Journal of Neuroscience Research 73:686–697 (2003)

Rapid Increase of Nurr1 Expression in the Substantia Nigra After 6-Hydroxydopamine Lesion in the Striatum of the Rat

Viviana Ojeda, José Antonio Fuentealba, Danny Galleguillos, and María Estela Andrés*
Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

Nurr1 is a transcription factor essential for the genesis of ventral dopaminergic neurons. In this study, we investigated the expression of Nurr1 protein and mRNA in the adult rat brain by using immunohistochemistry and in situ hybridization, respectively. Another aim of our study was to investigate Nurr1 expression in substantia nigra after dopamine depletion induced by the injection of 6-hydroxydopamine in the striatum. We observed that Nurr1 mRNA and protein are expressed in several brain regions, including cortex, hippocampus, substantia nigra, and ventral tegmental area, in agreement with previous reports using in situ hybridization. Additionally, we found that Nurr1 is expressed in brain regions that have not been previously reported, such as striatum, septum, and superior colliculus. Highest levels of expression were found in cortex, medial septum, dentate gyrus, some hypothalamic nuclei, and substantia nigra. Interestingly, we observed that, in the superior colliculus, Nurr1 protein is localized in the cytoplasm of cells, whereas, in other regions, it was localized mainly in the nuclei, suggesting that Nurr1 subcellular localization is regulated and may have functional implications. Dopamine depletion induced by an injection of 6-hydroxydopamine into the striatum produced an increase in the number of cells expressing Nurr1 mRNA and protein in both substantia nigra compacta and substantia nigra reticulata, ipsilateral and contralateral to the lesioned side, measured 24 hr after the 6-hydroxydopamine injection. These results suggest that Nurr1 may be involved in many neuronal functions in the adult central nervous system and, in particular, might be related to the compensation processes that take place in dopaminergic cells in order to normalize extracellular dopamine levels in the striatum.

Key words: Nurr1; dopamine; compensation; 6-hydroxydopamine

Nurr1 (for nuclear receptor-related factor 1, NR4A2) is an orphan member of the nuclear receptor superfamily (Law et al., 1992; Peña de Ortiz and Jamieson, 1996). Nurr1, together with the orphan receptors Nur77 and Nor-1, conforms the NUR subfamily, in which all members bind the same *cis*-acting consensus sequence (NBRE, NGFI-B-response element) to regulate the expression of target genes. NUR subfamily members are products of immediate-early genes, whose expression can be rapidly induced in response to a variety of stimuli, including growth factors, neurotransmitters, and depolarization (Law et al., 1992; Honkaniemi et al., 1995, 2000; Honkaniemi and Sharp, 1996; Nakki et al., 1996; Peña de Ortiz et al., 1996; Xing et al., 1997).

Nurr1 is expressed in several regions of the developing and adult central nervous system (CNS; Saucedo-Cardenas and Conneely, 1996; Xiao et al., 1996; Zetterström et al., 1996a,b). Expression of Nurr1 in mice is detected at embryonic day 10.5 in ventral midbrain, the region where mesencephalic dopaminergic neurons develop (Zetterström et al., 1996b). Various studies have revealed that Nurr1 is absolutely essential for the induction of dopaminergic neurons in mice midbrain (Zetterström et al., 1997; Castillo et al., 1998a;Saucedo-Cardenas et al., 1998). Mice lacking Nurr1 fail to generate dopaminergic neurons in midbrain and die soon after birth. Some reports have suggested that, in addition to the demonstrated role of Nurr1 in the acquisition of ventral dopaminergic phenotype, Nurr1 is important in the regulation of the ventral dopaminergic system in the adult. In fact, heterozygous Nurr1+/– mice are more vulnerable to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) than controls, showing a diminished capacity to recover dopamine (DA) levels in the striatum (CS) after the damage induced by MPTP (Le et al., 1999). On other hand, Tseng and coworkers (2000) reported that Nurr1 mRNA expression increases significantly in mesencephalic dopaminergic neurons in mice null for D2 receptors, suggesting a regulatory interaction between Nurr1 and this dopaminergic system. In

Contract grant sponsor: FONDECYT; Contract grant number: 1000722; Contract grant sponsor: Fundación Andes; Contract grant number: C-13680/3.

*Correspondence to: María Estela Andrés, Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia, Universidad Católica de Chile, Alameda 340, Santiago, Chile. E-mail: mandres@bio.puc.cl

Received 23 January 2003; Revised 2 May 2003; Accepted 15 May 2003

addition, a recent report showed a significant decrease of Nurr1 expression associated with a reduced expression of DA transporter (DAT) in ventral dopaminergic neurons of cocaine abusers (Bannon et al., 2002). It is unknown which processes are regulated by Nurr1 in vivo. However, several studies with cell cultures suggest that Nurr1 could regulate the expression of dopaminergic-specific genes, such as tyrosine hydroxylase (TH) and DAT. In this regard, it has been shown that forced Nurr1 expression in hippocampal neuronal precursors induces TH expression (Sakurada et al., 1999). Also, recombinant Nurr1 overexpressed in cell lines induces the expression of reporters coupled to TH and DAT promoters (Iwawaki et al., 2000; Sacchetti et al., 2001). Interestingly, mutations in the NR4A2 gene found in individuals affected with Parkinson's disease decrease both Nurr1 and TH mRNA levels (Le et al., 2003).

Various reports have suggested, in addition to the role of Nurr1 in the induction and regulation of ventral dopaminergic neurons in the brain, a wider functional influence of this transcription factor in other systems. Peña de Ortiz and her group (2000) found that Nurr1 expression increases in CA1 and CA3 subregions of the dorsal hippocampus during spatial discrimination learning, suggesting an important role for Nurr1 in long-term information storage. Also, it has been reported that Nurr1 expression increases transiently in the hippocampus after electroconvulsive seizures (Xing et al., 1997) and kainic acid-induced seizures (Crispino et al., 1998).

We were interested, in view of the important functions of Nurr1 in the ventral dopaminergic system, in studying the expression of Nurr1 mRNA and protein after 6-hydroxydopamine (6-OHDA) dopaminergic-terminal lesion in the CS of the rat. To this end, first, we characterized the regional and subcellular localization of Nurr1 mRNA and protein in the CNS of the rat. Second, we characterized the effect of 6-OHDA injections in the CS by measuring time-dependent changes in DA extracellular levels in the CS with microdialysis. Here we report that the injection of 6-OHDA in the CS induces a massive and rapid efflux of DA, followed by a continual decrease until the extracellular levels of DA become undetectable. This effect was associated with a bilateral increase of Nurr1 mRNA and protein expression in the substantia nigra (SN), measured 24 hr after the 6-OHDA injection. These results suggest that DA extracellular levels in the CS regulate the expression of Nurr1 in the SN of the rat and that the augmented expression of Nurr1 could be related to the compensation processes that occur in order to normalize DA extracellular levels in the CS.

MATERIALS AND METHODS

Rats were obtained from the Animal Service Unit, Catholic University of Chile, and were handled according to the regulations stipulated by the Catholic University of Chile and by The Animal Care and Use Committee of FONDECYT.

Animal Studies

Adult male Sprague Dawley rats (weighing 250–300 g) were deeply anesthetized intraperitoneally (i.p.) with chloral hydrate (400 mg/kg) and mounted in a stereotaxic apparatus (Stoelting). Twenty micrograms per four milliliters of 6-OHDA prepared in 0.02% ascorbic acid were injected in the right CS at a rate of 1 μl/min using a Hamilton microsyringe. Coordinates for the placement of the Hamilton syringe tip with regard to Bregma were A 1.2 mm, L 2.8 mm, and V 4.5 mm, according to the Paxinos and Watson atlas (1986). Sham-operated rats were stereotaxically injected with 0.02% ascorbic acid under the same conditions. Twenty-four hours after the lesion, rats were processed for histochemical studies as follows. Rats were deeply anesthetized with chloral hydrate and then killed by perfusion through the left cardiac ventricle with 0.9% NaCl, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 9.5. Brains were then removed from the skull, cut into blocks, postfixed in 4% paraformaldehyde for 1 hr, and finally maintained in 30% sucrose in 0.1 M PB containing 0.9% NaCl (PBS) during 48 hr at 4°C. Vibratome or cryostat serial brain coronal sections (30 μm) were collected and washed three times in 0.1 M PBS, pH 7.4, to be processed for in situ hybridization (ISH) and immunohistochemistry (IHC).

Microdialysis and DA Detection

Microdialysis and DA detection were performed essentially as described by Andrés and collaborators (1998). Briefly, rats were deeply anesthetized with chloral hydrate (400 mg/kg i.p.), and supplemental doses were administered as needed to maintain anesthesia during the experiment. A concentric microdialysis probe (MD-2262; CMA, Acton, MA) was implanted into the right CS. The probe was continuously perfused at a rate of 2 μl/min with a Krebs Ringer phosphate buffer (in mM: 120 NaCl, 2.4 KCl, 1.2 CaCl, 0.9 NaH_2PO_4, 1.4 Na_2HPO_4, pH 7.4) using a Harvard infusion pump (model 22). After a 30-min stabilization period, perfusion samples were collected every 5 min in 3 μl of 0.2 N perchloric acid and maintained on ice until DA determination by high-performance liquid chromatography (HPLC) coupled to electrochemical detection (EC). Then, 20 μg of 6-OHDA dissolved in 4 μl of 0.01% ascorbic acid were injected in CS through the lateral infusion tube of the microdialysis probe. After 1 hr, when the interfering signal of 6-OHDA was not detected in the HPLC-EC system, again 5-min perfusion samples were taken and assayed for DA content during the next 2 hr. The HPLC-EC system was configured with one piston pump (Shimadzu), a SepStick microbore column (BAS), and an amperometric detector LC4C (BAS). The mobile phase contained 0.1 M NaH_2PO_4, 1 mM EDTA, 1 mM 1-decanesulfonic acid, pH 3.2, and it was pumped at a flow rate of 70 μl/min. The potential of the amperometric detector was set at 650 mV. Under these experimental conditions, the retention time was 7 min for 6-OHDA and 13 min for DA. The routine limit of detection was 0.05 pg for DA.

ISH

ISH was performed according to Andrés and collaborators (1996). In brief, an oligonucleotide, "Nurr-44" (5′-gac ctc acc aac act gaa att act gcc acc act tct ctc cc-3′), complementary to nucleotides 133–173 of rat Nurr1 cDNA, that also recognizes Nurr1a but not Nurr2 cDNAs, was 3′-end labeled with digoxigenin (DIG)-dUTP using terminal transferase (Roche, Indianapolis, IN). Free-floating brain sections were prehybridized

during 1 hr at 62°C in 5× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate) and Denhardt's solution [Denhart's 100×: 2% ficoll; 2% polyvinyl pyrrolidone, and 2% bovine serum albumin (BSA)]. The hybridization was performed overnight at 38°C with 10 pmol/ml of the labeled probe in a solution containing 50% formamide, 1 mM dithiothreitol (DTT), 12 mM Tris HCl, 0.12 M NaCl, 0.8 mM EDTA, and 0.02 g/ml Sarcosyl, pH 7.5. After hybridization, tissue slices were rinsed with SSC (2×, 1×, 0.5×, and 0.1× for 10 min each) at 50°C. The presence of DIG oligonucleotide was detected with an anti-DIG antibody conjugated with alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN). Nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate (Gibco-BRL, Grand Island, NY) were used as enzyme substrates. Finally, sections were mounted on glass slides with gelatin (0.1%), dried, and coverslipped.

IHC

Endogenous peroxydase activity was exhausted by incubating the free-floating tissue sections in 0.5% H_2O_2 for 10 min. Tissue sections were then incubated in blocking solution (1.5% normal goat serum; 0.1% BSA, and 0.3% Triton X-100 in PBS) for 30 min, followed by incubation for 72 hr at 4°C with either of two rabbit polyclonal anti-Nurr1 antibodies (N-20 1:500 and M-196 1:100; Santa Cruz Biotechnology, Santa Cruz, CA). Tissue sections were washed in PBS with 0.1% Triton X-100 (3 × 10 min), incubated with biotinylated goat anti-rabbit IgG (Vectastain ABC method) in 1.5% normal goat serum in PBS for 2 hr, and washed in PBS with 0.1% Triton X-100 (3 × 10 min). The immunoreactivity was visualized with a standard ABC method (Vector Elite kit, 2 μl of avidin, 2 μl of biotin in 1 ml of PBS for 2 hr). Peroxidase was reacted with 0.05% diaminobenzidine (DAB; Sigma, St. Louis, MO) in 0.01% H_2O_2 for 5–10 min. Finally, the sections were mounted on gelatin-coated slides, air dried, and coverslipped. Photomicrographs were taken with a camera connected to a light microscope (Nikon Labophot-2).

Data Analysis

Brain sections from ISH and IHC were examined in a light microscope (Nikon Labophot-2) equipped with a video camera (Sony CCD-Iris) and coupled to a Macintosh computer. Quantification analyses were performed using a final magnification of 200×. Positive cells for immunostaining and hybridization signals were counted using NIH Image/ppc 1.61. From each animal, three independent areas of 0.9 mm^2 for the CS (Bregma: 0.48) and 0.15 mm^2 for SN compacta (SNc) and SN reticulata (SNr; Bregma: −6.04) of each of three coronal slides were counted. Data are presented as mean ± SEM of at least three independent experiments. Groups were compared using an unpaired Student's *t*-test. The null hypothesis was rejected at $P < .05$. Photomicrographs were taken with a camera (Nikon FDX-35) coupled to the Labophot-2 microscope.

Western Blot

The specificity of anti-Nurr1 antibody (N20; Santa Cruz Biotechnology) was evaluated on Western blot analysis of nuclear extracts from brain nuclei and HEK-293 cells transfected with a recombinant hemagglutinin (HA) epitope-tagged Nurr1 expression vector. The nuclear extracts were obtained essentially as described elsewhere (Dignam et al., 1983). Briefly, dissected brain nuclei or cultured cells were homogenized by syringe passing in buffer A [20 mM Hepes, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. After 2 min of centrifugation at 14,000*g*, the supernatant was saved as the cytoplasmic fraction. The pellet was then resuspended in buffer C (20 mM Hepes, pH 7.9, 1.5 mM $MgCl_2$, 0.2 mM EGTA, 20% glycerol, 0.42 M KCl, 1 mM DTT, and 1 mM PMSF) and kept on ice for 30 min with agitation. Finally, the suspention was centrifuged at 14,000*g* for 20 min, and the supernatant was saved as the nuclear fraction. The anti-Nurr1 antibody specificity was further evaluated on Western blot and IHC analysis using anti-Nurr1 antibody in the presence of equimolar amounts of the N-20 blocking peptide (Santa Cruz Biotechnology). HA-Nurr1 expression vector was generated by cloning a polymerase chain reaction (PCR) product containing the full-length Nurr1 cDNA (generously donated by Dr. G. Xing, National Institute of Mental Health, NIH) into pCGN vector (kindly donated by Dr. Jim Trimmer, SUNY Stony Brook). The construct was fully sequenced to check frame and fidelity of the sequence.

Reverse Transription-PCR

Total RNA from CS and SN was isolated with Trizol reagent (Gibco-BRL). One microgram of total RNA was reverse transcribed using oligo-$dT_{12–18}$ by MMLV reverse transcriptase, following the manufacturer's protocol (Gibco-BRL). PCR was performed using Nurr1 primers (390F: 5′-CCTGACTATCAGATGAGTGG-3′; 548R: 5′-CAGTTTGGACAGGTAGTTGG-3′) as follows: 35 cycles for 30 sec at 94°C, 30 sec at 50°C, 30 sec at 72°C, and a final extension at 72°C for 10 min. Primers were designed to amplify a region containing an alternative splice site, allowing us to distinguish Nurr1 of spliced variants Nurr1a/Nurr2 cDNAs by size.

RESULTS

Localization of Nurr1 Expression in the Brain

In situ localization of Nurr1 mRNA. Results from ISH studies using a DIG-oligonucleotide probe complementary to Nurr1 and to the spliced variant Nurr1a mRNAs showed that the distribution pattern of Nurr1 mRNA in adult rat brain is wider than previously reported. In fact, hybridization was detected in scattered cells with intense signal in the CS (Fig. 1D,F). Also, many cells with moderate signal were seen in the septum (Fig. 2C). Moderate intensity of the hybridization signal was seen in a high number of cells in SNr (Fig. 3D,F). Few cells with weak hybridization signal were detected in superior colliculus (Fig. 2D). Positive cells were also observed in areas already described (see Table I) as cerebral cortex and in scattered cells through CA1–CA3 and dentate gyrus of hippocampus, and many cells with moderate intensity signal were observed in SNc (Fig. 3D,E) and few cells in VTA (Table I). The specificity of the hybridization signal observed for the DIG-labeled Nurr1 probe was verified by tissue section hybridization with the corresponding DIG-oligonucleotide sense Nurr1 probe. As

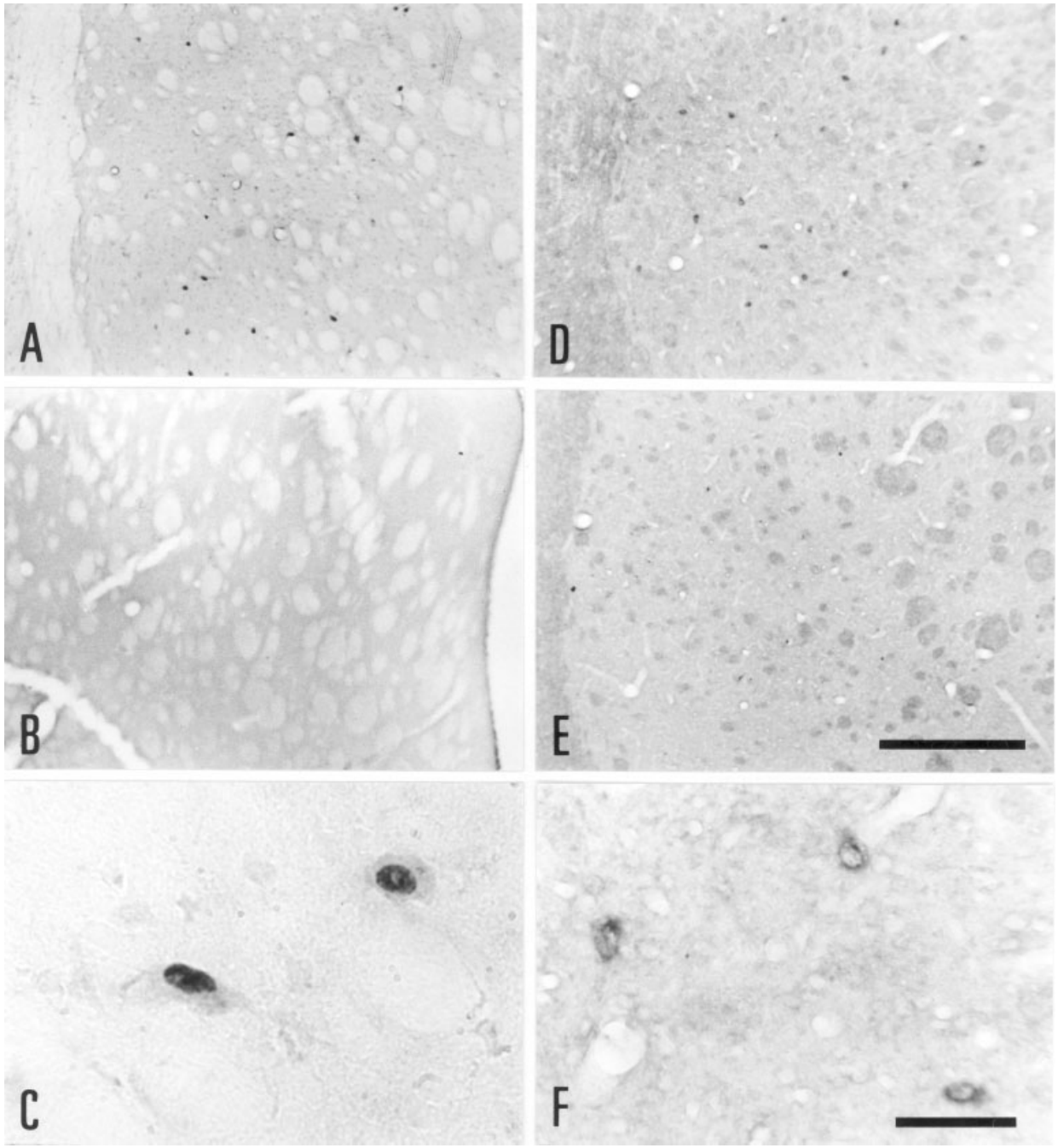

Fig. 1. Nurr1 expression determined by specific in situ hybridization and immunohistochemical detection. **A**: Expression of Nurr1 protein in CS. Strong nuclear immunoreactivity is observed in scattered cells in CS, which are shown in higher magnification in **C**. **B**: The immunoreactivity was completely blocked by coincubation of anti-Nurr1 antibody with an equimolar amount of the immunizing peptide. **D**: In situ hybridization signal shows a similar expression of Nurr1 mRNA in the CS; positive scattered cells are observed throughout CS, which are shown in higher magnification in **F**. Tissue sections hybridized with sense Nurr1 probe gave no hybridization signal above background (**E**). Scale bars = 200 μm in E (for A,D,E); 100 μm for B; 20 μm in F (for C,F).

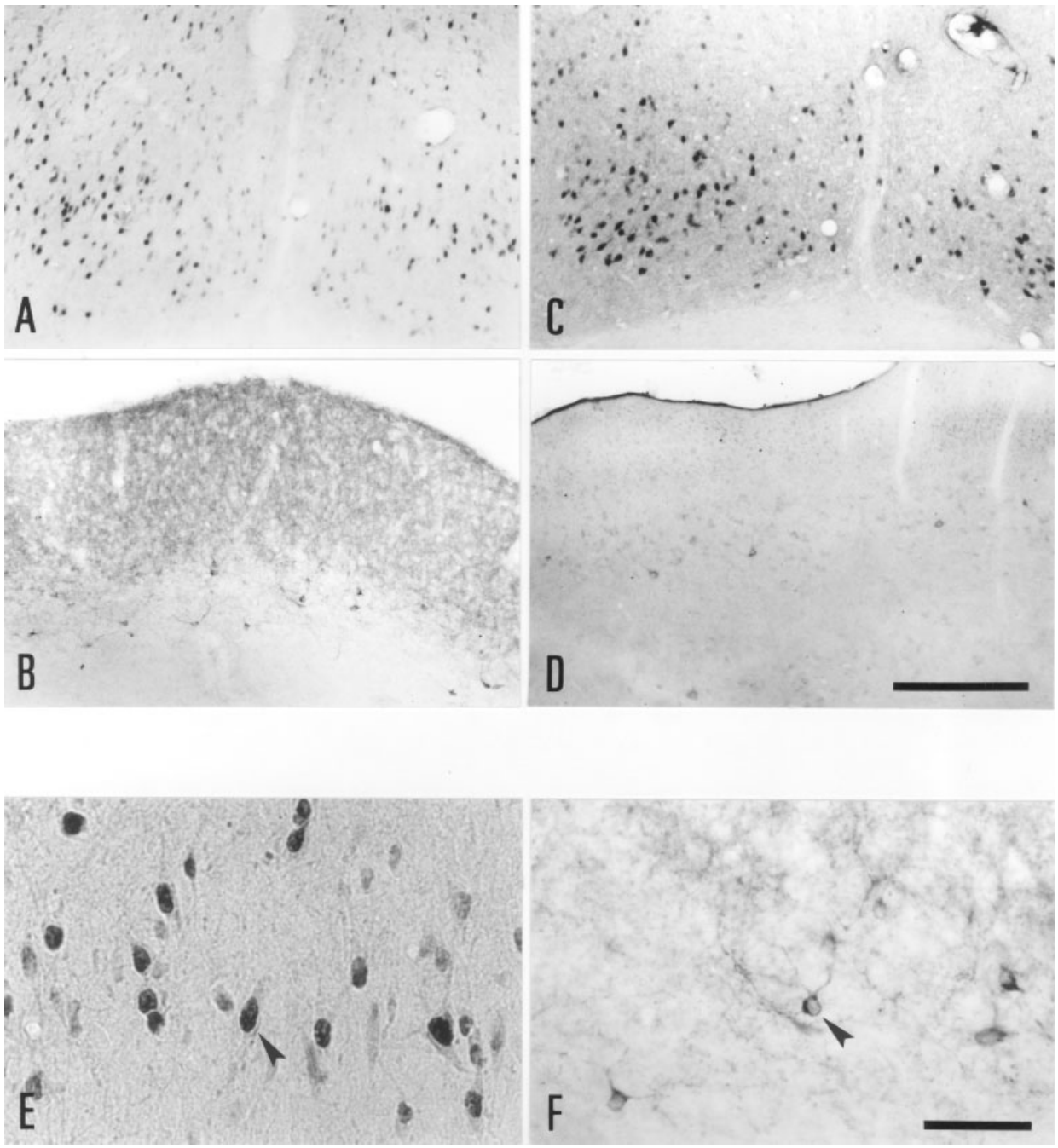

Fig. 2. Different subcellular distributions of Nurr1 in distinct nuclei of CNS. Nurr1 is expressed in septum, shown by a strong Nurr1-like immunostaining (**A**) and a similar distribution of cells positive for Nurr1 mRNA expression detected by ISH signal (**C**). A different pattern of staining was observed in superior colliculus. Scattered positive cells showing Nurr1-like immunopositive signal in the cytoplasm and processes (**B**). A similar distribution of scattered cells with expression for Nurr1 mRNA is shown in **D**. Higher magnification of septum cells with strong Nurr1-like immunostaining in the nuclei (arrowhead; **E**). Higher magnification of cytoplasmic and processes Nurr1-like immunostaining in superior colliculus cells (arrowhead; **F**). Scale bars = 100 μm in D (for A–D); 20 μm in F (for E,F).

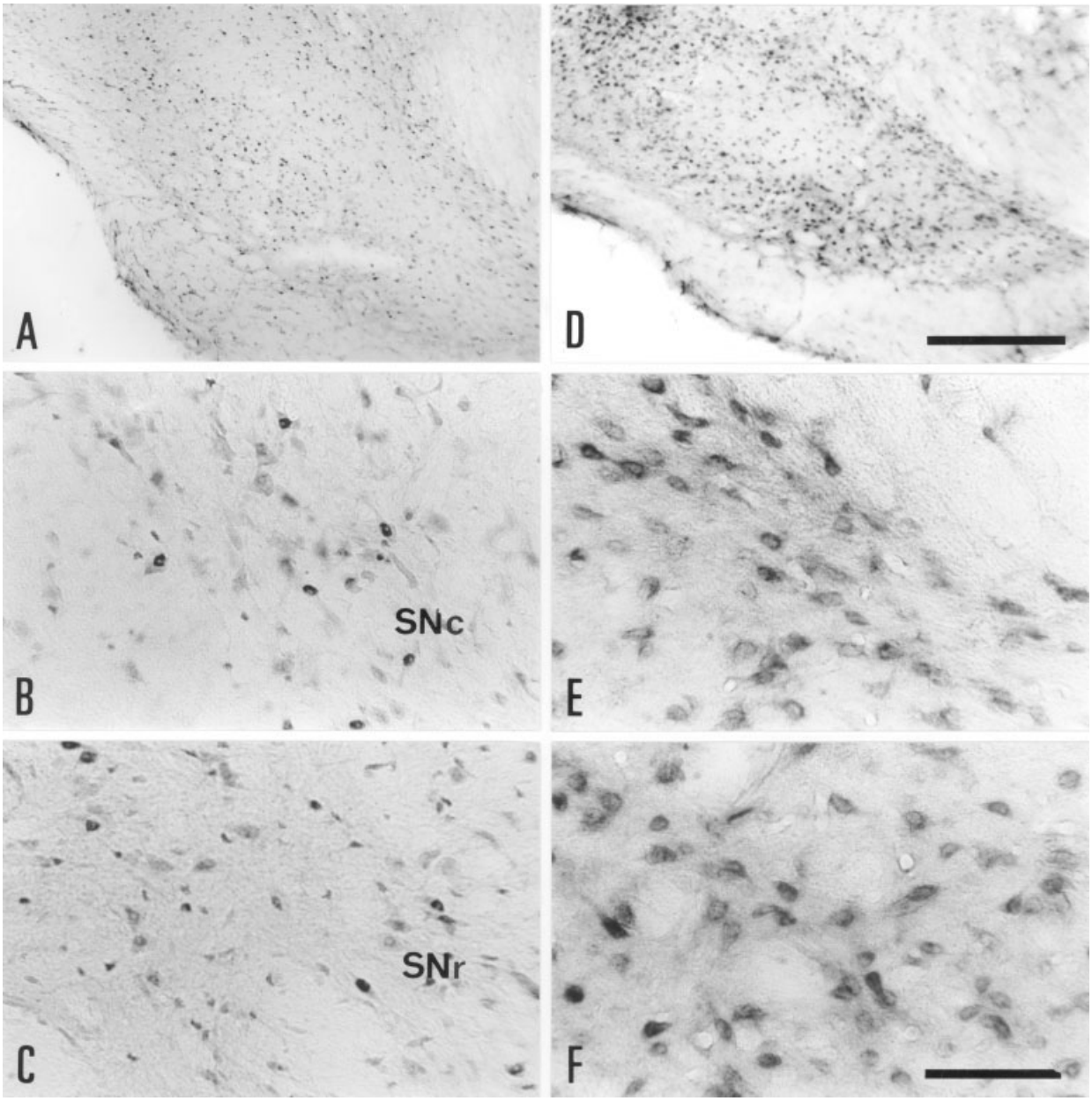

Fig. 3. Nurr1 is expressed in both SNc and SNr. A similar pattern of Nurr1-expressing cells was observed as assessed by IHC (**A–C**) and by ISH (**D–F**) in SNc and SNr. Strong nuclear staining was observed in several cells throughout SNc (A,B) and SNr (A,C). Cells with positive in situ hybridization signal show a comparable distribution in SNc (D,E) and SNr (D,F). Scale bars = 200 μm in D (for A,D); 50 μm in F (for B,C,E,F).

shown in Figure 1E, no signal was detected with the Nurr1 sense probe. The same result was obtained by using the DIG-oligonucleotide probe together with a 100-fold excess of unlabelled probe (data not shown).

Immunohistochemical localization of Nurr1 protein. In the present study, we used two commercial polyclonal anti-Nurr1 antibodies (N-20 and M-196; Santa Cruz Biotechnology) to evaluate the distribution of Nurr1-like immunoreactivity in the rat brain. Both antibodies gave similar results, although at different concentrations (see Materials and Methods). Because most of the experiments were carried out with N-20 antibody, the full-proof specificity tests were performed for N-20 antibody. The specificity of N-20 anti-Nurr1 antibody was tested by two approaches. First, anti-Nurr1 antibody recognized the recombinant HA-Nurr1 from transfected

TABLE I. Regional Expression of Nurr1 mRNA and Protein in the Rat Brain*

Brain region	ISH	IHC
Forebrain		
Cortex		
Frontal	+++	+++
Piriform	+++	+++
Cortical layer IV	+++	+++
Island of Calleja	+++	+++
CS	++	++
Globus Pallidus	−	−
Septum		
Lateral, dorsal part	+	+
Lateral, intermediate part	+	+
Medial	++	+++
Hippocampus		
CA1–CA3	+	++
Dentate gyrus	++	+++
Nucleus accumbens	−	−
BNST	+	+
Hypothalamus		
IP	+++	+++
Arc	+++	+++
LM	+++	++
SuM	+++	++
Thalamus		
Red nucleus	++	++
Oculomotor nucleus	+	+
Midbrain		
VTA	+	+
SNc	+++	+++
SNr	+++	+++
SuG of SC	+	++

*Distribution of Nurr1 mRNA (ISH) and protein (IHC) in the adult rat CNS. Relative abundance was assessed by estimating both staining and cell density. Signals are indicated as negative (−), weak (+), moderate (++), or strong (+++). Arc, arcuate; BNST, bed nucleus of stria terminalis; CA, Ammon's horn; CS, striatum; IP, interpeduncular; LM, lateral mammillary; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; SC, superior colliculus; SuG, superficial gray layer; SuM, supramammillary; VTA, ventral tegmental area.

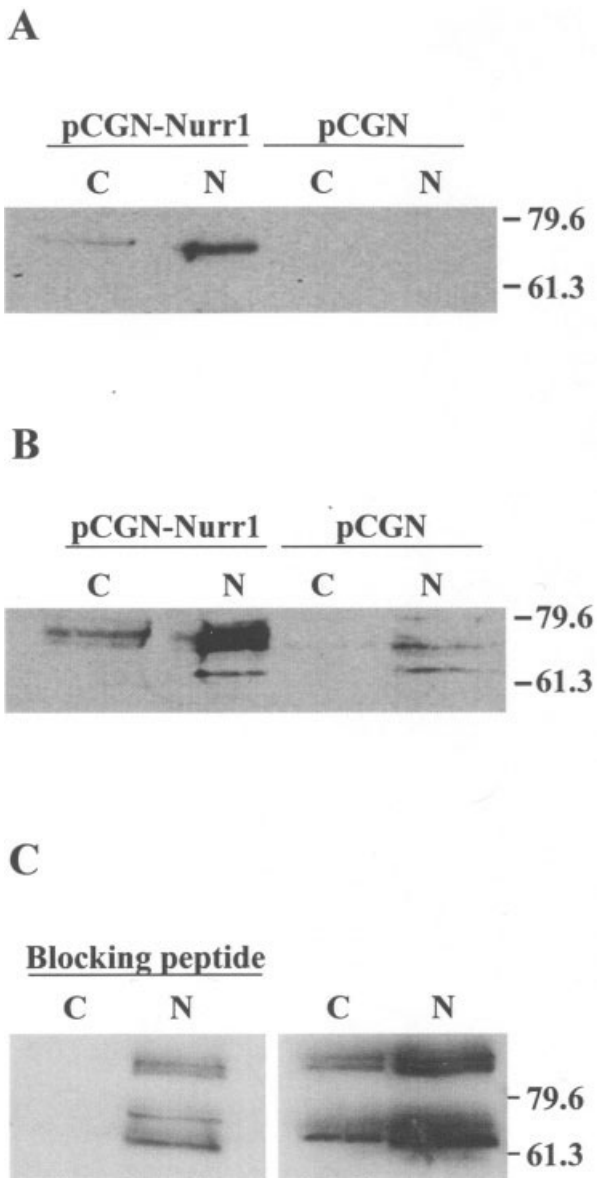

Fig. 4. Analysis of specificity of the anti-Nurr1 antibody by Western blotting. Nuclear (N) and cytoplasmic (C) extracts prepared from HEK293 transfected with the parent vector (pCGN) or transfected with recombinant HA-tagged Nurr1 (pCGN-Nurr1) were resolved on SDS-PAGE and blotted with a monoclonal anti-HA antibody (**A**) or with the polyclonal N-20 anti-Nurr1 antibody (**B**). **C**: Effect of coincubation of the anti-Nurr1 antibody with immunizing peptide on the detection of Nurr1. Nuclear (N) and cytoplasmic (C) extracts prepared from CS tissue were resolved on SDS-PAGE. Right: Western blot developed with anti-Nurr1 antibody (N-20). Left: Western blot developed with anti-Nurr1 antibody (N-20) in the presence of an equimolar amount of immunizing peptide. Positions of molecular weight markers are shown in kilodaltons.

HEK293 nuclear extracts as well as a faint signal of endogenous protein (compare Fig. 4A and B). Second, on Western blot analysis of nuclear and cytoplasm extracts from CS, Nurr1 antibody recognized two bands. One of the bands corresponded to the expected molecular mass of Nurr1 of 66 kDa; we suggest that the other band of higher molecular mass could correspond to a posttranslational modified form of Nurr1. It is noteworthy that Nur77, which shares high homology with Nurr1, is detected as a wide signal in Western blots because of heavy phosphorylation (Woronicz et al., 1995; Cheng et al., 1997). Both signals were efficiently blocked by the presence of equimolar amounts of the respective immunizing peptide (Fig. 4C). Moreover, Nurr1-like immunoreactive signal in brain was totally blocked when tissue slices were incubated with anti-Nurr1 antibody together with equimolar amounts of the immunizing peptide (Fig. 1B). The results of these studies revealed that the Nurr1 antibody used specifically recognizes Nurr1 protein. Because this antibody was developed using a peptide corresponding to the N-terminal of Nurr1 protein, it should recognize both Nurr1 and the reported spliced variant Nurr1a (Castillo et

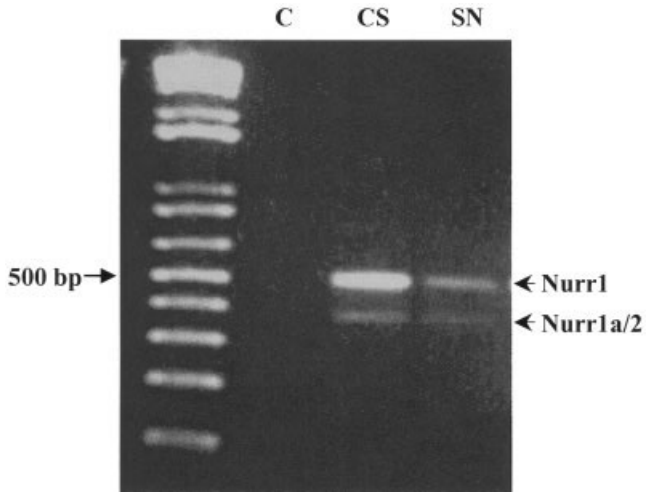

Fig. 5. Nurr1 and the spliced variants of Nurr1 are expressed in CS and SN. RT-PCR was performed on total RNA extracted from CS and SN. One microgram of total RNA was reverse transcribed using oligo-dT. Primer pair specific for Nurr1 was designed to amplify a region containing an alternative splice site, allowing us to discriminate between Nurr1 and the combination of the spliced variants Nurr1a/Nurr2 cDNAs by size. The expected bands of 494 bp for Nurr1 fragment and 374 bp for Nurr1a/Nurr2 fragment were detected in both CS and SN. C, control reaction was performed without reverse transcriptase.

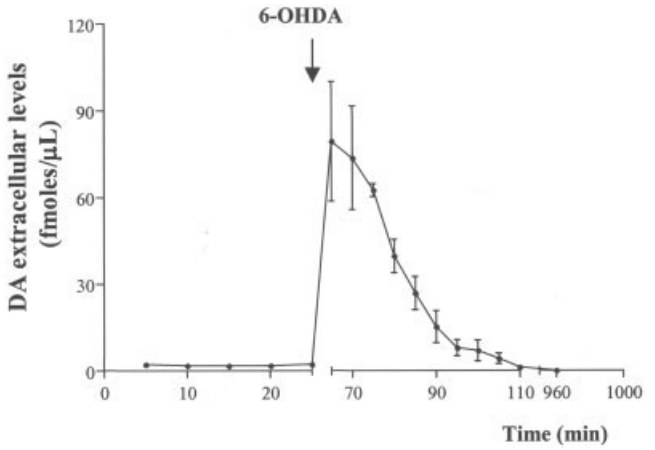

Fig. 6. Selective lesion of dopaminergic terminals with 6-OHDA in CS induces a massive increase, followed by a continuing decrease, of DA extracellular levels. The concentration of DA was measured by HPLC-EC from 5-min microdialysate samples. The arrow indicates time of the 6-OHDA injection in CS. The data correspond to the mean ± SEM of three independent experiments. At 110 min, DA extracellular levels were lower than DA extracellular levels before 6-OHDA injection (before 6-OHDA = 2.27 ± 0.5; 110 min = 1.15 ± 0.48). After 16 hr, DA extracellular levels were undetectable.

al., 1998b). We did not detect bands of lower molecular weight on Western blot analysis, suggesting that this isoform of Nurr1 is less expressed and undetectable by this technique. However, by RT-PCR, we detected a product corresponding to the expected size for the spliced variants of Nurr1, Nurr1a, and/or Nurr2 (Castillo et al., 1998b; Ohkura et al., 1999). To analyze whether Nurr1 spliced variants are expressed in rat brain, we designed primers across the alternative splicing site to distinguish by molecular weight the products in a PCR. As shown in Figure 5, two products were detected by RT-PCR: Nurr1, represented by a more abundant band of 494 bp, and a second, slighter product of 374 bp corresponding to the presence of Nurr1a and/or Nurr2 mRNAs in both SN and CS. This result indicates that the spliced variants of Nurr1 are less expressed in rat brain and suggests that the Nurr1-like immunostaining corresponds mostly to Nurr1 protein.

IHC studies of Nurr1 expression in rat brain revealed a pattern of staining similar to that seen on ISH. Nurr1-like immunostaining was localized into the nuclei of scattered but intensely labeled cells in the CS (Fig. 1A,C), in medial and lateral septum (Fig. 2A,E), and in several cells throughout SNc and SNr (Fig. 3A–C). A high level of staining was also seen in cortex, CA1–CA3 and dentate gyrus of hippocampus, and other areas (see Table I). Unexpectedly, in superior colliculus, staining was present in the cytoplasm and processes (Fig. 3B,F), but absent from the nuclei of the cells (compare Fig. 3E, septum, with F, superior colliculus). A similar pattern of staining was also seen in periductal central gray (data not shown). These results show that Nurr1 is widely expressed in the CNS of the adult rat, mostly in the nuclei of cells. The different subcellular distribution seen in other regions may have functional implications.

Effects of 6-OHDA Injections in the CS on DA Extracellular Levels and Nurr1 Expression

To explore the possibility that Nurr1 is important in the maintenance of the ventral dopaminergic phenotype in the adult, we have analyzed the expression of Nurr1 mRNA and protein in SN 24 hr after the injection of 6-OHDA in the CS. This model allowed us to study the early events after the decay of DA in the CS, before cell degeneration in SN. The DA extracellular levels in the CS, measured by in vivo brain microdialysis, showed a massive increase after injection of 6-OHDA, followed by a continual decrease. DA extracellular levels were undetectable at about 16 hr after the injection of 6-OHDA (Fig. 6). Twenty-four hours after the injection of 6-OHDA in the CS, a significant increase in the number of cells expressing Nurr1 mRNA was observed in both SNc and SNr contralateral and ipsilateral to the lesion, compared with sham rats (Fig. 7A,B). This increase in the number of cells expressing Nurr1 mRNA was accompanied by a significant increase in the number of cells positive for Nurr1-like immunostaining in the ipsilateral and contralateral SNr, as well as in contralateral SNc, compared with sham condition (Fig. 7C,D). Although not statistically significant, a substantial increase in the number of Nurr1-like immunoreactive cells was observed in the ipsilateral side of SNc of 6-OHDA-treated rats (Fig. 7C). No significant changes in the number of Nurr1 mRNA-

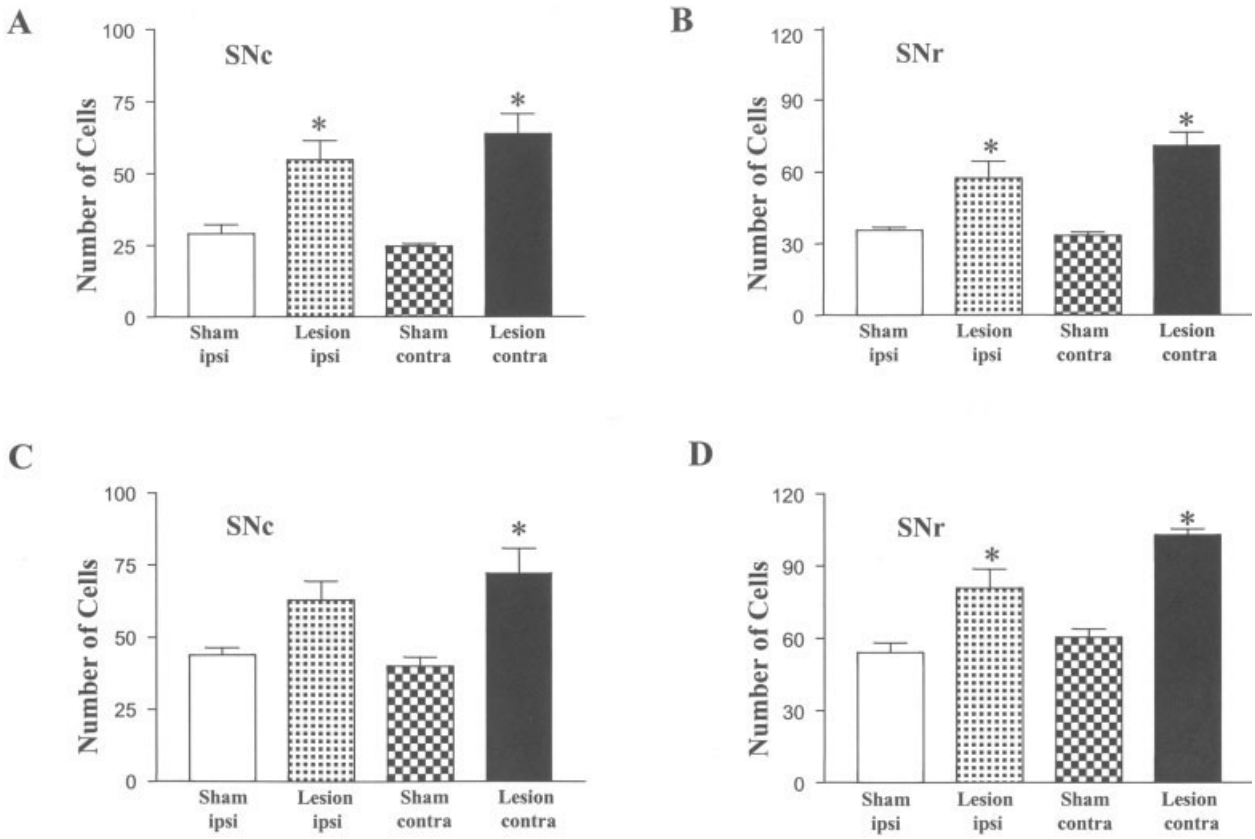

Fig. 7. Rapid bilateral increase of Nurr1-expressing cells in SNc and SNr after 6-OHDA injection in CS. Positive cells for Nurr1-like immunostaining and ISH signals were counted in sample areas of ipsilateral and contralateral SNc and SNr of sham- and 6-OHDA-treated rats. Number of positive cells from ISH experiments (**A,B**) and from IHC experiments (**C,D**). Data correspond to the mean ± SEM of at least three independent experiments. $\star P < .05$ compared with the sham condition.

and protein-expressing cells were seen in the CS after 6-OHDA lesion (data not shown).

DISCUSSION

Nurr1 Expression in Rat Brain

We observed high Nurr1 protein expression in areas previously described as positive for Nurr1 mRNA, including cortex, CA1–CA3 and the dentate gyrus of the hippocampus, and SNc (Xiao et al., 1996; Zetterström et al., 1996b;Bäckman et al., 1999; Baffi et al., 1999). Moreover, we observed Nurr1 mRNA and protein expression in CS and SNr, which have also been reported as positive for Nurr1 mRNA (Xiao et al., 1996). It is worthwhile noting that Xiao and collaborators also used a DIG-labeled probe to detect Nurr1 mRNA, suggesting that this type of signal gives a better signal/noise ratio, allowing the detection of ISH-positive cells with lower mRNA expression levels. Interestingly, we observed a high Nurr1 expression in lateral and medial areas of the septum, a limbic nucleus, and in the superficial gray layer of the superior colliculus, an important area in visual-motor integration. The wide distribution of Nurr1 in the CNS suggests an ample role for this transcription factor beyond the ventral dopaminergic system. In fact, it has been reported that Nurr1 is strongly overexpressed following seizure activity (Xing et al., 1997; Crispino et al., 1998; Honkaniemi and Sharp, 1999) and during spatial discrimination learning (Peña de Ortiz et al., 2000) in the hippocampus, suggesting that Nurr1 may play a role in plastic changes in the CNS. In general, most Nurr1-immunopositive cells were strongly stained in the nuclei, whereas staining in the cytoplasm was weak or absent. Cytoplasmic expression of Nurr1 was observed in the superior colliculus and in the periaqueductal gray nuclei. This cytoplasmic localization suggests that specific signals could regulate the traffic of Nurr1 to the nuclei of the cells in these regions of the CNS. It is tempting to suggest that Nurr1 could behave like a type I class of nuclear receptor in some areas and to be strictly nuclear in other areas, responding to different signals in distinct tissues.

DA Terminal Damage Increases Nurr1 Expression

In this work, we evaluated the early events induced by a rapid and massive decrease of DA in the CS. We found that, 24 hr after a unilateral 6-OHDA injection in the CS, a significant increase in the number of cells expressing Nurr1 occurs bilaterally in the SNc and SNr of the rat. The intrastriatal 6-OHDA injection induced an initial massive efflux of DA, followed by a continual decrease until DA extracellular levels were undetectable. The number of cells in the SNc remained normal 3 days after the injection of 6-OHDA in the CS, as assessed by Nissl staining (Iwata et al., 2001). Also, the number of neurons expressing TH in the SN showed no alteration 24 hr (Martí et al., 2002) or 3 days (Iwata et al., 2001) after the injection of 6-OHDA in the CS. Indeed, it has been reported that DA neuronal degeneration in the ipsilateral SN begins only 1 week after 6-OHDA intrastriatal injection (Sauer and Oertel, 1994; Lee et al., 1996). Therefore, we can exclude neuronal loss in the SN in the moment when positive cells for Nurr1-like immunoreactive and Nurr1 mRNA were counted. Our results support the hypothesis that DA levels in the CS could regulate the expression of Nurr1 in the ventral dopaminergic system, proposed initially by Tseng and coworkers (2000). In fact, these authors showed that Nurr1 mRNA is overexpressed in mesencephalic dopaminergic neurons in mice null for D2 class DA receptor. The decrease in DA extracellular levels in the CS induced by 6-OHDA resembles the incapacity of the dopaminergic terminals to detect extracellular levels of DA because of the absence of D2 class DA autoreceptors. In addition, a decrease of Nurr1 immunoreactivity has been shown in ventral neurons of cocaine abusers (Bannon et al., 2002). It is well documented that chronic treatment with cocaine or other abused drugs induces an increase of DA extracellular levels in the nucleus accumbens and in the CS (Di Chiara and Imperato, 1988). Taken together, these results argue in favor of the idea that DA extracellular levels in the CS may regulate Nurr1 expression in ventral dopaminergic neurons.

One interesting observation is the bilateral increase of Nurr1 expression in the SN after unilateral striatal DA decrease. Although unilateral lesions with 6-OHDA in the CS do not change the DA storage in the contralateral side (Matsuura et al, 1997), it has been reported that the stimulation of one nigrostriatal dopaminergic pathway influences the activity in the contralateral pathway (Nieoullon et al., 1977). Other authors have also reported bilateral changes in response to unilateral DA depletion. For instance, Delfs and coworkers (1996) have reported a bilateral increase of glutamate decarboxylase mRNA in the reticular thalamic nucleus. Porter and coworkers (1994) observed a bilateral decrease of MK-801 binding in CS, whereas our group showed a bilateral increase in N-methyl-D-aspartate (NMDA) NR1 mRNA in the CS (Andrés et al., 1996) after unilateral middle forebrain bundle lesion. Rodríguez-Puerta and coworkers (1999) have shown a bilateral increase in the levels of metabotropic glutamate receptor subtype 4 mRNA in both neocortex and neostriatum. Steiner and Kitai (2001) reported a bilateral reduction in cortical zif 268 expression 2 days after unilateral infusion of 6-OHDA into the midbrain. Electrophysiological, biochemical, and behavioral evidence supports the idea that these bilateral effects might be mediated through interhemispheric connections (Nieoullon et al., 1977; Leviel et al., 1979; Rodríguez et al., 1990; Roedter et al., 2001). The connection between one cerebral cortex and the contralateral CS and SN could thus be the final pathway by which changes in the activity of one dopaminergic system induce variations in the contralateral hemisphere.

The mechanism by which endogenous DA would regulate Nurr1 expression is not known. One possibility is an up-regulation of Nurr1 expression mediated by a CRE element located at the NR4A2 promoter (Castillo et al., 1997; Saucedo-Cardenas et al., 1997; Torii et al., 1999; Ichinose et al., 1999). D2 autoreceptors are coupled to Gi/Go and decrease cAMP. An increase in cAMP levels in the surviving dopaminergic terminals induced by a decreased DA level can activate protein kinase A, which phosphorylates the transcription factor cAMP response element-binding protein (CREB). Another possibility is that the increase of Nurr1 expression results from an increased dopaminergic cell firing produced by the decrease of DA extracellular levels. In fact, chronic K^+ and electrical stimulation of dissociated primary sensory neurons (Brosenitsch and Katz, 2001) and PC-12 cells (Law et al., 1992) induces a rapid increase of Nurr1 expression.

How might the increase in Nurr1 expression affect the dopaminergic system? It has already been demonstrated that Nurr1 is an essential factor in the acquisition of the ventral dopaminergic neuronal phenotype. In adults, Nurr1 may play a critical role in phenotype maintenance and survival of ventral dopaminergic neurons by regulating the expression of tyrosine hydroxylase (TH), DA transporter (DAT), and Ret, one component of the glial-derived neurotrophic factor (GDNF) receptors. The characterization of TH (Shimmel et al., 1999; Iwawaki et al., 2000) and DAT (Sacchetti et al., 1999) promoters showed the presence of NBRE elements, and, in fact, a body of evidence suggests that Nurr1 regulates the transcription of these dopaminergic genes (Sakurada et al., 1999; Iwawaki et al., 2000; Sacchetti et al., 2001). On the other hand, mice null for the Nurr1 gene showed deficient Ret mRNA expression in midbrain dopaminergic neurons (Wallen et al., 2001). It has been proposed that compensation mechanisms operate to maintain DA extracellular levels in the CS of animals with dopaminergic system damage (for review see Zigmond and Hastings, 1998). Increased discharge of dopaminergic neurons and increased DA synthesis and release are among the mechanisms proposed to play a role in compensation. Several authors have reported an increase in TH (Wolf et al., 1989; Blanchard et al., 1995) and in Ret expression (Noriega et al., 2000) in the SN of animals with nigrostriatal system damage. Marco and collaborators (2002) showed that mRNA levels of Ret and GFRα1 increase

transiently in SNc shortly after intrastiatal 6-OHDA injection, suggesting that the GFRα1/Ret complex is involved in the regulation of dopaminergic neuron survival. Our results support the hypothesis that a reciprocal regulation between Nurr1 and endogenous DA levels may exist in the ventral dopaminergic system. A decrease in DA levels induces an increase in Nurr1 expression (this study), which could result in a compensatory increase of TH transcription and a protective increase in Ret expression to compensate for the diminution of DA extracellular levels in CS. Le and coworkers (1999) have shown, in support of this hypothesis, an increased vulnerability to MPTP in Nurr1+/– mice. The increased Nurr1 expression in SN may be related to the increased TH and Ret expression observed after nigrostriatal denervation. Whether the increase in Nurr1 expression lasts longer in dopaminergic surviving neurons awaits clarification.

ACKNOWLEDGMENTS

We thank all members of the Biochemical-Pharmacology group for stimulating discussions and in particular Dr. Katia Gysling for critical reading of the manuscript. We thank Dr. G. Xing for the kind gift of the rat Nurr1 cDNA. J.A.F. is funded by a doctoral fellowship from FONDECYT.

REFERENCES

Andrés ME, Gysling K, Araneda S, Bustos G. 1996. NMDA-NR1 receptor subunit mRNA expression in rat brain after 6-OH-dopamine induced lesions: a non-isotopic in situ hybridization study. J Neurosci Res 46: 375–384.

Andrés ME, Gysling K, Bustos G. 1998. Differential regulation of dopamine release by N-methyl-D-aspartate receptors in rat striatum after partial and extreme lesions of the nigro-striatal pathway. Brain Res 797:255–266.

Bäckman C, Perlmann T, Wallen A, Hoffer BJ, Morales M. 1999. A selective group of dopaminergic neurons express Nurr1 in the adult mouse brain. Brain Res 851:125–132.

Baffi JS, Palkovits M, Castillo SO, Mezey E, Nikodem VM. 1999. Differential expression of tyrosine hydroxylase in catecholaminergic neurons of neonatal wild-type and Nurr1-deficient mice. Neuroscience 93:631–642.

Bannon MJ, Pruetz B, Manning-Bog AB, Whitty CJ, Michelhaugh SK, Sacchetti P, Granneman JG, Mash DC, Schmidt CJ. 2002. Decreased expression of the transcription factor NURR1 in dopamine neurons of cocaine abusers. Proc Natl Acad Sci USA 99:6382–6385.

Blanchard V, Chritin M, Vyas S, Savasta M, Feuerstein C, Agid Y, Javoy-Agid F, Raisman-Vozari R. 1995. Long-term induction of tyrosine hydroxylase expression: compensatory response to partial degeneration of the dopaminergic nigrostriatal system in the rat brain. J Neurochem 64:1669–1679.

Brosenitsch TA, Katz DM. 2001. Physiological patterns of electrical stimulation can induce neuronal gene expression by activating n-type calcium channels. J Neurosci 21:2571–2579.

Castillo SO, Xiao Q, Lyu MS, Kozak CA, Nikodem VM. 1997. Organization, sequence, chromosomal localization, and promoter identification of the mouse orphan nuclear receptor Nurr1 gene. Genomics 41:250–257.

Castillo SO, Baffi JS, Palkovits M, Goldstein DS, Kopin IJ, Witta J, Magnuson MA, Nikodem VM. 1998a. Dopamine biosynthesis is selectively abolished in substantia nigra/ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of the Nurr1 gene. Mol Cell Neurosci 11:36–46.

Castillo SO, Xiao Q, Kostrouch Z, Dozin B, Nikodem VM. 1998b. A divergent role of COOH-terminal domains in Nurr1 and Nur77 transactivation. Gene Expr 7:1–12.

Cheng LEC, Chang FKM, Cado D, Winoto A. 1997. Functional redundancy of the Nur77 and Nor-1 orphan steroid receptors in T-cell apoptosis. EMBO J 16:1865–1875.

Crispino M, Tocco G, Feldman JD, Herschman HR, Baudry M. 1998. Nurr1 mRNA expression in neonatal and adult rat brain following kainic acid-induced seizure activity. Brain Res Mol Brain Res 59:178–188.

Delfs JM, Ciaramitaro VM, Soghomonian JJ, Chesselet MF. 1996. Unilateral nigrostriatal lesions induce a bilateral increase in glutamate decarboxylase messenger RNA in the reticular thalamic nucleus. Neuroscience 71:383–395.

Di Chiara G, Imperato A. 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 85:5274–5278.

Dignam JD, Martin PL, Shastry BS, Roeder RG. 1983. Eukaryotic gene transcription with purified components. Methods Enzymol 101:582–598.

Honkaniemi J, Sharp FR. 1996. Global ischemia induces immediate-early genes encoding zinc finger transcription factors. J Cereb Blood Flow Metab 16:557–565.

Honkaniemi J, Sharp FR. 1999. Prolonged expression of zinc finger immediate-early gene mRNAs and decreased protein synthesis following kainic acid induced seizures. Eur J Neurosci 11:10–17.

Honkaniemi J, Sagar SM, Pyykonen I, Hicks KJ, Sharp FR. 1995. Focal brain injury induces multiple immediate early genes encoding zinc finger transcription factors. Brain Res Mol Brain Res 28:157–163.

Honkaniemi J, Zhang JS, Longo FM, Sharp FR. 2000. Stress induces zinc finger immediate early genes in the rat adrenal gland. Brain Res 877:203–208.

Ichinose H, Ohye T, Suzuki T, Sumi-Ichinose C, Nomura T, Hagino Y, Nagatsu T. 1999. Molecular cloning of the human Nurr1 gene: characterization of the human gene and cDNAs. Gene 230:233–239.

Iwata SI, Nomoto M, Fukuda T. 2001 Regulation of GAP-43 protein and mRNA in nigrostriatal dopaminergic neurons after the partial destruction of dopaminergic terminals with intrastriatal 6-hydroxydopamine. Synapse 39:16–22.

Iwawaki T, Kohno K, Kobayashi K. 2000. Identification of a potential nurr1 response element that activates the tyrosine hydroxylase gene promoter in cultured cells. Biochem Biophys Res Commun 274:590–595.

Law SW, Conneely OM, DeMayo FJ, O'Malley BW. 1992. Identification of a new brain-specific transcription factor, NURR1. Mol Endocrinol 6:2129–2135.

Le W, Conneely OM, He Y, Jankovic J, Appel SH. 1999. Reduced Nurr1 expression increases the vulnerability of mesencephalic dopamine neurons to MPTP-induced injury. J Neurochem 73:2218–2221.

Le WD, Xu P, Jankovic J, Jiang H, Appel SH, Smith RG, Vassilatis DK. 2003. Mutations in NR4A2 associated with familial Parkinson disease. Nat Genet 33:85–89.

Lee CS, Sauer H, Bjorklund A. 1996. Dopaminergic neuronal degeneration and motor impairments following axon terminal lesion by intrastriatal 6-hydroxydopamine in the rat. Neuroscience 72:641–653.

Leviel V, Cheramy A, Nieoullon A, Glowinski J. 1979. Symmetric bilateral changes in dopamine release from the caudate nuclei of the cat induced by unilateral nigral application of glycine and GABA-related compounds. Brain Res 175:259–270.

Marco S, Saura J, Pérez-Navarro E, Martí MJ, Tolosa E, Alberch J. 2002. Regulation of c-Ret, GFRα1 and GFRα2 in the substantia nigra pars compacta in a rat model of Parkinson's disease. J Neurobiol 52:343–351.

Martí MJ, Saura J, Burke RE, Jackson-Lewis V, Jiménez A, Bonastre M, Tolosa E. 2002. Striatal 6-hydroxydopamine induces apoptosis of nigral neurons in the adult rat. Brain Res 958:185–191.

Matsuura K, Makino H, Ogawa N. 1997. Cyclosporin A attenuates the decrease in tyrosine hydroxylase immunoreactivity in nigrostriatal dopaminergic neurons and in striatal dopamine content in rats with intrastriatal injection of 6-hydroxydopamine. Exp Neurol 146:526–535.

Nakki R, Sharp FR, Sagar SM, Honkaniemi J. 1996. Effects of phencyclidine on immediate early gene expression in the brain. J Neurosci Res 45:13–27.

Nieoullon A, Cheramy A, Glowinski J. 1977. Interdependence of the nigrostriatal dopaminergic systems on the two sides of the brain in the cat. Science 198:416–418.

Noriega V, Escobar J, Casar JC, Abarca J, Gysling K, Bustos G. 2000. Differential expression of GDNF, GDNFR alfa, and RET mRNA in rat substantia nigra after partial lesion of the nigrostriatal dopamine pathway. Biocell 24(Suppl):57.

Ohkura N, Hosono T, Maruyama K, Tsukada T, Yamaguchi K. 1999. An isoform of Nurr1 functions as a negative inhibitor of the NGFI-B family signaling. Biochem Biophys Acta 1444:69–79.

Paxinos G, Watson C. 1986. The rat brain in stereotaxic coordinates. Sydney: Academic Press.

Peña de Ortiz S, Jamieson GA Jr. 1996. HZF-3, an immediate-early orphan receptor homologous to NURR1/NOT: induction upon membrane depolarization and seizures. Brain Res Mol Brain Res 38:1–13.

Peña de Ortiz S, Maldonado-Vlaar CS, Carrasquillo Y. 2000. Hippocampal expression of the orphan nuclear receptor gene hzf-3/nurr1 during spatial discrimination learning. Neurobiol Learn Mem 74:161–178.

Porter RH, Greene JG, Higgins DS Jr, Greenamyre JT. 1994. Polisynaptic regulation of glutamate receptors and mitochondrial enzyme activities in the basal ganglia of rats with unilateral dopamine depletion. J Neurosci 14:7192–7199.

Rodriguez M, Castellano MA, Palarea MD. 1990. Interhemispheric regulation of dopaminergic ascending systems. Life Sci 47:377–384.

Rodriguez-Puertas R, Herrera-Marschitz M, Koistinaho J, Hokfelt T. 1999. Dopamine D1 receptor modulation of glutamate receptor messenger RNA levels in the neocortex and neostriatum of unilaterally 6-hydroxydopamine-lesioned rats. Neuroscience 89:781–797.

Roedter A, Winkler C, Samii M, Walter GF, Brandis A, Nikkhah G. 2001. Comparison of unilateral and bilateral intrastriatal 6-hydroxydopamine-induced axon terminal lesions: evidence for interhemispheric functional coupling of the two nigrostriatal pathways. J Comp Neurol 432:217–229.

Sacchetti P, Brownschidle LA, Granneman JG, Bannon MJ. 1999. Characterization of the 5′-flanking region of the human dopamine transporter gene. Brain Res Mol Brain Res 74:167–174.

Sacchetti P, Mitchell TR, Granneman JG, Bannon MJ. 2001. Nurr1 enhances transcription of the human dopamine transporter gene through a novel mechanism. J Neurochem 76:1565–1572.

Sakurada K, Ohshima-Sakurada M, Palmer TD, Gage FH. 1999. Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. Development 126:4017–4026.

Saucedo-Cardenas O, Conneely OM. 1996. Comparative distribution of NURR1 and NUR77 nuclear receptors in the mouse central nervous system. J Mol Neurosci 7:51–63.

Saucedo-Cardenas O, Kardon R, Ediger TR, Lydon JP, Conneely OM. 1997. Cloning and structural organization of the gene encoding the murine nuclear receptor transcription factor, NURR1. Gene 187:135–139.

Saucedo-Cardenas O, Quintana-Hau JD, Le WD, Smidt MP, Cox JJ, De Mayo F, Burbach JP, Conneely OM. 1998. Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. Proc Natl Acad Sci USA 95:4013–4018.

Sauer H, Oertel WH. 1994. Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neuroscience 59:401–415.

Schimmel JJ, Crews L, Roffler-Tarlov S, Chikaraishi DM. 1999. 4.5 kb Of the rat tyrosine hydroxylase 5′ flanking sequence directs tissue specific expression during development and contains consensus sites for multiple transcription factors. Brain Res Mol Brain Res 74:1–14.

Steiner H, Kitai ST. 2001. Unilateral striatal dopamine depletion: time-dependent effects on cortical function and behavioral correlates. Eur J Neurosci 14:1390–1404.

Torii T, Kawarai T, Nakamura S, Kawakami H. 1999. Nucleotide organization of the human orphan nuclear receptor Nurr1 gene. Gene 230: 225–232.

Tseng KY, Roubert C, Do L, Rubinstein M, Kelly MA, Grandy DK, Low MJ, Gershanik OS, Murer MG, Giros B, Raisman-Vozari R. 2000. Selective increase of Nurr1 mRNA expression in mesencephalic dopaminergic neurons of D2 dopamine receptor-deficient mice. Brain Res Mol Brain Res 80:1–6.

Wallén A, Castro DS, Zetterström RH, Karlen M, Olson L, Ericson J, Perlmann T. 2001. Orphan nuclear receptor Nurr1 is essential for Ret expression in midbrain dopamine neurons and in the brain stem. Mol Cell Neurosci 18:649–663.

Wolf ME, Zigmond MJ, Kapatos G. 1989. Tyrosine hydroxylase content of residual striatal dopamine nerve terminals following 6-hydroxydopamine administration: A flow cytometric study. J Neurochem 53:879–885.

Woronicz JD, Lina A, Calnan BJ, Szychowski S, Cheng L, Winoto A. 1995. Regulation of the Nur77 orphan steroid receptor in activation-induced apoptosis. Mol Cell Biol 15:6364–6376.

Xiao Q, Castillo SO, Nikodem VM. 1996. Distribution of messenger RNAs for the orphan nuclear receptors Nurr1 and Nur77 (NGFI-B) in adult rat brain using in situ hybridization. Neuroscience 75:221–230.

Xing G, Zhang L, Zhang L, Heynen T, Li XL, Smith MA, Weiss SR, Feldman AN, Detera-Wadleigh S, Chuang DM, Post RM. 1997. Rat nurr1 is prominently expressed in perirhinal cortex, and differentially induced in the hippocampal dentate gyrus by electroconvulsive vs. kindled seizures. Brain Res Mol Brain Res 47:251–261

Zetterström RH, Solomin L, Mitsiadis T, Olson L, Perlmann T. 1996a. Retinoid X receptor heterodimerization and developmental expression distinguish the orphan nuclear receptors NGFI-B, Nurr1, and Nor1. Mol Endocrinol 10:1656–1666.

Zetterström RH, Williams R, Perlmann T, Olson L. 1996b. Cellular expression of the immediate early transcription factors Nurr1 and NGFI-B suggests a gene regulatory role in several brain regions including the nigrostriatal dopamine system. Brain Res Mol Brain Res 41:111–120.

Zetterström RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. 1997. Dopamine neuron agenesis in Nurr1-deficient mice. Science 276: 248–250.

Zigmond MJ, Hastings TG. 1998. Neurochemical responses to lesions of dopaminergic neurons: implications for compensation and neuropathology. Adv Pharmacol 42:788–792.