a reticule (Friedemann and Gerhardt, 1992). The solution volume was adjusted in order to obtain DA signals of equivalent amplitudes, which were subsequently analyzed for clearance kinetics (Cass and Gerhardt, 1995, Cass, et al., 1993). When the resulting peak from DA application had returned to a stable baseline, the recording assembly was then lowered 400 μm and the recordings were allowed to baseline for a minimum of 5 minutes before DA application at the next recording depth. All DA signals used for analysis exhibited mean reduction/oxidation ratios of 0.78 ± 0.01 (mean ± SEM, N = 107 signals), consistent with the normal redox profile for DA (Gerhardt and Hoffman, 2001, Gerhardt, et al., 1986).

After electrochemical recordings, animals were decapitated while under anesthesia and the brains were dissected and frozen until sectioning (40 μm) on a cryostat (Microm, Zeiss, and Thornwood, NY, USA). Sections were mounted on glass slides, stained with cresyl violet acetate and coverslipped to confirm the microelectrode placements in the targeted striatal regions. Figure 1 illustrates the electrode placements in schematics of the striatum (A) and a typical dopamine chronoamperometric signal (B).

2.4 Semiquantitative *in situ* hybridization histochemistry

Semi-quantitative In situ hybridization of the dopamine D_2 receptor was performed as previously described (Wang and McGinty, 1995). Briefly, sections were cut at 12 μm with a cryostat through the striatum of each mouse (n=4 for $Gdnf^{+/-}$ and n=5 for WT) and thaw-mounted onto Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA, USA). These sections were pretreated to fix and defat the tissue and block non-specific hybridization. A 1:1 mixture of synthetic cDNA oligodeoxynucleotide probes complementary to the long form of D2 receptor mRNA ($D2_{244;}$ corresponding to bases 728–763) and the short form of D2 receptor mRNA ($D2_{415}$; corresponding to bases 704–739) was labeled using alpha-[35S]-dATP (Amersham Biosciences, Piscataway, NJ, USA) and terminal deoxynucleotidyl transferase (Roche Diagnostics Corporation, Indianapolis, IN, USA). Sections were immersed in 5.0×10^5 c.p.m./20 ml hybridization buffer/section overnight (15 h) at 37°C in a humid environment and then washed and air dried before being placed into a film cassette with ^{14}C standards (American Radiolabeled Chemicals, St Louis, MO, USA) and Kodak Biomax film (Rochester, NY, USA) for 2 weeks. Quantitation of the mRNA hybridization signals was performed using NIH image 1.62 software as previously described (Wang and McGinty, 1995).

2.5 Immunoblotting

Striatal samples from 12 month old WT and *Gdnf*$^{+/-}$ mice (20μg) were loaded in duplicate and separated on 4–12% NuPAGE Bis Tris gels (Invitrogen, Carlsbad, CA) at 150V for 45 minutes (N=7 per genotype). After separation onto the gel, the gel was transferred via wet transfer onto a nitrocellulose membrane for one hour at 30V. The membrane was then removed from the cassette and blocked for one hour in 5% non-fat milk in PBS at room temperature and subsequently incubated overnight at room temperature in a dopamine D_2 receptor primary antibody (1:200, mouse monoclonal, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, sc-5303). After 24 hours, blots were washed in PBS-T (0.1% Tween-20) then incubated in secondary antibody (1:5000, donkey anti-mouse IgG, Jackson Immunoresearch, West Grove, PA, 715-035-151) in 5% non-fat milk in PBS for one hour at room temperature. Blots were then washed in PBS-T then imaged on a Kodak Image Station 4000 (4 exposures, 15 sec each) using Immobilon chemiluminescent reagent (Millipore, Bellerica, MA). For loading control, blots were incubated with anti-actin antibody for one hour at room temperature, then incubated in secondary antibody, then finally re-imaged under the same settings. The samples were normalized to actin. Integrated density of the bands was

analyzed using AlphaEaseFC (FluorChem 9900) and results are reported as percent of wildtype.

2.6 Tissue studies of dopamine and metabolites and serotonin and metabolites using high performance liquid chromatography coupled with electrochemical detection (HPLC-EC)

Striatal tissue was collected from a separate cohort of 12 month old *Gdnf*$^{+/-}$ and WT mice (N=8 per genotype) to mimic the general recording tracks from the chronoamperometry measures by obtaining rostral and caudal tissue samples. Mice used for neurochemistry were anesthetized with isoflurane and euthanized by decapitation. Brains were quickly removed and placed in an ice cold mouse brain mold (ASI instruments, Warren, MI). Rostral and caudal striatal tissue punches from the dorsal striata were obtained bilaterally and placed into pre-weighed microcentrifuge tubes and stored on dry ice. The tubes were weighed again before being stored at −70° C until HPLC-EC analysis.

In preparation for HPLC-EC analysis, samples were sonicated in cold mobile phase (pH = 4.1) with dihydroxybenzylamine (DHBA) as an internal standard. Samples were centrifuged at 16,000 × *g* for 10 minutes. The supernatants (50 μL) were injected into the HPLC system using dual-coulometric electrochemical detectors (Hall, et al., 1989). The HPLC system detection limits were < 1 ng/g tissue wet weight for the analytes of interest. DA, 3–4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were identified by peak retention times and analyzed by peak areas using standard concentrations of analytes. Striatal tissue neurochemical content was expressed as ng/gram wet weight of tissue.

2.7 Statistics

2.7.1. Locomotor activity—Basal locomotor activity data collected following saline injections were analyzed with a 2(Genotype) × 4(15-Min Interval) mixed factor ANOVA with repeated measures on the Interval factor. Because of the genotype difference in basal activity data collected following the nomifensine injections were analyzed with a 2(Genotype) × 8 (Time Interval) ANOVA with repeated measures on the interval factor.

2.7.2. *In vivo* electrochemistry—High–speed chronoamperometric data were analyzed using custom MATLAB®-based software (The MathWorks™, Inc.). Several signal parameters were obtained from each DA signal (shown in Fig. 1B). Signal amplitude (nM) was calculated as the change from baseline after local application of DA. The first order exponential decay constant, k_{-1} (sec^{-1}), was calculated from the decay portion of the DA signal fitted to the slope of the linear regression of the natural log transformation of the data (Sabeti, et al., 2002). This is indicated (Fig. 1B) by the dashed line on the decay portion of the signal after local application of DA. The first order decay constant, k_{-1} (sec^{-1}), is a quantitative measure that can be multiplied by the signal amplitude (nM) to yield the uptake rate (nM/sec) (Stephens, et al., 2009). For our purposes we analyzed the raw k_{-1} as signal amplitude was controlled. A single value for k_{-1} was obtained from a single peak from each recording depth using peak amplitudes that were matched (Cass, et al., 1992,Cass, et al., 1993). The volume of solution applied to obtain signals with amplitudes ranging from 500–1000 nM was recorded and used to calculate the total amount (pmol) of DA applied. Adjusting the volume of DA applied enables modulation of signal amplitude such that physiologically appropriate concentrations are analyzed and the inherent Michaelis-Menten properties of the DAT are not altered by varied extracellular concentrations of substrate (Hebert and Gerhardt, 1999). In addition, the amplitude of the DA signal (nM) per amount of DA applied (pmol) (Fig. 1B) is a measure of the surface expression of the DAT (Cass and Gerhardt, 1995,Hebert and Gerhardt, 1999). Because prior work demonstrated heterogeneous properties of DA uptake and DAT density, which varies most notably along

the dorsal-ventral orientation of the striatum (Hebert and Gerhardt, 1999,Hebert, et al., 1999), uptake data (k_{-1} and amplitude per picomole DA applied) from the striatum were analyzed from dorsal and ventral subregions at each recording site. Five recording depths separated by 400 μm were used in each recording site. For each animal, data from the two most dorsal recording depths were used to characterize the dorsal striatum (Str) (sites 1 and 2) and the two most ventral sites were used similarly to characterize the ventral striatum/ nucleus accumbens (NAc) of the rostral-medial recording site (site 1) or ventral Str of the caudal-lateral recording site (site 2). Data from the dorsal and ventral subregions of all animals were combined, averaged and analyzed by a two-tailed unpaired t-test to compare differences at the same recording subregion between genotypes. Data from the respective dorsal and ventral subregions remained separated by recording site.

2.7.3. Neurochemistry HPLC-EC, In situ hybridization immunohistochemistry, & Immunoblotting—Neurochemical contents of brain tissues from striatal regions were compared between genotypes and analyzed by a two-tailed unpaired t-test.

For all measures, statistical significance was defined by $P < 0.05$.

3. Results

3.1 Nomifensine-Induced locomotor activity

Locomotor activity of the 12-month-old *Gdnf*$^{+/-}$ and WT mice was investigated to study potential age-related changes in the movement capabilities of the *Gdnf*$^{+/-}$ mice and the effects of a catecholamine uptake inhibitor on locomotion in these mice. As seen in Fig. 2A, the spontaneous locomotor activity following saline (vehicle) injection was diminished in the 12-month-old *Gdnf*$^{+/-}$ mice compared to that of WT mice. A 2(Genotype) × 4(15-Min Interval) mixed factor ANOVA with repeated measures on the interval factor provided statistical support for reduced motor activity observed for the *Gdnf*$^{+/-}$ mice [Genotype: $F_{1,12}$= 15.179, $P < 0.01$]. This result confirms results of a previous study which also established a positive correlation for reductions in motor activity and TH-positive neurons in the SNpc (Boger, et al., 2006). Following the habituation period, mice were injected with nomifensine; a known DAT inhibitor that increases locomotor activity (Altar and Marshall, 1988,Hebert and Gerhardt, 1998). Because *Gdnf*+/− mice were less active than WT controls during the habituation period, data generated following the nomifensine injections are expressed as changes in motor activity relative to the final 15-min base interval after the saline injections. The activity increase relative to basal levels is noted in Fig. 2B and reflects a greater nomifensine-induced increase in locomotor activity for the 12-month-old *Gdnf*$^{+/-}$ than age-matched WT control mice. A Genotype × Interval ANOVA on these data indicated a significant Genotype × Interval interaction [$F_{7,84}$=3.119, P<0.01] with greater nomifensine-induced locomotor activity for the *Gdnf*$^{+/-}$ mice during the earlier intervals of activity.

3.2 Studies of dopamine clearance and uptake

Studies of DA clearance using *in vivo* electrochemical methods coupled with local application of DA from micropipettes were carried out in the striata of *Gdnf*$^{+/-}$ and WT mice to further investigate the functional properties of DAT *in vivo*. These methods have previously been shown to reliably determine the relative density of DAT and its kinetic properties in mice and rats (Thomas, et al., 2007). Local application of DA solution was used to obtain DA signals of equivalent amplitude (771 ± 16, mean ± SEM (nM), N = 107 signals) for DA uptake analysis. Genotypes were compared using k_{-1} (sec^{-1}) (Fig. 1B), which is the first-order rate constant of the decay of the DA clearance signals. *Gdnf*$^{+/-}$ mice and WT mice did not show significant differences in the k_{-1} in the dorsal striatum of either

recording site: rostral-medial (site 1) [$P = 0.36$]; caudal-lateral (site 2) [$P = 0.67$] (Figs. 3A, 3B). However, increased DA uptake kinetics measured by k_{-1} were significantly increased in the ventral subregions of both recording sites of the 12 month old *Gdnf*$^{+/-}$ mice as compared to WT mice [rostral-medial [(site 1): $t_{15} = 2.280$, $P = 0.04$; caudal-lateral (site 2): $t_{22} = 2.437$, $P = 0.02$] (Figs. 3A, 3B). The ventral Str/NAc (recording site 1) of *Gdnf*$^{+/-}$ mice showed a 96% increase in mean k_{-1} compared to WT counterparts [WT: 0.0023 ± 0.00045, N = 12; *Gdnf*$^{+/-}$: 0.0045 ± 0.0011, N = 5; mean ± SEM (sec^{-1})]. Similarly, the analogous ventral striatal subregion of recording site 2 showed a 100% increase in DA uptake [WT: 0.0019 ± 0.00016, N = 12 *Gdnf*$^{+/-}$: 0.0038 ± 0.00076, N = 12; mean ± SEM (sec^{-1})].

A second series of studies compared the amplitudes of the DA signals achieved per amount of DA solution locally applied, to investigate the surface expression of DAT in the striatal subregions. We have previously shown that the amplitude of the DA signals per amount applied increases as the surface expression of DAT decreases (Cass and Gerhardt, 1995, Hebert and Gerhardt, 1999). The resulting amplitude per picomole varied according to striatal subregion and genotype (Fig. 4). Increased dopamine uptake in the *Gdnf*$^{+/-}$ mice was evident as the resulting amplitude per picomole of applied DA solution was smaller in the *Gdnf*$^{+/-}$ mice. This is evident in both dorsal recording subregions in *Gdnf*$^{+/-}$ mice (Figs. 4A, 4B). There was a 74% decrease in amplitude per unit volume of ejected DA in dorsal striatum of *Gdnf*$^{+/-}$ mice compared to WT mice (site 1): WT: 167.7 ± 35.6, N = 14; *Gdnf*$^{+/-}$: 43.35 ± 11.3, N = 10; mean ± SEM (nM/pmol) ($t_{22} = 2.86$, $P < 0.001$) (Fig. 4A). *Gdnf*$^{+/-}$ mice and WT mice did not show significant differences in the amplitude per picomole in the ventral Str/NAc (site 1) [$P = 0.14$] (Fig. 4A). Recording site 2 had a 82% decrease in amplitude per picomole in the dorsal striatum (WT: 168.6 ± 29.7, N = 12; *Gdnf*$^{+/-}$: 30.23 ± 7.312, N = 10; mean ± SEM (nM/pmol) ($t_{20} = 4.153$ $P < 0.001$) and a 69% decrease in the ventral striatum (WT: 206.2 ± 45.2, N = 12; *Gdnf*$^{+/-}$: 64.39 ± 26.25, N = 10; mean ± SEM (nM/pmol) (Fig. 4B; $t_{20} = 2.575$, $P = 0.01$). Thus, a significant decrease in the amplitude per picomole of DA that was locally applied, combined with an increase in k_{-1} indicate increased clearance of exogenously applied DA in *Gdnf*$^{+/-}$ mice *versus* WT counterparts and/or a change in the surface expression of DAT on the plasma membrane of DA nerve terminals and varicosities in the striatum of the *Gdnf*$^{+/-}$ mice.

3.3 Striatal dopamine D_2 receptor mRNA and protein expression

In a separate cohort of mice, striatal dopamine D2 receptor mRNA and protein expression was assessed in 12 month old WT and *Gdnf*$^{+/-}$ mice to determine a potential connection between altered D_2 receptor expression and DAT activity (Figure 5). Using *in situ* hybridization histochemistry, striatal dopamine D_2 receptor mRNA expression was determined. At 12 months of age, *Gdnf*$^{+/-}$ mice had significantly higher dopamine D_2 receptor mRNA in the dorsal striatum than WT mice (Figure 5A; $t_7 = 2.463$, $P = 0.04$). In addition to an increase in striatal dopamine D_2 receptor mRNA, immunoblotting for the D_2 receptor protein in the striatum was also increased in 12 month old *Gdnf*$^{+/-}$ mice compared to WT mice (Figure 5B, $t_{12} = 2.645$, $P = 0.02$).

3.4 Striatal tissue measures of dopamine and metabolites and serotonin and metabolites by HPLC-EC

To determine if there were concurrent alterations in DA synthesis or metabolism in addition to altered DA uptake, analysis of DA and its metabolites (DOPAC, HVA) was performed on tissue samples harvested from rostral (site 1: AP+1.0) and caudal (site 2: AP+0.1) sections of the mouse striatum in a separate cohort of 12 month old male *Gdnf*$^{+/-}$ and WT mice (N = 8 per genotype; Table 1). The 12 month old *Gdnf*$^{+/-}$ mice had lower striatal tissue levels of DA than WT mice in both sets of brain coordinates [site 1: AP+1.0, $t_{13} = 3.541$, $P < 0.01$;

site 2: AP+0.1, t_{13} = 2.281, P = 0.04]. The genotypes did not differ in tissue content of DOPAC or HVA at either set of coordinates (Table 1). DA turnover was significantly elevated at both recording sites in *Gdnf*$^{+/-}$ mice as indicated by the increase in ratios of HVA:DA [site 1: AP+1.0, t_{13} = 3.672, P = 0.0028; site 2: AP +0.1, t_{13} = 3.835, P <0.01] and (DOPAC + HVA):DA [site 1: AP+1.0, t_{13}=2.504, P = 0.03; site 2: AP+0.1, t_{13} = 4.099, P <0.01]. In addition, 12-month-old *Gdnf*$^{+/-}$ mice differed in the DOPAC:DA ratio compared to WT mice in the caudal striatum [site 2, AP+0.1, t_{13} = 2.264, P = 0.04]. Thus, *Gdnf*$^{+/-}$ mice demonstrated decreased DA tissue storage, which likely contributed to the decreases see in the DA turnover ratios.

Whole tissue levels of 5-HT and its metabolite 5-HIAA were also investigated by HPLC-EC from striatal tissues as outlined above (Table 1). The genotypes did not differ in tissue levels of 5-HT or the metabolite, 5HIAA, at either recording site (p>0.05). However, the ratio of 5-HIAA:5-HT in the caudal region of the striatum (site 2) was significantly greater in *Gdnf*$^{+/-}$ mice compared to WT mice [t_{13}=2.388, P = 0.03].

4. Discussion

The present studies reveal dynamic alterations in DA neurotransmission in the striatum of middle-aged *Gdnf*$^{+/-}$ mice. Locomotor experiments indicated lower spontaneous locomotor activity of the 12-month-old *Gdnf*$^{+/-}$ mice than in WT mice that confirms our previous report (Boger, et al., 2006). By contrast, nomifensine produced greater relative increases in locomotor activity of the *Gdnf*$^{+/-}$ mice, suggesting alterations in DAT function and/or postsynaptic DA signaling and furthermore demonstrating that a partial *Gdnf* deletion did not cause inability to move in these behavioral tasks. *In vivo* electrochemical recordings of DA uptake indicated a higher rate, k_{-1}, of DA uptake in the ventral striatal subregions of *Gdnf*$^{+/-}$ mice compared to WT mice. In addition, *Gdnf*$^{+/-}$ mice showed significant decreases in the amplitude per picomole DA applied in multiple striatal subregions compared to WT mice. Thus, the chronoamperometric studies confirm that *Gdnf*$^{+/-}$ mice have increased high-affinity DA uptake *in vivo* consistent with an increase in the relative B_{max}. Assessment of striatal tissue levels of DA and DA metabolites indicated lower DA levels for the *Gdnf*$^{+/-}$ mice, which is consistent with the lower spontaneous locomotor activity and lower number of TH-positive neurons in the SNpc of these mice (Boger, et al., 2006). Taken together, these data indicate robust functional changes in DA neurons of middle-aged *Gdnf*$^{+/-}$ mice, supporting a role for GDNF in age-related changes in DA neuron function (Yurek and Fletcher-Turner, 2001, Yurek, et al., 1998).

4.1 Middle-aged *Gdnf*$^{+/-}$ mice show enhanced behavioral sensitivity to DAT inhibition by nomifensine

The diminished spontaneous locomotor activity baseline activity observed for the 12-month-old *Gdnf*$^{+/-}$ mice confirms results of a previous study on *Gdnf*$^{+/-}$ mice (Boger, et al., 2006). Consistent with previous reports, nomifensine injections increased spontaneous motor activity in both genotypes, which is consistent with the DAT inhibition produced by nomifensine (Altar and Marshall, 1988, Marshall and Altar, 1986). Although the DAT inhibitor elevated the activity of both genotypes, the response was greater for the *Gdnf*$^{+/-}$ mice. Interestingly, the enhanced response to nomifensine seen for middle-aged *Gdnf*$^{+/-}$ mice in the current study was not observed in previous experiment on younger (3 months of age) *Gdnf*$^{+/-}$ mice compared to their age-matched WT mice (Boger, et al., 2007). The combined results of the two experiments show that GDNF and the aging process interact to influence progressive alteration in DA systems, an interpretation consistent with the increasing DAT activity observed in aging (from 3 to 12 months of age) *Gdnf*$^{+/-}$ mice and not WT counterparts (Boger, et al., 2007). Diminished DA uptake is evident in aged rats (Friedemann, 1992, Hebert and Gerhardt, 1999) and is accompanied by an attenuated effect

of nomifensine on locomotor behavior (Stanford, et al., 2002) and reductions in DAT density (Hebert, et al., 1999). Considering these previous studies in our model, the increased sensitivity to nomifensine suggests an increase in high affinity DA uptake by the DAT in the *Gdnf*$^{+/-}$ mice, which was confirmed by the *in vivo* chronoamperometric studies discussed below.

Previous work showing both comparatively weak inhibition of the serotonin transporter by nomifensine (Tuomisto, 1977) and a lack of effect of serotonin uptake inhibition on locomotor activity (Hebert and Gerhardt, 1998) support a minor if any contribution from serotonergic systems in these studies. Further evidence is provided by the striatal tissue analysis for 5-HT and 5-HIAA discussed below.

4.2 Middle-aged *Gdnf*$^{+/-}$ mice show increased DA uptake *in vivo*

Aged rats are reported to have changes in DA uptake and release kinetics in the dorsal striatum, which are postulated to contribute to age-related motor deficits in these animals (Hebert and Gerhardt, 1998). In the current study, the increase in the decay constant, k_{-1}, in the ventral striatum (caudal-lateral, site 2) and ventral Str/NAc (rostral-medial, site 1) indicates a more selective effect on mesoaccumbens (A10) DA projections *versus* nigrostriatal (A9) DA terminals, which are more widespread in the dorsal striatum (Bentivoglio and Morelli, 2005). We would expect limbic-related behaviors, particularly those influenced by DAT modulation, to be differentially affected in *Gdnf*$^{+/-}$ mice. Our findings of mesoaccumbens modulation in 12-month-old *Gdnf*$^{+/-}$ mice are interesting in light of prior findings that GDNF supplementation attenuates behavioral adaptations following administration of addictive drugs(Ghitza, et al., 2009, Messer, et al., 2000) (Messer, et al., 2000). Although reward and reinforcing behaviors are traditionally attributed to mesolimbic DA projections (Di Chiara and Imperato, 1988), DA neurotransmission in the NAc, and surrounding ventral striatum may influence motor activity as well (Koob, et al., 1981, Pijnenburg, et al., 1976), a function historically associated with nigrostriatal DA projections (Kirik, et al., 1998). With consideration of placement variation between animals, striatal heterogeneity (Glynn and Yamamoto, 1989), and the diffuse anatomical boundaries of mesolimbic and nigrostriatal projections, it is unlikely either of the 'ventral' subregions studied reflect mesoaccumbens projections exclusively (Beckstead, et al., 1979, Fallon and Moore, 1978). We believe DA innervation from the SNpc likely contributes to the uptake changes seen in the ventral subregions. Indeed, analysis of the amplitude per picomole of DA solution applied indicates uptake changes in both dorsal subregions (sites 1 and 2) and the ventral striatum (site 2). Previous work has shown that the positive relationship between DA signal amplitude from increasing amounts of locally applied DA solution is linear (Cass and Gerhardt, 1995, Hebert and Gerhardt, 1999). The relationship between the amount of locally applied DA and the resulting amplitude is associated with normal DAT function, and was previously shown to reflect an *in vivo* B_{max}, which allows for comparison of the relative number of functional DA uptake sites (Cass and Gerhardt, 1995, Cass, et al., 1993, Hoffman and Gerhardt, 1998). The increased striatal DA uptake for *Gdnf*$^{+/-}$ mice indicated by the chronoamperometric clearance experiments provides *in vivo* confirmation of our earlier *in vitro* work indicating increased DAT activity in striatal synaptosomes from *Gdnf*$^{+/-}$ mice (~30% increase in DA uptake compared to age-matched WT mice) (Boger, et al., 2007). Since the *in vitro* experiments indicated increased DAT activity in tissue from *Gdnf*$^{+/-}$ mice with no overall change in DAT protein expression, upregulation of DAT expression doesn't likely account for the current findings. An increase in B_{max} due to increased membrane expression of the DAT supports the enhanced sensitivity to DAT inhibition by nomifensine seen in the locomotor data and chronoamperometric studies. Indeed, increased surface expression of the DAT in *Gdnf*$^{+/-}$ mice would explain how DAT protein levels are not different between genotypes despite increased DAT activity *in vitro* (Boger, et al., 2007).

Future studies will be conducted to determine the surface expression of the DAT in *Gdnf*$^{+/-}$ vs. WT mice. Underlying mechanisms of interest regarding DAT surface expression include decreased internalization of the transporter via attenuated activity of protein kinase C (PKC), which has been implicated in molecular control of endocytosis of the DAT (Sorkina, et al., 2005).

Altered DA uptake is common to aging studies of rats (Hebert and Gerhardt, 1998, Yurek, et al., 1998) and the *Gdnf*$^{+/-}$ mice. The former involves diminished uptake while our model examined here appears to show increased DA uptake with an enhanced response to nomifensine rather than the attenuated response observed in prior studies in aged rats (Hebert and Gerhardt, 1998). Changes in DAT activity have been postulated to help maintain DA signaling in lesioned (Hoffman and Gerhardt, 1998, van Horne, et al., 1992) and aging (Hebert and Gerhardt, 1999, Hebert, et al., 1999) animal models of DA dysfunction; perhaps modulating signal duration by increasing the amount of DA and/or time synaptic DA is available. We postulate that increased DAT activity observed for *Gdnf*$^{+/-}$ mice is accompanied by relative decreases in tonic levels of synaptic and extracellular DA resulting in decreased post-synaptic DA receptor activation.

4.3 Middle-aged *Gdnf*$^{+/-}$ mice show increased dopamine D_2 receptor mRNA and protein

Decreased DA receptor activation would support diminished spontaneous motor activity in Gdnf$^{+/-}$ mice reported in this study and previous work (Boger, et al., 2006). However, here we show an increase in DA D_2 receptor mRNA as well as protein in the striatum of 12 month old *Gdnf*$^{+/-}$ mice compared to WT mice(Boger, et al., 2004). Therefore, if the reduced availability of tonic levels of synaptic DA in *Gdnf*$^{+/-}$ mice is accompanied by an increase in DA D_2 receptor activity, the exaggerated motor activity response to nomifensine might by accounted for by the drug-induced increases in synaptic DA. Thus, the effective stimulus response is exacerbated in the *Gdnf*$^{+/-}$ mice due to 1) enhanced sensitivity to nomifensine and 2) increased D_2 receptor activation. Definitive conclusions about the involvement of the D_2 autoreceptor in uptake changes cannot be made from protein and mRNA assays, which detect primarily post-synaptic D2 receptors (Levey, et al., 1993). The contribution of D_2 autoreceptors is possible and needs to be directly investigated in the future. However, local inhibition of DA D_2 receptors has been shown to produce a more modest ~40% decrease in DA clearance in amp/pmol (Cass and Gerhardt, 1994) as compared to the ~80% decrease in amp/pmol for DA clearance in the middle-aged *Gdnf*$^{+/-}$ mice. Additionally, PKC mediated regulation of the DAT membrane trafficking discussed previously can function independently of DA D2 receptors (Furman, et al., 2009). Although protein and mRNA levels are significantly increased, profound receptor changes are unlikely considering the modest decline (30%) in tissue DA levels which do not fulfill required deficits (>90%) for receptor supersensitivity phenomena (Hefti, et al., 1980). Future studies will be conducted to further investigate striatal D_2 receptor surface expression and activation as well as tonic extracellular DA levels in the *Gdnf*$^{+/-}$ mice, which will prove useful to evaluate receptor activation.

Boger et al., (2007) showed increased susceptibility to methamphetamine-induced toxicity in *Gdnf*$^{+/-}$ mice and attributed the susceptibility to increased DAT activity. Although the mechanisms of increased DAT activity have not been clearly defined, we would predict increased DAT activity and/or surface expression of DAT to render the *Gdnf*$^{+/-}$ mouse more susceptible to other DA neurotoxins like MPTP and 6-OHDA, which confer toxicity largely through the DAT (Gainetdinov, et al., 1997, Storch, et al., 2004). In 6-hydroxydopamine (6-OHDA) lesioned rats, high affinity DA uptake by DAT is diminished due to a profound loss of striatal DA terminals following 6-OHDA lesioned rats (Hoffman and Gerhardt, 1998). However, our previous work showed decreases in TH-positive fiber density and TH-positive neurons in the SN of *Gdnf*$^{+/-}$ mice (Boger, et al., 2006), with increased DAT activity

(current data). An increase in fiber density is also not supported by the overall decrease in striatal DA content discussed below. Regarding potential involvement of serotonergic systems in the electrochemistry clearance studies, prior work shows the clearance properties of locally applied DA in the striatum to be unchanged following selective inhibition of 5-HT transporters (Cass and Gerhardt, 1995). Tissue neurochemistry, however, includes analysis of 5-HT and 5-HIAA discussed below.

Altogether, these data support an increased DA clearance capacity in middle-aged *Gdnf*$^{+/-}$ mice, which may be explained by changes in the DAT activity, affinity and/or density of DAT membrane expression as seen also in the aged rat (Hebert, et al., 1999) and human brain (Volkow, et al., 1994). It is unclear if the GDNF depletion in this model varies within the striatum as previous characterization studies have focused on the entire striatum, excluding the NAc (Boger, et al., 2006). A differential effect of GDNF on DA terminals in the striatal subregions and/or differential spatial expression in this model could contribute to the current findings. These results require further investigation to determine the definitive mechanism(s), which underlie the apparent uptake changes.

4.4 Middle-aged *Gdnf*$^{+/-}$ mice demonstrate decreased DA synthesis and increased DA turnover without regional alterations in 5-HT

HPLC-EC analyses of DA and its metabolites were conducted to determine if DA synthesis and metabolism are altered in the striatum of *Gdnf*$^{+/-}$ mice. Tissues from rostral-medial and caudal-lateral striatal regions (recording sites 1 and 2) showed decreases in DA tissue levels (average ~25%), indicative of decreased DA synthesis. Decreased DA synthesis is not surprising given the loss of TH-positive cell bodies in the SN of *Gdnf*$^{+/-}$ mice (Boger, et al., 2006). Although there is evidence for decreased striatal TH staining density (Boger, et al., 2007), it is also possible that the relative activity of TH is reduced due to dephosphorylation, an aspect of TH activity shown to be enhanced by GDNF supplementation *in vivo* (Salvatore, et al., 2009, Salvatore, et al., 2004). Given the nature of the global partial depletion of GDNF protein in the brain in this model, both nigral and striatal TH phosphorylation may be diminished. The increased ratio of metabolites to DA indicate increased DA metabolism, but this is likely due to the decreased levels of DA, as the DA metabolites DOPAC and HVA were not significantly different in the *Gdnf*$^{+/-}$ mice.

HPLC-EC quantification of 5-HT and its metabolites were carried out to examine serotonergic changes in *Gdnf*$^{+/-}$ mice. There were no changes in whole tissue 5-HT and metabolite (5-HIAA) levels ($p>0.05$) at the two recording sites which support little contribution of the serotonergic systems in the outlined studies.

4.5 Conclusions

Motor and pathological manifestations associated with this *Gdnf*$^{+/-}$ mouse model become apparent later in life and progress gradually (Boger, et al., 2006). This slower time course contrasts with other DA lesion models in which the onset of neurodegeneration is comparatively rapid and severe (Ichitani, et al., 1994, Kirik, et al., 1998, Lee, et al., 1996, Sauer and Oertel, 1994), and deficits may recover with time (Yuan, et al., 2005). The *Gdnf*$^{+/-}$ mouse provides a novel model of DA dysfunction, which uniquely models subtle aspects of the human DA dysfunction seen in aging. The regulation of GDNF in aging and DA dysfunction in general has yet to be clearly defined. There are contradictory results regarding the regulation of GDNF in DA dysfunction and in age-associated movement disorders like PD. It has been suggested that GDNF isoforms may be differentially expressed in PD with an increase in isoform I (Backman, et al., 2006). Conversely, decreases in GDNF levels have been reported in the SN of PD patients (Jenner and Olanow, 1998). In animal models of DA dysfunction, both increased (Nakajima, et al., 2001) and

unchanged levels of striatal GDNF (Smith, et al., 2003) have been reported following 6-OHDA administration. Our studies reveal changes in DA neurochemistry, uptake, and consequential behavioral implications in a mouse model with a chronic and partial genetic reduction of GDNF, which appears to manifest age-related changes in DA function at 12 months of age. Taken together, these studies support a unique role for GDNF in the regulation of DA and DA neuron development and maintenance. Further studies are necessary to determine the specific mechanisms which underlie the apparent neurochemical and kinetic changes in DA regulation as well as the implications of these changes.

Acknowledgments

This work was supported by USPHS grants AG023630, IT32 DA022738, NS39787, DA017186, AG13494 and NSF grant EEC-0310723.

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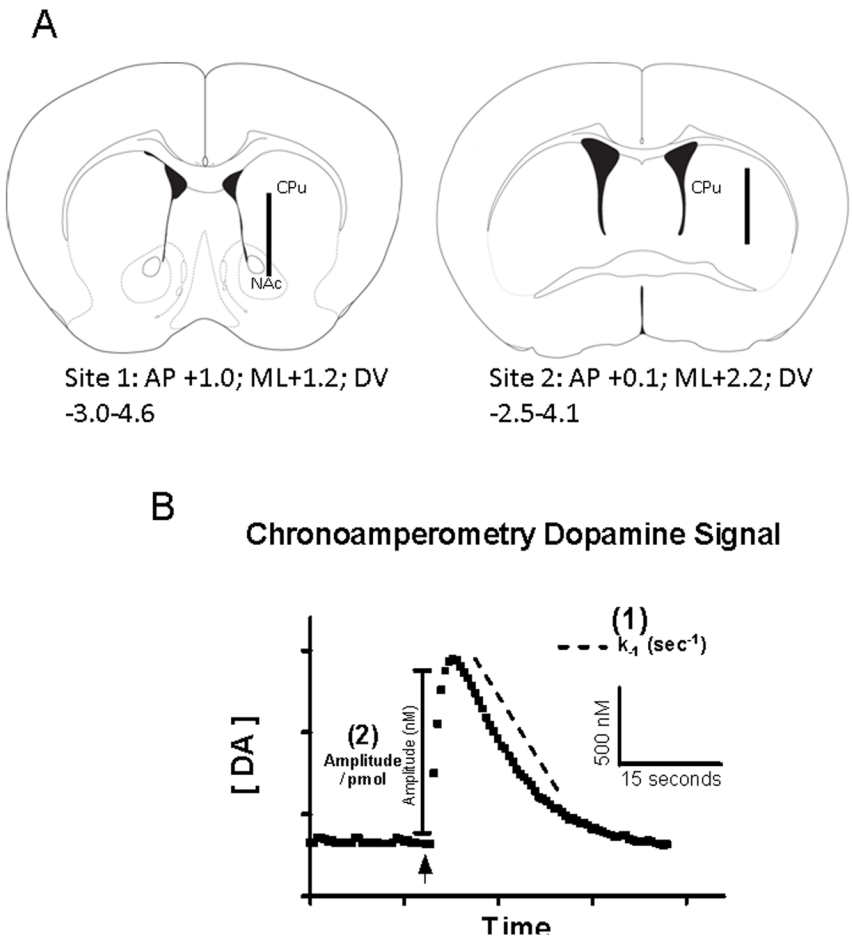

Figure 1.

(A) Microelectrode placement through the mouse dorsal striatum and nucleus accumbens. The diagrams show the approximate location of the microelectrode at site 1: AP+1.0 and site 2: AP+0.1 (relative to *bregma*; modified from Franklin and Paxinos (2001)). The microelectrode was moved in increments of 400 μm throughout the dorsal and ventral parts of the striatum. (B) Analysis parameters of the *in vivo* electrochemical DA signals. Following local application of DA solution (indicated by arrow), the amplitude (nM) is determined by calculating the change from a stable baseline using the calibration curves generated from each microelectrode prior to their use. The first order exponential decay constant, k_{-1} (sec^{-1}) (1), is calculated from the decay portion of the DA signal fitted to the slope of the linear regression of the natural log transformation of the data. By dividing the resulting DA signal amplitude (nM) by the amount (picomole) of DA solution applied the amplitude per picomole (nM/pmol) (2) is calculated.

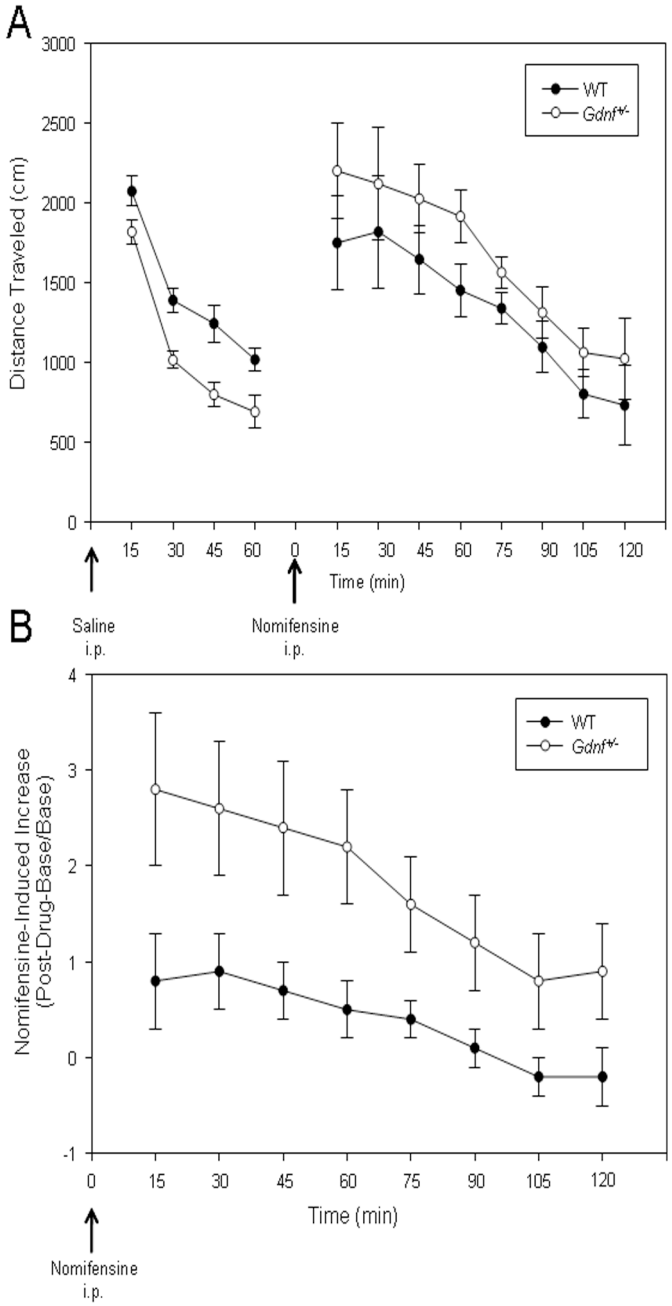

Figure 2.

Spontaneous and nomifensine-induced locomotor activity in 12 month old $Gdnf^{+/-}$ and WT mice. (A) Saline-injected (indicated by arrow, 0.01ml/g body weight) $Gdnf^{+/-}$ mice showed significantly decreased total distance traveled as compared to saline-treated WT mice [$P < 0.01$]. (B) $Gdnf^{+/-}$ mice showed greater nomifensine-induced (indicated by arrow, 7.5mg/kg, i.p.) locomotor activity, compared to WT mice [$F_{7,84} = 3.119$, $P < 0.01$].

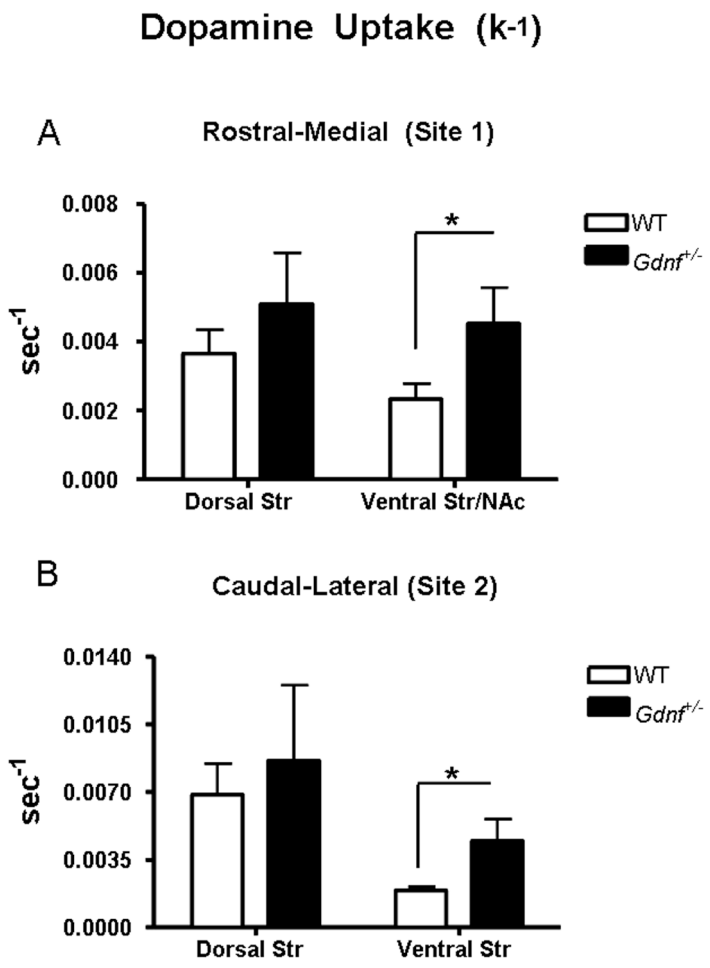

Figure 3.

Average k_{-1} (sec^{-1}) from dorsal and ventral striatal recording subregions of *Gdnf*$^{+/-}$ and WT mice (expressed as mean ± SEM). Local application of DA solution was used to obtain peaks rangeing from 500–1000 nM in amplitude for uptake analysis. The k_{-1} was analyzed to compare *Gdnf*$^{+/-}$ mice and WT mice. The k_{-1} was not significantly changed between genotypes in the dorsal striatum of the rostral-medial recording site 1 (Coordinates: AP +1.0; ML+1.2; DV −3.0–4.6) or caudal-lateral recording site 2 (Coordinates: AP +0.1; ML +2.2; DV −2.5–4.1). The k_{-1} did show genotype differences exclusive to the ventral Str/NAc of the rostral-medial recording site 1 [**P* = 0.0377] (A) and ventral striatum of the caudal-lateral recording site 2 [**P* = 0.0233] (B) with significant increases in DA uptake in the *Gdnf*$^{+/-}$ mouse.

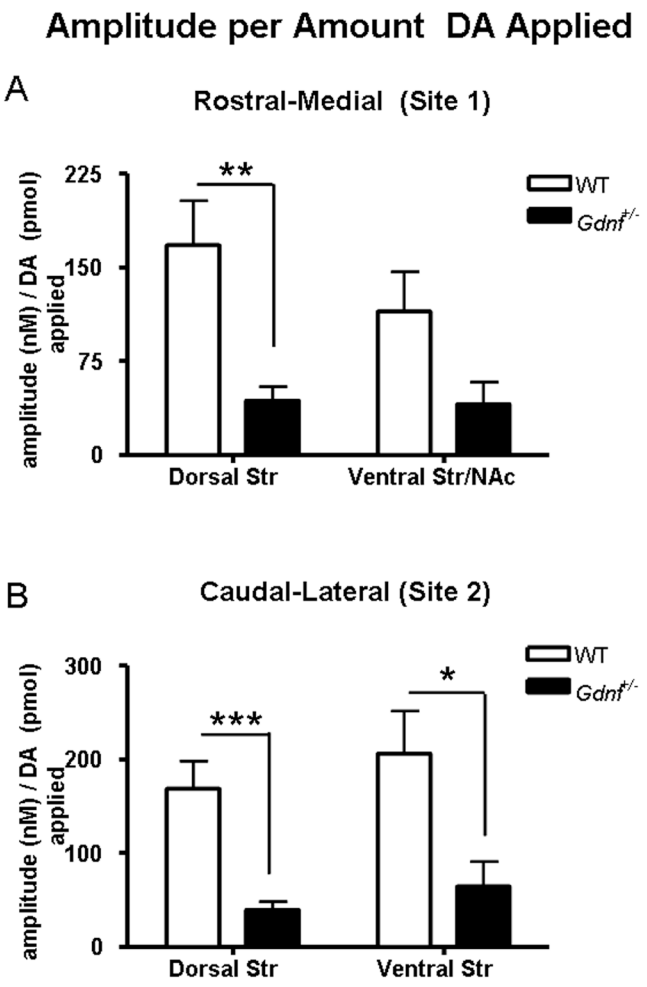

Figure 4.

Average amplitude per amount of locally applied DA solution (nM/pmol) from dorsal and ventral striatal recording subregions of *Gdnf*$^{+/-}$ and WT mice (expressed as mean ± SEM). The volume of solution was controlled in order to obtain peaks of amplitudes ranging from 500–1000 nM for uptake analysis. The resulting amplitude per picomole was analyzed to compare *Gdnf*$^{+/-}$ *versus* WT mice. The amplitude per picomole was not significantly different between genotypes in the ventral Str/NAc of the rostral-medial recording site 1 (A) (Coordinates: AP +1.0; ML+1.2; DV −3.0–4.6). The dorsal striatum of recording site 1 (A) however showed a significant difference between genotype [$^{**}P = 0.0091$]. Both recording subregions of recording site 2 (B) (Coordinates: AP +0.1; ML+2.2; DV −2.5–4.1) showed a significant change in amplitude per volume [$^{***}P = 0.0005$] (dorsal striatum) and [$P = 0.0181$] (ventral striatum). All striatal subregions with significant genotype differences showed a decrease in the average amplitude per picomole applied in the *Gdnf*$^{+/-}$ mice.

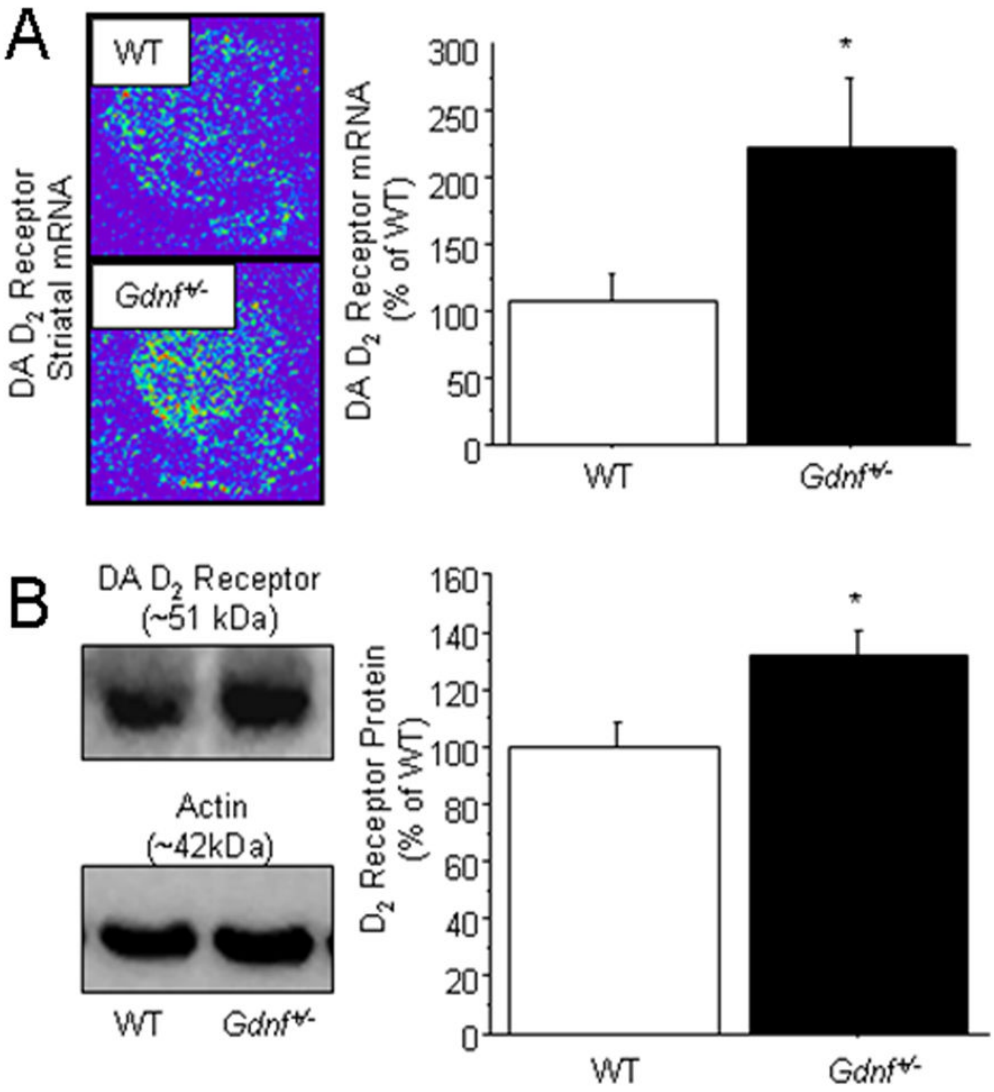

Figure 5.

Striatal dopamine D2 receptor mRNA and protein expression in $Gdnf^{+/-}$ vs. WT mice. (A) *In situ* hybridization immunohistochemical detection revealed a significantly greater expression of the dopamine D2 receptor mRNA in the dorsal striatum of $Gdnf^{+/-}$ mice compared to WT controls [$*P = 0.0433$]. (B) Protein levels of the dopamine D_2 receptor were significantly greater in the dorsal striatum of $Gdnf^{+/-}$ mice compared to WT mice [$*P = 0.0214$].

Table 1

Dorsal Striatum Tissue Levels of Dopamine (DA) & metabolites (DOPAC, HVA). Serotonin (5-HT) & metabolite 5-Hydroxyindoleacetic acid (5HIAA) and turnover ratios in 12 month old WT and *Gdnf*$^{+/-}$ male mice.

	Striatal Region	DA (ng/g)	DOPAC (ng/g)	HVA (ng/g)	DOPAC: DA	HVA: DA	(DOPAC +HVA): DA	5-HT (ng/g)	5-HIAA (ng/g)	5-HIAA:5-HT
WT	AP: +1.0	19509±141	3198±73	2087±29	0.164±0.011	0.107±0.004	0.272±0.014	874±10	590±6	0.680±0.030
Gdnf$^{+/-}$	AP: +1.0	15749±1044*	2907±250	2050±96	0.184±0.007	0.132±0.005*	0.316±0.010*	854±51	635±19	0.757±0.043
WT	AP: +0.1	8656±253	1796±47	1359±25	0.210±0.010	0.162±0.011	0.372±0.019	903±23	653±17	0.726±0.025
Gdnf$^{+/-}$	AP: +0.1	6026±924*	1487±244	1297±61	0.244±0.011*	0.222±0.010*	0.466±0.010*	867±53	755±56	0.879±0.053*

* $p < 0.05$, WT versus *Gdnf*$^{+/-}$, same striatal region

Values shown are mean ± SEM