

Research report

Glial cell line-derived neurotrophic factor receptor GFR α 1 is expressed in the rat striatum during postnatal development

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Abstract

Dopamine neurons of the substantia nigra (SN) undergo a natural cell death event which is biphasic, with peaks at postnatal days (PNDs) 2 and 14. There is growing evidence that GDNF functions as a striatal target-derived neurotrophic factor to regulate the first phase. It has been unknown whether the GDNF receptor, GFR α 1, may play a role in regulating either phase. To evaluate a possible role for GFR α 1 we have examined its expression throughout postnatal development in the SN and particularly in the striatum, where its expression has been uncertain. GFR α 1 mRNA is highly expressed in SN, as previously shown, with highest levels at PND14–28. We find that it is also expressed in striatum with a similar time course, but with a more discrete period of maximal expression between PND10 and PND14. The cellular basis of this maximum of expression is an increased number of GFR α 1 mRNA-positive medium-sized neurons evenly distributed within the striatum. Immunostaining reveals GFR α 1 protein-positive neurons with a similar morphology and distribution. We conclude that GFR α 1 is expressed in striatum maximally late in postnatal development. In this location it may act in trans to influence the viability and development of nigral dopamine neurons.

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1. Introduction

Like most developing neuronal populations, the dopamine (DA) neurons of the SN undergo an apoptotic natural cell death (NCD) event [14,13,27]. This event is largely postnatal, and it is biphasic with peaks at PND2 and 14. As envisioned by classic neurotrophic theory [1,8], this event is likely to be regulated by interactions with the target of these neurons, the striatum, because disruption of such interactions by target lesion [22], DA neuron terminal lesion [23] or axotomy [9] augments the NCD event. However, the

trophic factors mediating this striatal target-derived trophic support have been unknown.

One candidate neurotrophic factor for such a role has been GDNF, which was first identified on the basis of its ability to support the development of embryonic mesencephalic DA neurons in culture [20]. In support of this possibility, mRNA for GDNF is highly expressed in the striatum postnatally [3,7,33,34], and mRNA for its receptor, GFR α 1, and its signaling kinase, Ret, are highly expressed in SNpc [36,40]. In addition, we have shown that GDNF suppresses apoptotic cell death in DA neurons during the postnatal period both in vitro [5] and in vivo [28], and that intra-striatal injection of neutralizing antibodies to GDNF augments the NCD event in vivo [28]. Neutralization of GDNF within the striatum is able to induce NCD in DA neurons only during the first postnatal week, indicating that it regulates only the first phase [28]. In support of this concept, we have shown that overexpression

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of GDNF in mesencephalic target structures in double transgenic mice augments the number of surviving DA neurons after the first phase of NCD, but not into adulthood [16]. Therefore, while GDNF appears to be both necessary and sufficient to regulate NCD during the first phase, other factors must become necessary between the termination of the first phase and adulthood.

While many factors may play a regulatory role in the later phase of development, it is possible that the GDNF receptor, GFR α 1, itself becomes limiting, either in its presynaptic localization in DA neurons or postsynaptically, in trans [11,26,29,40]. Regarding the latter possibility, it has recently been shown that GFR α 1 can function in a non-cell-autonomous fashion to influence neurite outgrowth in the presence of non-limiting concentrations of GDNF [19]. Such neurite outgrowth could influence DA neuron target interactions during later phases of development and thus the magnitude of the NCD event.

However, it has been controversial whether GFR α 1 is expressed in striatum. While Nosrat et al. [26] identified some minimal ventral striatal expression in newborn mice, Yu et al. [40] identified very little expression in 1-week-old rats. Golden et al. identified no GFR α 1 expression in the striatum of adult mice [12]. Therefore, to determine whether GFR α 1 is expressed in striatum during development, and thus could play a role in trans for the developing DA nigro-striatal projection, we have examined its mRNA expression in striatum in comparison to SN throughout the NCD period. In addition, we have examined its protein expression in striatum by immunohistochemistry at PND14, the peak of the second phase of NCD, when mRNA levels were at their highest.

2. Materials

2.1. Animals

Timed pregnant Sprague–Dawley rats (Charles River Labs, Wilmington, MA) were used to obtain striatum and SN at PND2, 6, 10, 14, 28, and adult. The day of delivery was defined as PND1. SN and striatum tissue were obtained by microdissection as previously described [10]. The tissues were then frozen on dry ice and stored at -80°C until RNA isolation. This protocol has been approved by the Institutional Animal Care and Use Committee at Columbia-Presbyterian Medical Center.

2.2. Northern analysis

Northern analysis was performed as previously described [10]. Briefly, RNA was isolated using Qiagen RNeasy Mini kit. The RNA concentration of each sample was determined by measuring absorption at 260 nm on a GenQuant spectrophotometer (Amersham Pharmacia Biotech, Piscataway, NJ). Twenty micrograms of each RNA was electrophoresed

in 1.4% agarose-formaldehyde gel and transferred onto an Immobilon (+) membrane (Millipore, Bedford, MA).

To create a probe for Northern analysis, oligonucleotide primers for rat GFR α 1 mRNA were designed based on published sequences (Accession No. U97142) and they were synthesized by GenLink (Hawthorne, NY). The primers were as follows: the forward primer was 5'-GAACAGAGCTG-CAGCACCAA-3' (bp 371–390) and the reverse primer was 5'-GTCTTGCAGGAGTCTTGCAGA-3' (bp 1011–991). One microgram of the SN or striatum RNA was used for cDNA synthesis with a Promega reverse transcription system using the recommended conditions. RT-PCR was performed in a Mastercycler (Eppendorf Scientific) with Roche Taq polymerase. PCR products were cloned in pGEM-T vector (Promega), and the 641-bp sequence confirmed. Plasmids were used for generation of a ^{32}P -labeled antisense riboprobe with an in vitro transcription system (Promega).

The hybridization was performed overnight at 68°C in Ultrahyb buffer from Ambion (Austin, TX). The membrane was then exposed to phosphor imager cassettes, scanned and analyzed by Image Quant software (Molecular Dynamics, Indianapolis, IN). For the developmental studies, all developmental ages were represented by a single animal on each membrane, except PND2 which was represented by two. Eight independent hybridizations were performed, for an $N=16$ for PND2, and $N=8$ at all other developmental time points. Radioactive bands were expressed as a relative percentage of the radioactivity at PND2 on each membrane. We used quantitative analysis of total RNA and inspection of ethidium bromide stained gels to ensure equal loading of lanes. We have previously shown that GAPDH mRNA expression, often used as an indicator of total mRNA loading, is developmentally regulated [10], so that normalization by GAPDH mRNA levels results in a spurious, decreased apparent level of expression of the mRNA of interest between postnatal weeks 2 and 4. Similarly, α - and β -tubulin [2] and β -actin [18,24] mRNA are developmentally regulated. For these reasons, we did not normalize mRNA determinations by the mRNA for these genes. For the studies of the relative levels of expression in SN and striatum at the two peaks of NCD (PND2 and 14), two animals representing each region and each time point were represented on each blot. Four independent hybridizations were performed, for an $N=8$ for each region at PND2 and 14.

2.3. Immunohistochemistry

PND14 rats were anesthetized with halothane and perfused with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.1. Each brain was carefully removed and postfixed in the same fixative for 3 h. The brain was then cryoprotected in 20% sucrose for overnight and then rapidly frozen in 2-methylbutane on dry ice. The brain was cut at 30 μm on a cryostat, and striatum sections were incubated with anti-rat GFR α 1 goat polyclonal antibody (R&D systems, AF560) at 1:50 in PBS–5% normal horse

serum for 48 h at room temperature with agitation. Following washes, sections were incubated with biotinylated horse anti-goat antibody (Vector Laboratories, Burlingame, CA) at 1:200 in PBS–5% normal horse serum for 2 h at room temperature. Sections were then treated with avidin biotinylated horseradish peroxidase complexes (ABC; Vector Laboratories), followed by incubation with diaminobenzidine as chromogen. Sections were mounted onto gelatin-coated slides, dehydrated and coverslipped. The monospecificity of the anti-rat GFR α 1 goat polyclonal antibody was verified by Western analysis. For Western analysis, coronal sections containing striatum and cortex of PND14 rat were collected. Protein (100 μ g/lane) was electrophoresed on a 12% SDS-polyacrylamide gel, and proteins were transferred onto Hybond-P membrane (Amersham) using semidry electroblotting (E&K Scientific Products). The membranes were blocked in TBS with 0.1% Tween 20 containing 5% dry milk for 1 h at room temperature and then incubated with the GFR α 1 antibody (1:500) overnight at 4 °C. Membranes were then washed at room temperature with blocking solution, incubated with horseradish peroxidase-conjugated rabbit anti-goat IgG (Pierce, 1:5000), and stained with Supersignal West Dura substrate (Pierce). Chemiluminescent signals were detected using the FluorChem imaging system (Alpha Innotech, CA). This analysis showed that the anti-GFR α 1 antibody recognized a single distinct band at approximately 62 kDa (Fig. 1), which is within the range of previous reports [17,38,39]. In addition, the monospecificity of this antibody has previously been confirmed for immunohistochemistry in rodent tissues [37,30]; specifically, complete absence of staining is observed in GFR α 1 homozygous null tissues [30].

For immunofluorescence double-labeling of GFR α 1 and tyrosine hydroxylase (TH), animals were perfused and their brains sectioned as described. Sections were then incubated

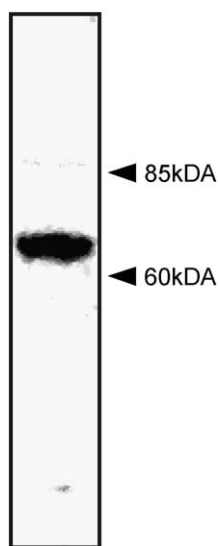


Fig. 1. Characterization of the anti-GFR α 1 antibody. Western blot on PND14 rat brain homogenate shows that the anti-GFR α 1 antibody recognized one band at a molecular weight of approximately 62 kDa.

with a mouse monoclonal anti-TH antibody at 1:40 (Chemicon; MAB5280) in addition to anti-GFR α 1 at 1:50 in PBS/5% horse serum/5% donkey serum overnight at room temperature. After washes, the sections were incubated with donkey anti-goat-AlexaFluor594 at 1:250 (Molecular Probes) and fluorescein-conjugated horse anti-mouse at 1:75 (Vector Laboratories) for 2 h at room temperature. Sections were rinsed and then mounted on gelatin-coated slides, dried, and coverslipped with DAKO anti-fade medium. The sections were then examined with appropriate filters by epifluorescence on a Nikon Eclipse 800 microscope.

2.4. *In situ* hybridization

Brains were rapidly removed from PND6 and PND14 rats, and rapidly frozen in OCT (Tissue-Tek) on dry ice. Sections (14 μ m) were thaw-mounted on glass slides (Superfrost Plus, Fisher). For hybridization, sections were warmed to room temperature and fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate-buffered saline. After washing, sections were acetylated by treatment with acetic anhydride in triethanolamine. After another wash, sections were treated with a pre-hybridization solution as previously described [4] for 2 h at room temperature. Sections were then covered with hybridization solution and incubated overnight at 68 °C. Hybridization solution contained a GFR α 1 probe labeled with digoxigenin-UTP, prepared as per the manufacturer's instructions (Roche Diagnostics, Penzberg, Germany). The size and integrity of labeled probe were confirmed by gel electrophoresis. The same probe used for Northern analysis was used for the *in situ* hybridization. After washes in $0.2 \times$ SSC at 68 °C, sections were incubated with an anti-digoxigenin antibody (Roche) at 1:5000 overnight at 4 °C. After additional washes, sections were then incubated with a developing solution containing BCIP/NBT (Promega) overnight at room temperature in the dark. Sections were then washed and coverslipped with DAKO aqueous mounting medium. To determine the number of GFR α 1 mRNA-positive profiles in the striatum at PNDs 7 and 14, the entire striatum was scanned at $600 \times$, and results were expressed as neurons/section.

2.5. Statistics

One-way ANOVA (with a Tukey post hoc analysis) and Student's *t*-test were performed for statistical analysis (SigmaStat, SPSS Science).

3. Results

3.1. Developmental expression of GFR α 1 mRNA in striatum and SN

Northern analysis of striatal and SN GFR α 1 mRNA revealed two major bands, at about 4.0 and 8.0 kb (Fig.

2A,D), as previously reported for rat tissues [31]. In both tissues, a very faint band was also observed just below the 4.0-kb band, as also previously reported by Sanicola et al. [31], although that mRNA species was barely detectable in brain in their report. In striatum, the expression of both major mRNA species was biphasic, with high levels at PND2, and a second, broader (and higher) maximum at

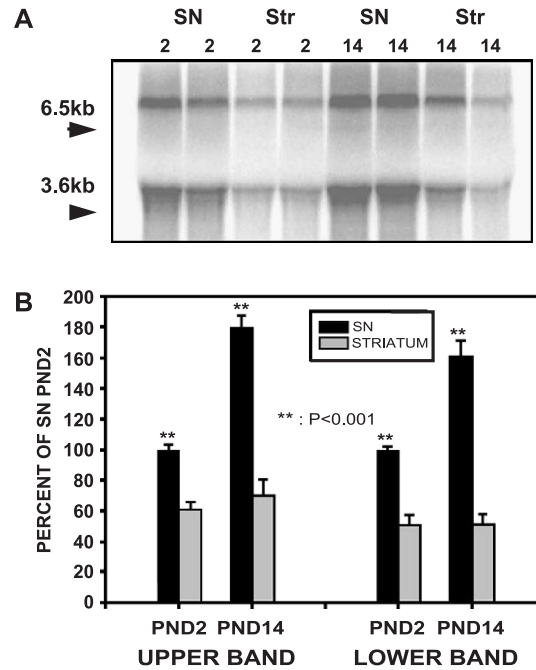
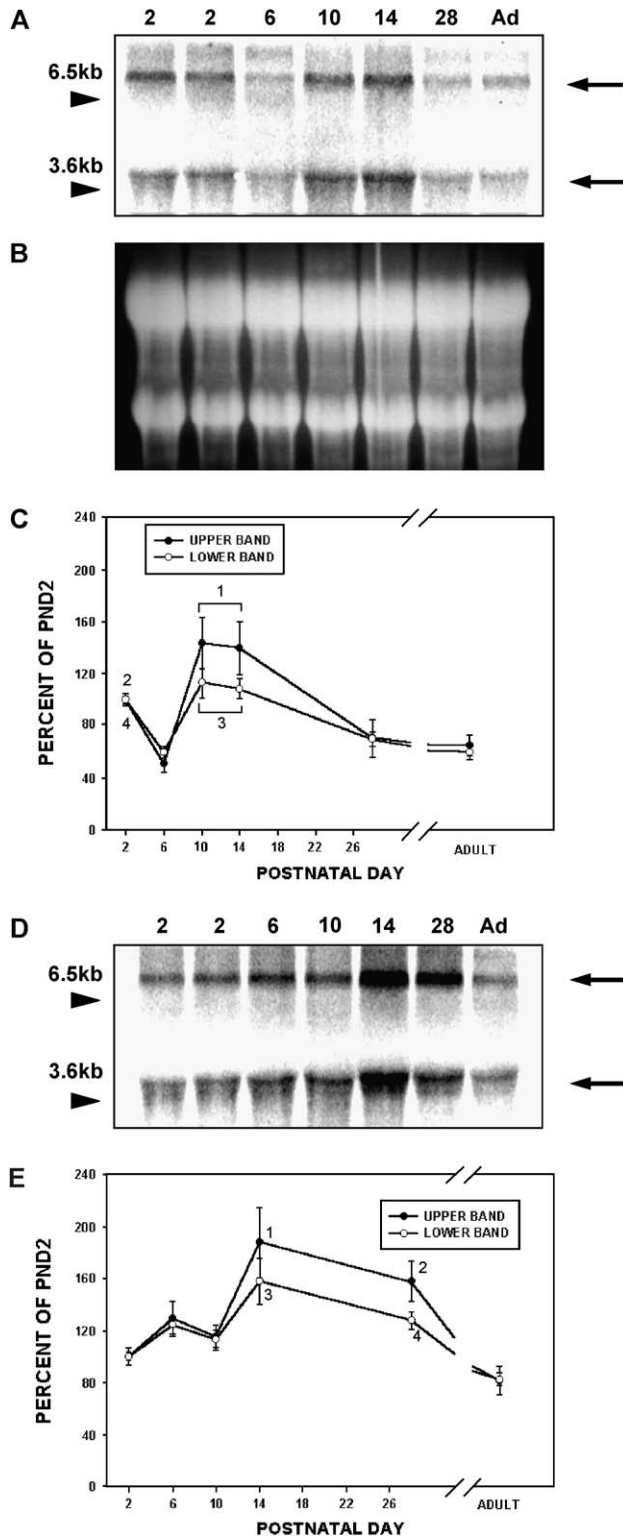


Fig. 3. Relative levels of GFR α 1 mRNA in striatum and SN at the two peaks of NCD in SN. (A) Representative Northern analysis of GFR α 1 mRNA expression in striatum and SN at PND2 ($N=2$ for each region; RNA from two different animals is loaded in adjacent lanes) and PND14 ($N=2$ for each region). (B) Quantitative analysis of GFR α 1 mRNA expression in striatum and SN at the two phases of NCD in SN ($N=8$ for each condition). Note that GFR α 1 mRNA levels were higher in the SN than in striatum, at both PND2 and 14 for both bands ($p<0.001$, Student's t -test).

PND10–14 (Fig. 2C). GFR α 1 mRNA was also detected in SN (Fig. 2D), confirming previous studies by in situ hybridization [36,40]. As for striatum, two major mRNA

Fig. 2. Developmental time course of GFR α 1 mRNA expression in striatum and in SN. (A) Representative Northern blot of GFR α 1 mRNA expression in striatum. Twenty micrograms of total RNA was loaded per lane, and the blot was probed with a riboprobe generated from 641 bp cDNA of GFR α 1. The blot revealed two major transcripts, at 4.0 (lower band) and 8.0 kb (upper band) (arrows). (B) An ethidium bromide stain of the gel demonstrates equal loading of the lanes. (C) Developmental time course of GFR α 1 mRNA expression in striatum. The radioactive bands are plotted as a relative percentage of the average radioactivity at PND2 ($N=16$ for PND2 [$N=2$ per blot, eight blots were examined]; $N=8$ for all other time points [$N=1$ per blot]). Each point represents the mean, and the error bars the standard error of the mean. Note that expression of GFR α 1 in the striatum is biphasic, with high levels at PND2 and PND10–14. Analysis of upper band: ANOVA, $p<0.001$. Post hoc analysis: (1) $p<0.01$ PND10 and 14 vs. PND6, 28 and adult; (2) $p<0.05$ PND2 vs. PND6. Analysis of lower band: ANOVA, $p<0.001$. Post hoc analysis: (3) $p<0.005$ PND10 and 14 vs. PND6, 28 and adult; (4) $p<0.01$ PND2 vs. PND6, 28 and adult. (D) Representative Northern analysis of GFR α 1 mRNA expression in SN from PND2 to adult. (E) Developmental time course of GFR α 1 mRNA expression in SN ($N=16$ for PND2 [$N=2$ per blot, eight blots were examined]; $N=8$ for all other time points [$N=1$ per blot]). Note that expression of GFR α 1 is highest between PND14 and PND28 and then decreases in adulthood. Analysis of upper band: ANOVA, $p<0.001$. Post hoc analysis: (1) $p<0.01$ PND14 vs. PND2, 10 and adult; (2) $p<0.05$ PND28 vs. PND2, adult. Analysis of lower band: ANOVA, $p<0.001$. Post hoc analysis: (3) $p<0.005$ PND14 vs. PND2, 10 and adult; (4) $p<0.005$ PND28 vs. adult.

species were detected in approximately equal abundance throughout development. Expression levels in SN, like those in striatum, were highest later in postnatal development, from PND14 to 28 (Fig. 2E).

In separate experiments directly comparing the relative abundance of GFR α 1 mRNAs in SN and striatum, both mRNA species were found to be more abundant in SN (Fig. 3A,B), as would be expected for the receptor of a striatal target-derived neurotrophic factor. Nevertheless, substantial expression of GFR α 1 mRNA was observed in striatum at both PND2 and 14, at the two peaks of NCD.

3.2. Cellular expression of GFR α 1 mRNA in the striatum

Because high levels of GFR α 1 mRNA are expressed in the endopiriform nucleus and the lateral septal area [40], both closely adjacent to our striatal punches, we sought to confirm its striatal expression and to perform a cellular analysis by *in situ* hybridization. This analysis confirmed high levels of expression in the endopiriform nucleus, lateral

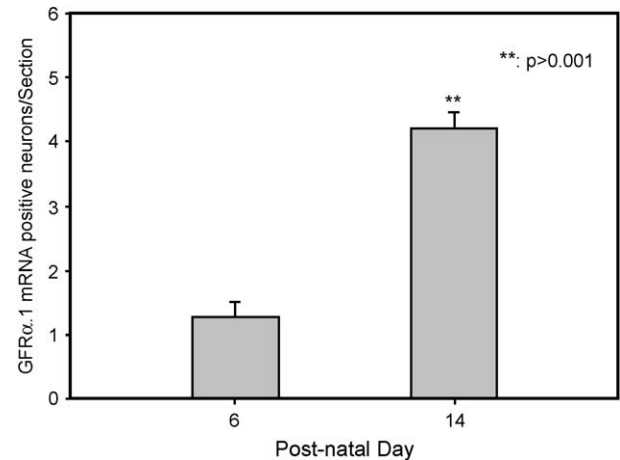


Fig. 5. Counts of neuronal profiles identified by non-radioactive *in situ* hybridization for GFR α 1 in the striatum at PND6 and 14 ($N=29$ sections from four animals for PND6; $N=22$ sections from four animals for PND14). The number of neurons positive for GFR α 1 was significantly higher at PND14 than PND6 ($p<0.001$).

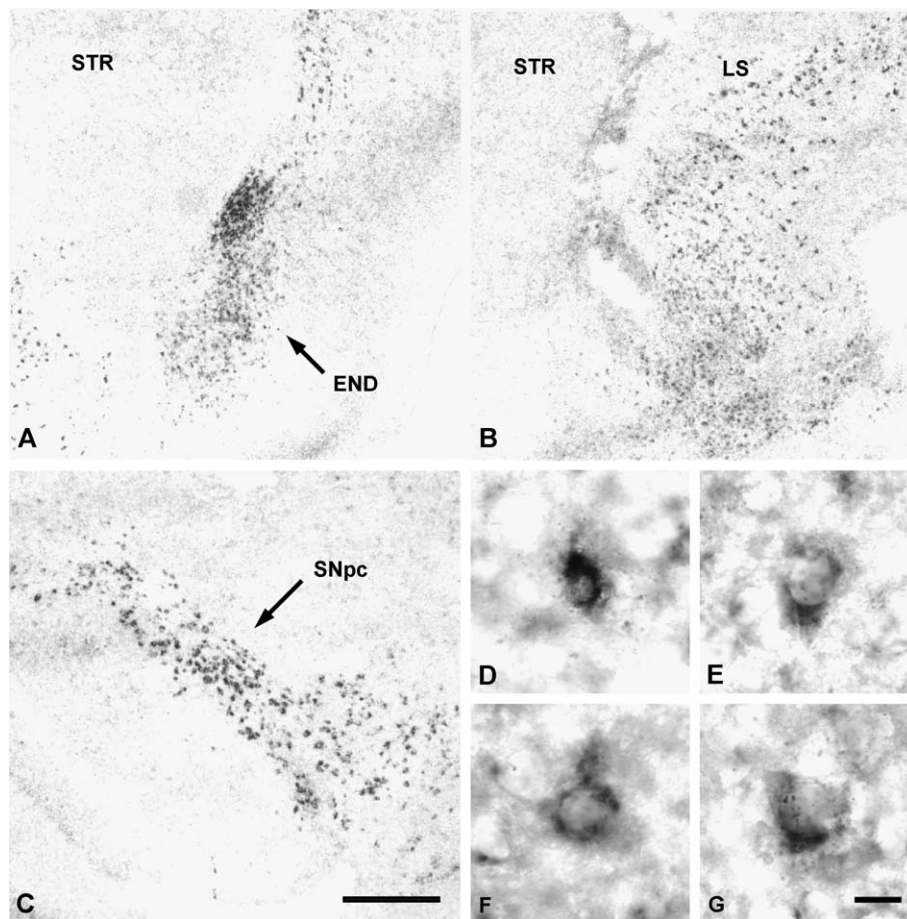


Fig. 4. *In situ* hybridization analysis of GFR α 1 mRNA expression at PND14. Positive staining for GFR α 1 mRNA is demonstrated in endopiriform nucleus (END) (A), the lateral septal nucleus (LS) (B), and the SNpc (C) as previously described [40]. At a regional level of analysis, the striatum (STR) in (A) and (B) shows little staining (bar=500 μ m; A–C). At higher power, staining of individual neurons in the striatum is observed, as shown in four examples (D–G) (bar=10 μ m; D–G).

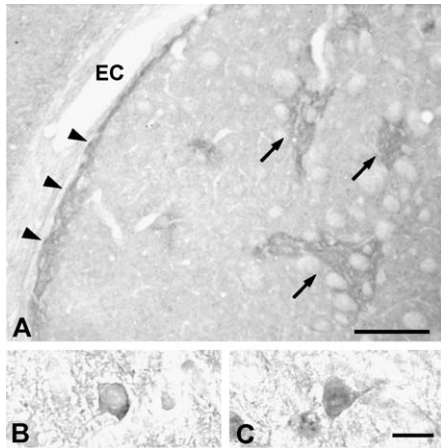


Fig. 6. Immunostaining for GFR α 1 in striatum at PND14. (A) A low power photomicrograph demonstrates stained striosome patches within the striatum (arrows). A rim of positive staining is observed laterally in the striatum (arrowheads), adjacent to the external capsule (EC) (bar = 200 μ m). At higher power, staining of individual neurons is observed in two examples (B) and (C) (bar = 10 μ m; B,C).

septal area, and SNpc as previously reported (Fig. 4A–C) [36,40], and it confirmed striatal GFR α 1 mRNA expression at PND14 in individual striatal neurons (Fig. 4D–G). Positive neurons were evenly distributed throughout the striatum without any apparent sub-regional organization in striosomes or gradients. All of the profiles identified were in the medium size (10–20 μ m) class of striatal neurons; none was of the less numerous large neuron (20–60 μ m) type. Hybridization with a sense probe revealed no signal above background (not shown). In order to confirm the difference

in striatal mRNA expression between PND6 and 14 as revealed by Northern analysis, we performed counts of GFR α 1 mRNA-positive profiles in striatal sections. This analysis confirmed that GFR α 1 mRNA-positive profiles are much more numerous on PND14 (Fig. 5).

3.3. Immunohistochemical analysis of GFR α 1 protein expression in striatum

In order to confirm the functional significance of GFR α 1 mRNA expression in the striatum, we performed immunohistochemistry for protein expression. At a regional level, at PND14, GFR α 1 immunostaining was most prominent in striosomal patches (Fig. 6A) and in a lateral crescent just subjacent to the external capsule. Interestingly, this regional pattern of expression is identical to that previously described for GDNF during development [21]. The cellular basis of this striosomal staining was a mixture of positive cellular somata and punctate elements, but predominantly the latter. Distinctively positive neuronal cellular profiles (Fig. 6B,C) were observed evenly distributed within the striatum, as was the case for in situ-labeled profiles. At a cellular level, positive profiles were 10–20 μ m in size, as were the in situ-labeled cells. Thus, expression of GFR α 1 in individual striatal neurons at PND14 was confirmed by analysis of both protein and mRNA.

Because the cellular basis of the striosomal pattern of staining was predominantly due to neuropil, and because GFR α 1 mRNA is highly expressed in neurons of the SNpc, we sought to determine whether some of the striosomal GFR α 1-positive neuropil might be derived from the dopa-

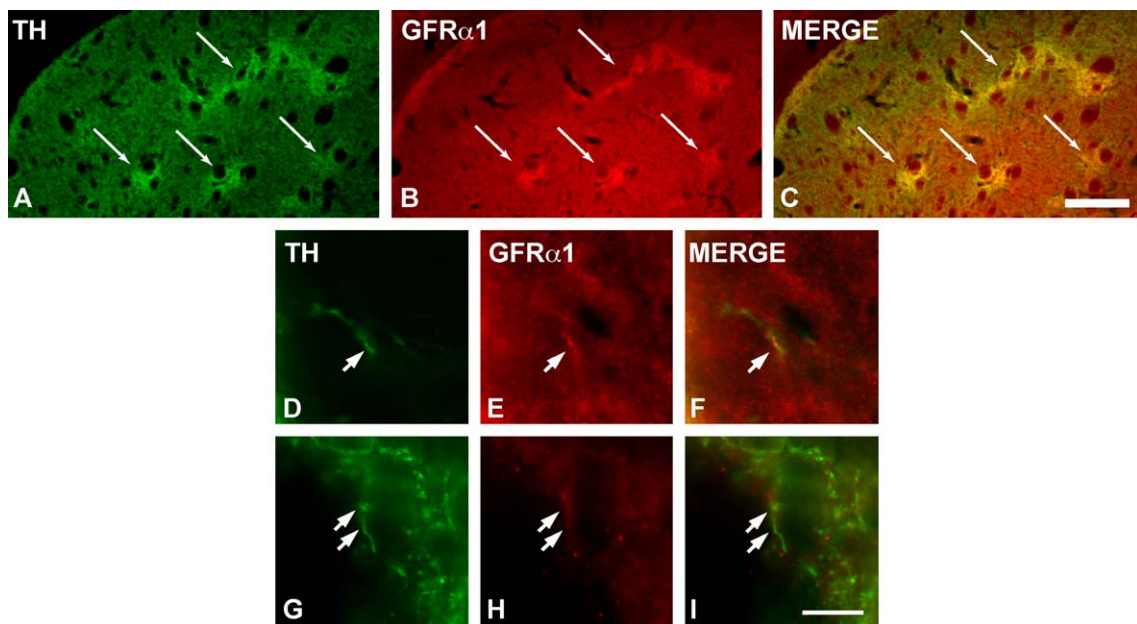


Fig. 7. Double immunofluorescence staining for GFR α 1 and TH in rat striatum at PND14. (A) TH immunostaining of the striatum, shown here at low power, demonstrates four striosomal patches (long arrows). (B) These four striosomes are also stained for GFR α 1, as demonstrated in the merged image (C) (bar = 200 μ m; A–C). A representative TH-positive fiber, co-labeled for GFR α 1, is shown in panels D–F, and a second example is shown in panels G–I (bar = 10 μ m; D–I).

minergic nigro-striatal afferent projection. We therefore performed double immunofluorescence labeling for GFR α 1 and TH. Double-labeling revealed a precise correspondence between striosomes identified by TH labeling, and those identified by GFR α 1 (Fig. 7A–C). At a cellular level, GFR α 1 immunostaining was identified within individual TH-positive fibers (Fig. 7D–I).

4. Discussion

Previous investigations using *in situ* hybridization [36, 32,40] have shown that GFR α 1 mRNA is highly expressed in SN. We have confirmed this expression by Northern analysis and demonstrate that it is due to two major mRNA species of approximately equal abundance. Previous investigations by Sanicola et al. [31] had demonstrated these two species in other rat tissues. Their studies demonstrated the 4.0-kb species to be predominant in whole brain, whereas in our analysis the two were of approximately equal abundance in SN throughout development. This may be due to a regional or developmental difference between the two studies. Like previous investigators, we show by *in situ* hybridization that, at a cellular level, GFR α 1 mRNA expression in SN is predominantly in neurons of the SNpc. No glial profiles were identified. We conclude that our developmental studies, performed at the tissue level, are primarily a reflection of expression in SNpc neurons.

The high levels of GFR α 1 mRNA expression in SN during development are consistent with the concept that it may serve as a receptor for striatal target-derived GDNF in DA neurons during the NCD period. We have shown that intrastriatal injection of GDNF is able to suppress NCD in DA neurons during the first phase of NCD during the first postnatal week [28]. During the first phase, but not during the second phase, which occurs on PND14–28, intrastriatal injection of neutralizing antibodies to GDNF augments NCD [28]. In support of the hypothesis that GDNF regulates NCD during the first phase, we have shown that overexpression of GDNF in the striatum in double transgenic mice results in an increased surviving number of DA neurons after this phase [16]. Thus, during the first phase of NCD in DA neurons, striatal GDNF is both necessary and sufficient to maintain the survival of DA neurons. It is during this development period, when striatal GDNF levels are limiting for the survival of DA neurons, that striatal GDNF mRNA levels are highest [6]. This observation may suggest that mRNA levels for neurotrophic factors are highest when they play a critical regulatory role. Similar observations have been made, for example, for NGF support of striatal cholinergic neurons. During the postnatal period when NGF regulates striatal cholinergic neuron development [35], mRNA levels are at their highest [25].

This relationship between mRNA levels and a critical regulatory period may apply to neurotrophic factor receptors

as well. Ledda et al. [19] have demonstrated that GFR α 1 mRNA levels are highest in target structures when GFR α 1 exerts a more pronounced non-cell autonomous effect. In relation to GFR α 1, its expression in SN is at lower levels during the first postnatal week than the second, and yet it clearly is not limiting for the mediation of GDNF effects. In this developmental period, intrastriatal injection of GDNF suppresses apoptosis in DA neurons [28]. The higher levels of expression of GFR α 1 in SN during later development may suggest that it may play a more critical limiting regulatory role, as yet undefined, during that time.

We have shown that GFR α 1 mRNA is expressed in the postnatal striatum. As in the SN, GFR α 1 mRNA in the striatum consists of two major species which are expressed in approximately equal abundance and regulated in parallel throughout development. In the striatum GFR α 1 mRNA is expressed at highest levels in development, as it is in the SN, but with a narrower time frame of maximal expression between PND10 and PND14. Based on the considerations outlined above, this may be the period in which striatal GFR α 1 plays a critical limiting regulatory role during NCD of DA neurons. Our *in situ* hybridization analysis demonstrates that the cellular basis for peak levels of GFR α 1 mRNA expression in striatum at this developmental period is an increased number of GFR α 1 mRNA-positive, medium-sized striatal neurons. These positive neurons are evenly distributed throughout the striatum, without a regional pattern of localization in striosomes or gradients. No glial profiles were observed. We therefore conclude that, as for SN, our developmental studies of striatal GFR α 1 expression, performed at a tissue level, are primarily a reflection of expression in striatal neurons.

This pattern of GFR α 1-positive neuron expression was confirmed at the protein level by immunohistochemistry. Like GFR α 1 mRNA-positive neurons, GFR α 1 protein-positive neurons were medium in size and evenly distributed throughout the striatum. At a regional level, GFR α 1-positive immunostaining in the striatum revealed positive striosomes and a distinct lateral crescent of staining subjacent to the external capsule. At a cellular level, while the striosomes occasionally contained GFR α 1-positive neurons, they were rare. Most of the immunopositive structures within the striosomes were punctate or fibrillar neuropil. We have shown by double immunofluorescence labeling that some of this neuropil arises from the dopaminergic nigro-striatal projection. Given that we have shown that GFR α 1 mRNA is more abundant in SN than striatum, it is likely that the GFR α 1 protein observed in TH-positive afferents arises from dopaminergic cell bodies and is transported anterograde to the striatum. Nevertheless, given that we have also shown that GFR α 1 is synthesized locally in the striatum, it is possible that some observed in TH-positive fibers has been taken up following in trans release within the striatum and is transported retrograde.

Our results demonstrate that GFR α 1 is expressed in striatal target neurons, primarily late in development during

the second phase of NCD in DA neurons. It is therefore possible that GFR α 1 in striatum may play a role in regulating DA neuron development in trans during that period. GFR α 1 has been proposed to act in trans the lateral septum, the superior colliculus and other structures which express GFR α 1, but little Ret [40]. The lateral septum, for example, receives a Ret-expressing projection from the CA3 region of the hippocampus, and GFR α 1 has been proposed to influence incoming Ret-positive axons by acting in trans [40]. It has been shown in vitro that GFR α 1 can be released by neurons to modulate downstream signaling and potentiate neurite outgrowth, neurite guidance, and neuronal survival [29]. Further, in the presence of uniform and saturating concentrations of GDNF, immobilized, exogenous GFR α 1 acting in trans can influence the strength and direction of neurite outgrowth [19]. Thus the GFR α 1 expressed in striatum may be capable of exerting effects on the strength and direction of the DA nigrostriatal projection. Such effects could strengthen target interactions during late development and thereby influence DA neuron viability late in the NCD period. While developing DA neurons appear to depend on striatal GDNF only through PND7 [28], they depend on continued striatal interactions through PND14. Ablation of striatal target [15] or lesion of DA nerve terminals [23] augments the NCD event through PND14. Thus, striatal GFR α 1, expressed at peak levels at that time, is a candidate factor for regulation of this target dependence late in the NCD period.

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