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Research report

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

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Abstract

Nurr1 and NGFI-B are closely related orphan members of the steroid-thyroid hormone receptor family involved in immediate early responses to stimuli such as growth factors. In-situ hybridization in the developing and adult mouse and rat demonstrated Nurr1 mRNA in several regions during early central nervous system (CNS) development. Expression persisted through the pre- and postnatal periods and was also found in several areas in the adult CNS. Positive areas include the olfactory bulb, parts of the cortex, the hippocampal formation and substantia nigra where Nurr1 and tyrosine hydroxylase mRNAs were co-expressed. 6-Hydroxydopamine-induced degeneration of mesencephalic dopamine neurons led to a corresponding loss of Nurr1 mRNA, demonstrating a link between Nurr1 and dopaminergic neurons. NGFI-B mRNA was not found in the prenatal CNS but was highly expressed in the adult brain in many areas including the olfactory bulb, cortex, basal ganglia and hippocampus. The spatiotemporal distribution of Nurr1 and NGFI-B mRNAs suggests that these transcription factors are involved in the development and maturation of specific sets of CNS neurons. The experimental data imply that one of these functions may be to control gene regulatory events important for development and function of those neurons that degenerate in patients with Parkinson's disease.

Keywords: Nurr1; NGFI-B; In-situ hybridization; Immediate early gene; Transcription factor; Orphan receptor

1. Introduction

Nurr1 (nur-related factor 1, also known as RNR1, NOT, HZF-3) and NGFI-B (nerve growth factor-inducible B, also known as Nur77, NAK1/TR3, N10) are two closely related members of the steroid-thyroid hormone receptor family of ligand-activated transcription factors [6,11,15,22]. Since Nurr1 and NGFI-B lack identified ligands they are also referred to as 'orphan receptors'. Additional receptors with similarity to Nurr1 and NGFI-B were recently identified in rat, *C. elegans* and *Drosophila* [10,16,23]. The latter invertebrate orphan receptors demonstrate a remarkable evolutionary conservation of the Nurr1/NGFI-B subclass of steroid-thyroid hormone receptors.

The Nurr1/NGFI-B subgroup is unique within the large family of nuclear receptors by being encoded by immediate early genes that are rapidly induced by various stimuli, e.g., growth factors. Other immediate early genes, including *c-fos* and *c-myc*, are involved in critical processes such as growth, differentiation and apoptosis in response to external stimuli, suggesting that also Nurr1 and NGFI-B might play similar central roles in cell signaling. Indeed, NGFI-B has recently been implicated in T cell apoptosis and adenosteroidogenesis [14,25,26], even though recent results indicate that there is no absolute requirement for the presence of NGFI-B for apoptosis to take place [12]. No function has yet been described for Nurr1.

Nurr1 and NGFI-B bind to specific DNA sequences (response elements) in the vicinity of genes that they regulate [4,15,20]. Several nuclear receptors, such as the all-*trans* retinoic acid (RA) receptor (RAR), bind to such DNA sequences as heterodimers with the receptor for 9-*cis* RA (RXR) [13]. Nurr1 and NGFI-B have been shown to interact with DNA as monomers [25] but can also form heterodimers with RXR and bind to similar response ele-

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ments as those recognized by RXR–RAR heterodimers [20]. However, whereas RAR prevents RXR from being activated, RXR-Nurr1/NGFI-B heterodimers are highly responsive to the RXR ligand, 9-*cis* RA [3,20]. Thus, heterodimerization with Nurr1 or NGFI-B shifts RXR from a silent to an active heterodimerization partner. Being encoded by growth factor-inducible immediate early genes, the interaction of Nurr1 and NGFI-B with RXR thus provides a mechanism for cross-talk between vitamin A and growth-factor signaling pathways.

As a first step towards understanding the function of Nurr1 and NGFI-B we have used in-situ hybridization to analyze the spatiotemporal cellular distribution of their mRNAs during embryonic and postnatal development and in the adult. We find that these immediate early genes are expressed in specific regions of the developing and adult CNS. The data suggest that Nurr1 plays a role in developing and adult CNS neurons including the dopaminergic neurons of substantia nigra.

2. Materials and methods

2.1. Animals

A total of 22 mice (NMRI, Bomholt Breeding and Research Center, Copenhagen, Denmark) and 33 rats (Sprague–Dawley, B&K Laboratories, Sollentuna, Sweden) from different developmental stages (E13, E15, E18, P0, P7, P14 and adult) were used. Pregnant females were killed by cervical dislocation and embryos were removed, staged (E15 and E18 decapitated) and fixed immediately in

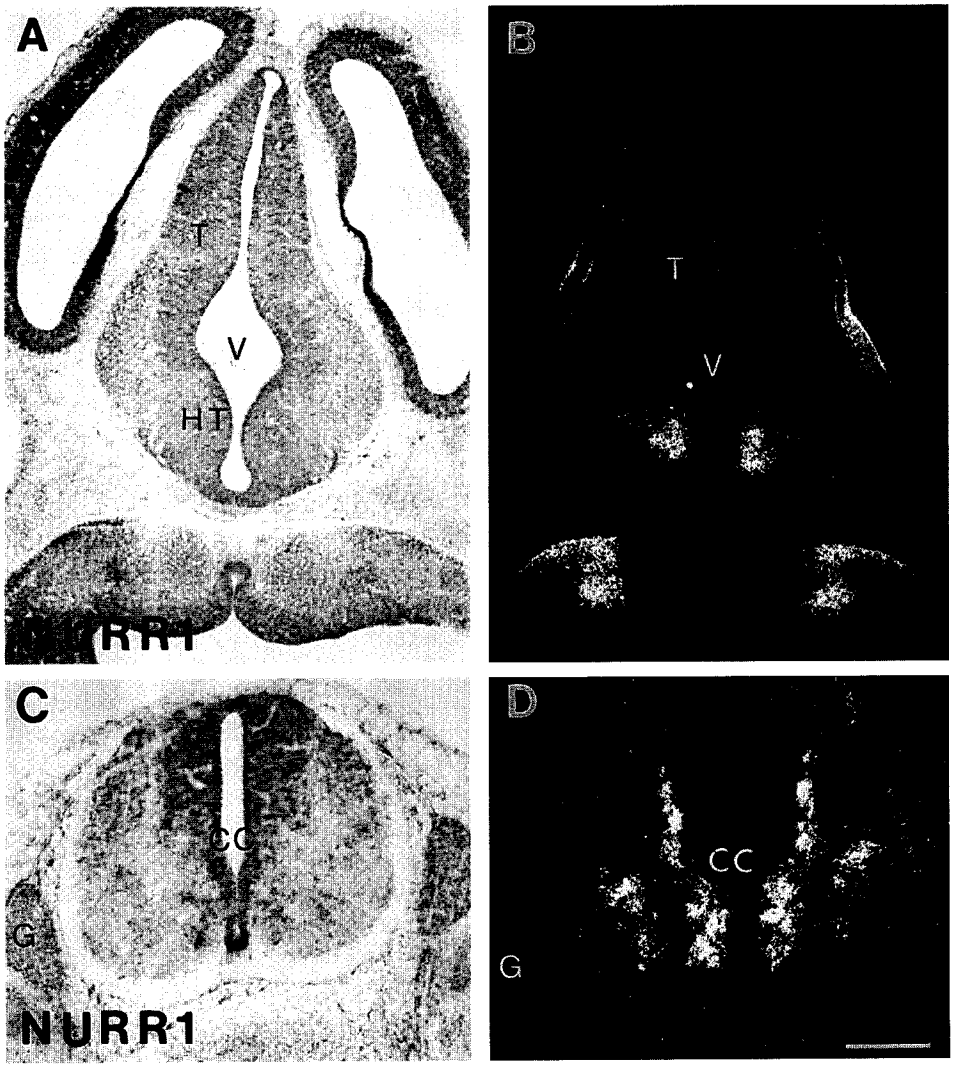


Fig. 1. Bright- (A,C) and dark-field (B,D) photomicrographs of transverse sections showing Nurr1 mRNA expression in the E13 mouse CNS. A,B: Strong Nurr1 mRNA expression is seen within the medial neuroepithelium of the posterior neocortex, in ventral nuclei of the hypothalamus (HT) and in the differentiating field of the developing pons. The distribution in this region suggests that the Nurr1 message is specifically excluded from the trigeminal and facial motor nuclei. C,D: Nurr1 mRNA is found to be heavily expressed in the spinal cord. The dorsal root ganglion (G) is also included in this section, and like all other peripheral ganglia, is negative. Thalamus (T), 3rd ventricle (V), central canal (CC). Scale bar = 350 μm.

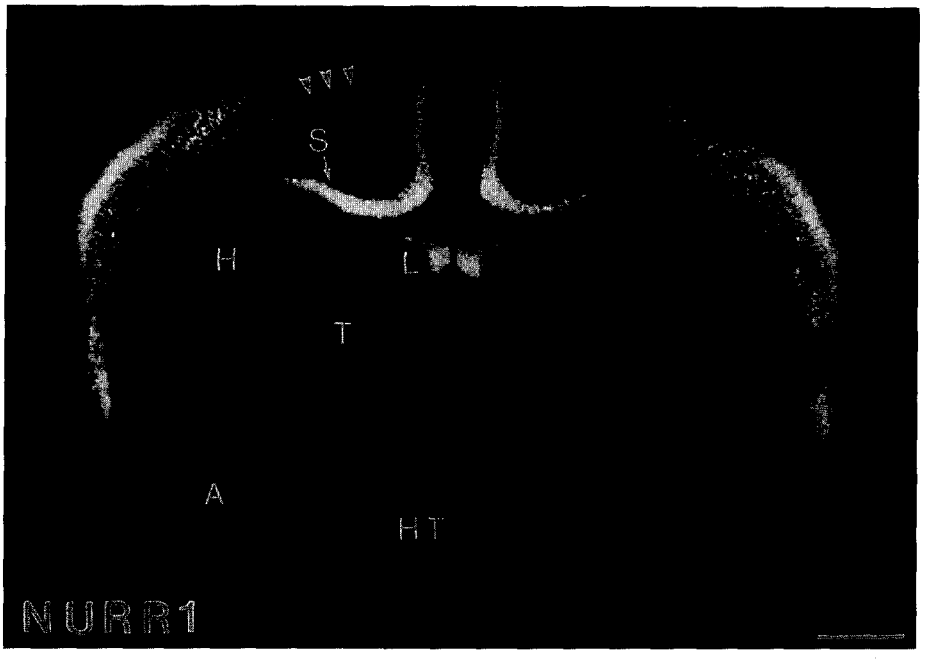


Fig. 2. Frontal section of a newborn rat brain showing Nurr1 mRNA expression. Strong expression of Nurr1 mRNA is seen in deep layer VI neurons in cortex close to the developing corpus callosum (arrowheads). More laterally in cortex Nurr1 mRNA-positive cells are also seen in the other cortical layers. Very strong expression is also seen in subiculum (S) and the medial habenular nucleus. In thalamus (T) no expression of Nurr1 mRNA is seen but a group of cells in the area of the periventricular hypothalamic nucleus in hypothalamus (HT) are Nurr1 mRNA positive. Additionally, Nurr1-expressing cells are found in CA1 of the hippocampal formation (H) and scattered Nurr1 mRNA-positive cells are seen in the lateral habenular nucleus (L) and the amygdaloid complex (A). Scale bar = 1 mm.

4% paraformaldehyde, cleared overnight in phosphate buffered saline (PBS) and infiltrated with 20% sucrose prior to cutting. Animals from postnatal stages were killed by decapitation and tissues were rapidly frozen. Tissues were sectioned at 10 μm (embryonic) or 14 μm (postnatal) and thaw-mounted onto slides (ProbeOn, Fischer).

2.2. Probe preparation and specificity controls

Oligonucleotide probes complementary to nucleotides 1430–1477 in Nurr1 mRNA [11], nucleotides 1191–1238 in NGFI-B mRNA [15] and 1441–1478 in tyrosine hydroxylase (TH) mRNA [5] having no similarities to known sequences, were synthesized (Scandinavian Gene Synthesis AB) and radiolabeled with [^{35}S]deoxyadenosine 5′-[α-thio]triphosphate (NEN) at the 3′-end using terminal deoxynucleotidyl transferase (Amersham). Specificity controls included: (1) use of a second probe complementary to another part of the mRNA strand (1334–1381 for Nurr1 mRNA and 1272–1319 for NGFI-B mRNA). The patterns observed with these complementary probes were always identical; (2) Inclusion of a 100-fold excess of unlabeled probe in the hybridization solution, in which case the labeling normally obtained was completely absent; (3) use of a 50 bp random probe, unique compared to known sequences in Genbank, labeled with the same amount of ^{35}S. This probe did not show any localized or specific binding to tissue components. As a positive control we used the fact that hybridization with a TH-specific probe in all cases generated the expected pattern of mRNA distribution.

2.3. In-situ hybridization

In-situ hybridization was performed according to Dagerlind et al. [2]. In brief, sections were hybridized for 16–18 h at 42°C in humidified boxes and rinsed 5 × 15 min in 1 × SSC at 55 or 60°C. Sections were then dehydrated, air-dried, dipped in photographic emulsion (Kodak NTB-2, diluted 1 : 1 in deionized water), exposed for 4–6 weeks at −20°C, developed 4 min (D-19, Kodak), fixed 6 min (mixture of Kodak 3000A and 3000B) and rinsed 30 min in water before being lightly counterstained with Cresyl Violet or Toluidine Blue and mounted (Entellan, Merck). Sections were analyzed using light- and dark-field microscopy (Zeiss, Axiophot) and photographed (Kodak T-Max 100). The identification of CNS areas was guided by published atlases [1,9,18,19]. Amount of specific labeling was semiquantitively recorded using a scale with 4 steps: −, (+), + and + +. Reliability and validity of this scale was ascertained by the positive correlation obtained between individual animals, between rats and mice as well as between observers.

Table 1
Distribution of Nurr1 and NGFI-B mRNA in the postnatal rat CNS

Brain area	Nurr1 mRNA				NGFI-B mRNA			
	P0	P7	P14	Adult	P0	P7	P14	Adult
Olfactory bulb *	+	+	+	+	++	++	++	++
Neocortex *	++	++	++	++	−	−	++	++
Claustrum	++	++	++	++	−	−	+	+
Caudate/putamen *	−	−	−	−	+	+	++	++
Accumbens nucleus	−	−	−	−	−	−	(+)	(+)
Olfactory tubercle	−	−	−	−	−	−	(+)	(+)
Septum ‡	+				−			(+)
Diagonal band ‡	+			(+)	−			−
Globus pallidus	−	−	−	−	−	−	−	−
Hippocampal formation CA1	+	+	+	+	−	−	++	++
CA2	−	+	+	+	−	−	++	++
CA3	−	+	+	+	−	−	+	+
Dentate gyrus	−	(+)	(+)	(+)	−	−	(+)	(+)
Subiculum	++	++	++	++	−	−	++	++
Medial habenular nucleus	++	++	++	++	−	−	(+)	(+)
Lateral habenular nucleus	(+)	(+)	(+)	(+)	−	−	(+)	(+)
Thalamus	−	−	−	−	−	−	−	−
Hypothalamus *	++	++	++	++	−	−	(+)	(+)
Amygdala	(+)	(+)	(+)	(+)	−	−	(+)	(+)
Ventral tegmental area *	++	++	++	++	−	−	−	−
Substantia nigra p. compacta *	++	++	++	++	−	−	−	−
Substantia nigra p. reticulata	−	−	−	−	−	−	−	−
Interpeduncular nucleus	−	−	−	−	−	−	−	−
Red nucleus	−	−	−	−	−	−	−	−
Locus coeruleus	−	−	−	−	−	−	−	−
Motor nucleus of vagus nerve				++				−
Cerebellum †			+	++			+	++
Spinal cord *	+	+	−	−	−	−	−	−

−, no expression; (+) scattered mRNA-positive cells; +, expression; ++, strong expression.
* See results for a further description.
† possibly striate pattern in granular layer.
‡ individually variable and scattered expression.

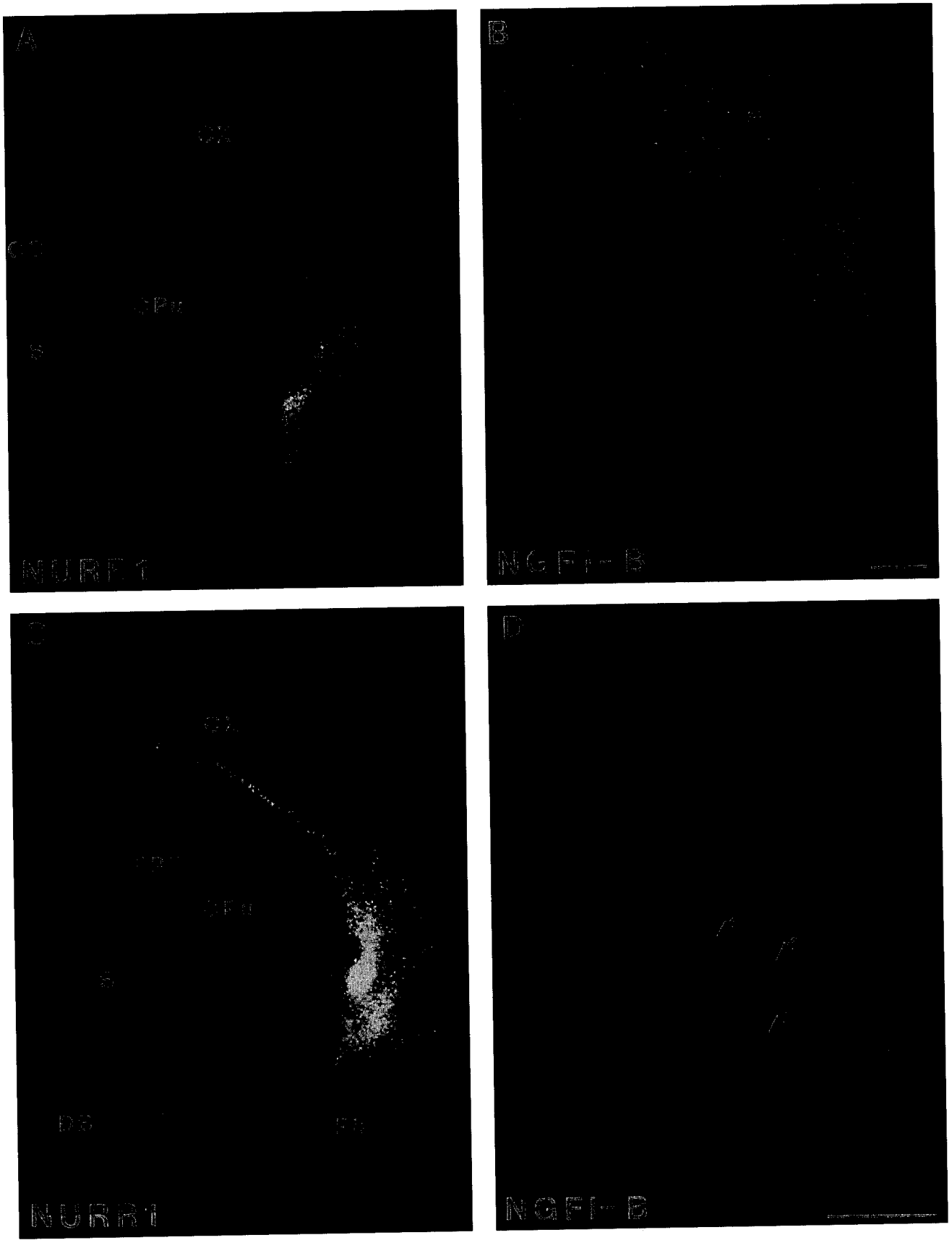
2.4. 6-Hydroxydopamine lesions

Rats were anesthetized and placed in a stereotaxic frame. Eight micrograms of 6-hydroxydopamine (6-OHDA) in 4 μl of Ringer's solution with 80 μg of ascorbic acid was unilaterally injected into the nigro-striatal pathway [24]. Two rats were killed each after 1, 3 and 7 days and an additional six animals were taken 9 months after surgery. The rats taken after 9 months were also tested in a rotometer for apomorphine-induced (0.05 mg/kg, s.c.) rotational behavior to assure a complete dopamine denervation [24].

3. Results

The expression of Nurr1 mRNA was high in many regions of the developing rat and mouse CNS and the expression remained high in most of these areas into adulthood. In contrast, only limited expression of NGFI-B

Fig. 3. Coronal sections of the newborn (C,D) and adult (A,B) rat brain at the level of caudate/putamen. Strong Nurr1 mRNA expression is found in cortical layer VI and in the area of claustrum and dorsal endopiriform nucleus, both in the newborn (C) and adult (A) brain. In (C) also note the scattered Nurr1 mRNA-positive cells in septum (S) and diagonal band (DB). B,D: Corresponding sections labeled with the NGFI-B-specific probe. In cortex (CX), no NGFI-B mRNA expression is seen in the newborn animal (D) whereas a strong in-situ signal is found in most cortical layers in the adult rat brain (B). The labeling in caudate/putamen (CPu) is also strikingly different between the newborn and adult animals. Within the newborn caudate/putamen, expression is seen in clusters (arrows in D) while in the adult rat striatum, NGFI-B-positive cells are scattered throughout the entire area. Scattered NGFI-B mRNA-labeled cells are also seen in the piriform cortex (Pir) in the newborn rat brain. Due to the special oblique light illuminator (Zeiss) used instead of an ordinary dark-field condensor to visualize Nurr1 and NFGI-B mRNA expression in low-power magnifications, areas where cells are densely arranged, as in the caudate/putamen neuroepithelium cpu, tend to refract light and appear labeled. This is also true for white matter like corpus callosum (CC). However, no Nurr1 or NGFI-B mRNA expression was observed in those areas. Scale bar = 500 μm.


A
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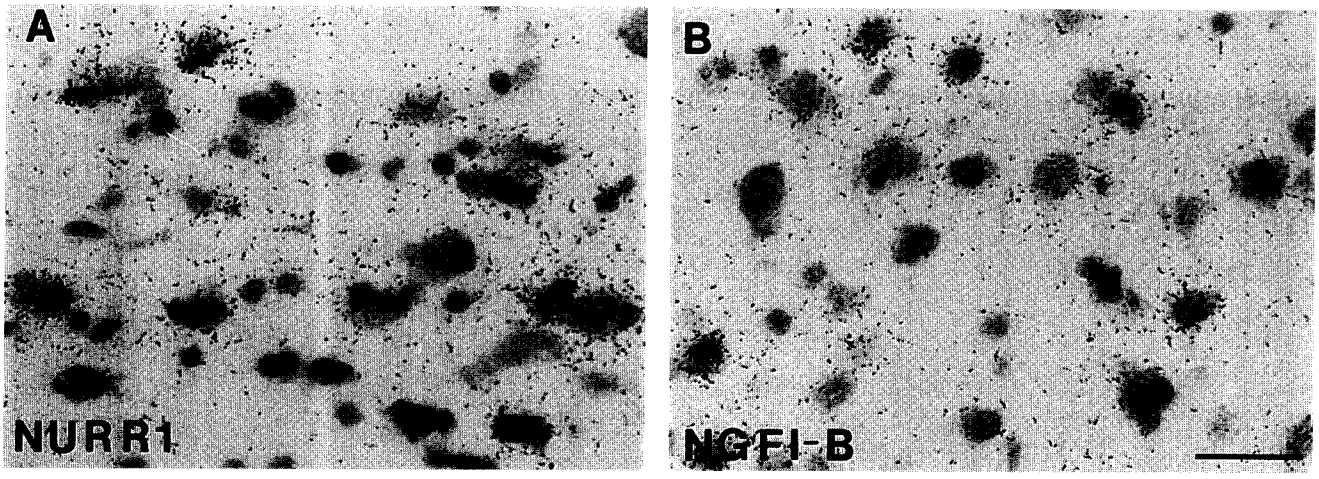


Fig. 4. Nurr1 (A) and NGFI-B mRNA (B) in adult cortical neurons. Bright-field microscopy. Label is found over selected cell somas. Scale bar = 50 μm.

mRNA was seen in the prenatal CNS. At the time of birth low NGFI-B mRNA expression appeared in small groups of cells in striatum. The level of expression increased postnatally in striatum and the transcript also appeared in other regions. In the adult animal, NGFI-B mRNA was found in several parts of the brain. Most cells that were Nurr1 or NGFI-B mRNA positive in the CNS appeared to be neurons rather than glial cells. Nurr1 mRNA was mainly seen in the CNS, but the transcript was also found in salivary glands and seminiferous tubules of the testis. In contrast, NGFI-B mRNA was observed in several places outside the CNS including: seminiferous tubules of the testis, adrenal glands, glomeruli of the kidney, lung, salivary glands, skeletal muscle and nerves. Areas where neither Nurr1- nor NGFI-B transcripts could be seen included liver and intestine. There were no major differences between rats and mice except a somewhat lower labeling using the NGFI-B probe in mice.

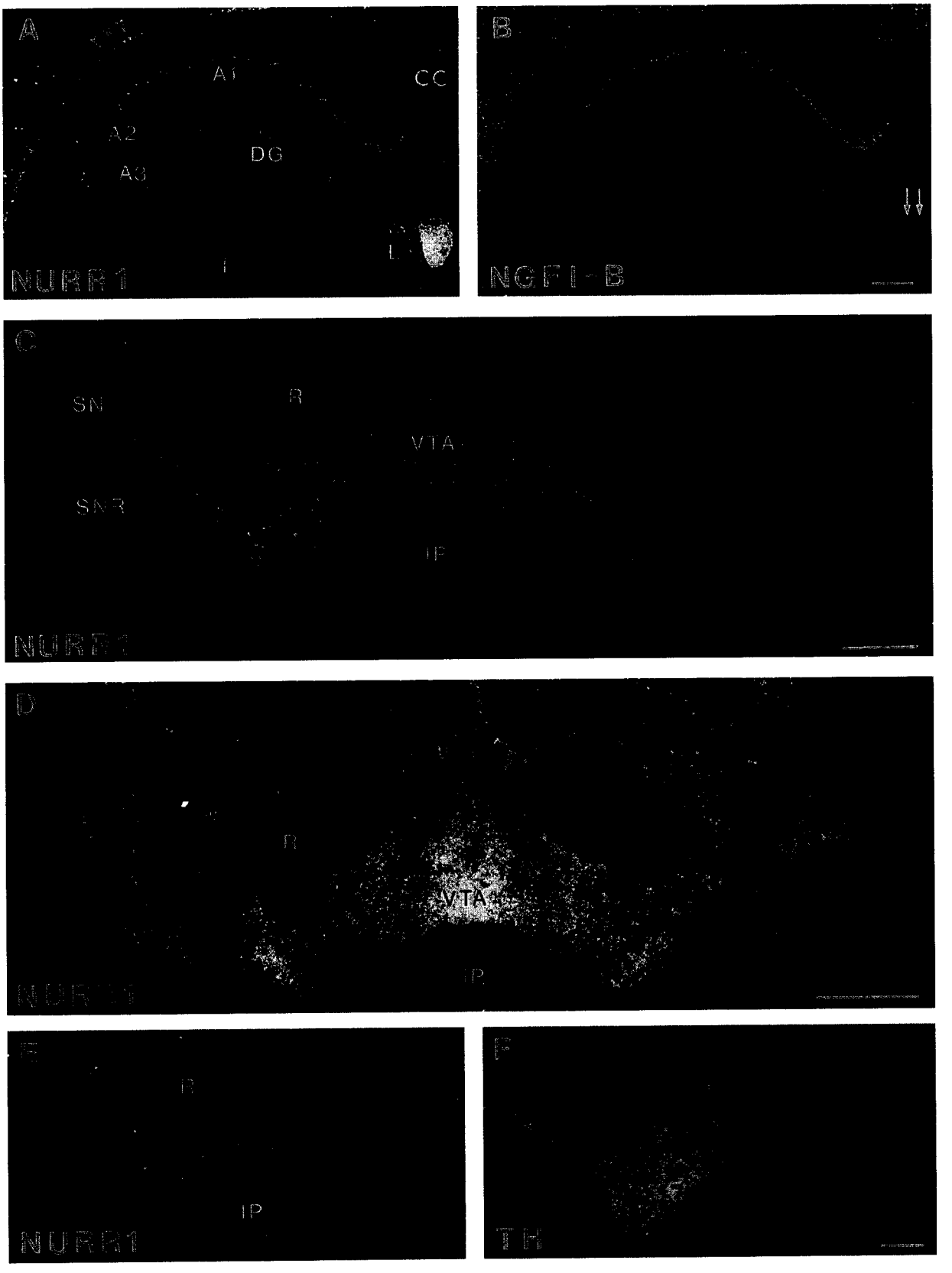
3.1. Rich prenatal expression of Nurr1 mRNA

Nurr1 mRNA was expressed across many regions of the developing CNS, although as development continued, the patterns of expression became progressively restricted to more specific regions. However, where Nurr1 mRNA continued to be expressed, strong labeling was always observed. At E13, the Nurr1 probe hybridized to cells in the differentiating fields of thalamus, hypothalamus, the insular cortex, rhinencephalon and pretectum and labeling extended down through isthmus, the pontine area, medulla and spinal cord. There was also strong labeling in the medial neuro-epithelium of both posterior neocortex and medial pons (Fig. 1A,B). In the latter case, labeling did not extend to the neurons within the most ventricular layers. In the spinal cord, labeling was divided into an extensive area of expression within the marginal zone of the neuroepithelium and a more lateral labeling in the ventral region (Fig. 1C,D). No labeling for Nurr1 mRNA was observed within the cerebral cortex or hippocampus at this age, nor in the peripheral nervous system at any prenatal age examined.

Within thalamus, labeling at E15 was restricted to the medial and lateral habenular nuclei and became further localized to the medial habenular nucleus by E18 although scattered cells could still be seen in the lateral nucleus. Labeling within the insular cortex began to adopt its postnatal pattern of being confined to regions of the future claustrum and endopiriform cortex. At E15, Nurr1 mRNA expression was also found in the developing anterior cortex and was largely confined at this, and subsequent embryonic ages, to the deepest layer of neurons of the infragranular part of the cortical plate. There was no labeling in the embryonic hippocampal formation until

Fig. 5. Coronal sections of the newborn (D) and adult rat brain (A–C and E–F) at the level of mid hippocampus (A,B) and substantia nigra (C–F). A,B: Two adjacent sections demonstrating Nurr1- (A) and NGFI-B mRNA (B) expression in hippocampus and associated areas. Nurr1- and NGFI-B mRNA expressing cells are found in CA1 (A1), CA2 (A2) and CA3 (A3) and there are also scattered Nurr1- and NGFI-B mRNA-positive cells in the dentate gyrus (DG). In (B), note the different levels of NGFI-B transcripts seen in CA1 and CA2 compared to CA3. A strong labeling with the Nurr1-specific probe is also seen in the medial habenular nucleus (lower right area of A). Note the lack of NGFI-B expression in this nucleus (arrows in B). Scattered Nurr1 mRNA-positive cells are also seen in the lateral habenular nucleus (L). In cortex, note the difference in expression patterns of the two gene products. NGFI-B mRNA is seen in all layers, except layer I, while Nurr1 mRNA expression mainly is restricted to cells in deep layer VI, close to corpus callosum (arrowheads). C,D: Basal expression of Nurr1 mRNA in the ventral tegmental area (VTA) and substantia nigra. There is also a group of scattered Nurr1 expressing cells in a region close to the red nucleus (R). E,F: Two adjacent sections of a unilaterally 6-OHDA denervated rat hybridized with a Nurr1 mRNA-specific probe (E) and a TH probe (F). Note the perfect match of Nurr1- and TH mRNA expression in VTA and substantia nigra pars compacta on the intact side and the corresponding matching absence of expression on the lesioned side 9 months after 6-OHDA treatment. Interpeduncular nucleus (IP), Substantia nigra pars compacta (SN), pars reticulata (SNR). Scale bar = 500 μm.

E18, when labeling was observed in subiculum and in scattered cells within the medial CA1. At E18, there was also intense labeling within the ventral tegmental area and over various brainstem nuclei, although matching of Nurr1 mRNA expression to specific individual nuclei was not possible.



3.2. Almost no prenatal expression of NGFI-B

Prenatally, weak NGFI-B mRNA labeling was observed within the choroid plexus and cranial nerves at E18. At all other investigated ages and regions, no expression was detected.

3.3. Postnatal expression of Nurr1 and NGFI-B

Major findings are listed in Table 1 and/or shown in Figs. 2–6. A more precise description of selected observations follows below.

3.3.1. Olfactory bulb

Nurr1 mRNA was seen in olfactory bulbs of newborn pups in scattered cells in the glomerular layer. From P7, Nurr1 mRNA was also found in the mitral cell layer and in the internal granular layer. Expression of NGFI-B mRNA was found in this region in all cellular layers.

3.3.2. Neocortex

In the upper/medial parts of cortex at P0, P7 and P14 the transcript for Nurr1 was found in neurons in the developing layer VI and this expression persisted into adulthood (Fig. 2, Fig. 3A,C and Fig. 5A). More laterally, in the caudal parts of parietal cortex area 1 (Par1), in insular cortex, in the whole area Par2 and in the perirhinal and entorhinal cortex, the message was also found in the other cortical layers. In the area of claustrum and the dorsal endopiriform nucleus there was a strong labeling which was also found later during development and in adulthood. Thus, the neocortical Nurr1 expression was confined to deep layers in cranial, dorsomedial areas while present in most layers of caudal, ventrolateral areas. NGFI-B mRNA was not found in cortex until postnatal day 14, at which time marked expression was found in many evenly distributed neurons in all the cortical layers except in layer I. The piriform cortex was also found to contain NGFI-B expressing neurons. This pattern of expression was also seen in the adult (Fig. 3B,D and Fig. 5B).

3.3.3. Caudate / putamen

NGFI-B was expressed in groups of cells in striatum in a patch/marginal zone manner in the newborn brain. This 'patchy' distribution disappeared later and in the P14 and adult striatum NGFI-B mRNA-positive cells were found evenly distributed throughout the whole caudate/putamen (Fig. 3B,D). The transcript for NGFI-B was also found in the bed nucleus of stria terminalis in the adult brain.

3.3.4. Hypothalamus

Nurr1 mRNA-positive cells were found in a few cell groups in postnatal rats. Most of these groups of neurons were not confined to known neuroanatomical entities. The labeling included parts of the posterior and lateral hypothalamic area, the dorsomedial hypothalamic nucleus and the supramamillary nucleus. Additionally, Nurr1 mRNA was found in the adults in what appeared to be the premamillary nucleus.

3.3.5. Substantia nigra / ventral tegmental area

Within this area Nurr1 mRNA was found both during development and in the adult animals (Fig. 5C,D). The adult distribution of Nurr1 mRNA in this region resembled the distribution of the dopamine-containing neurons and additional experiments with 6-OHDA denervated animals supported this conclusion; 1 day after 6-OHDA, a decrease in TH and Nurr1 mRNA expression was seen on the

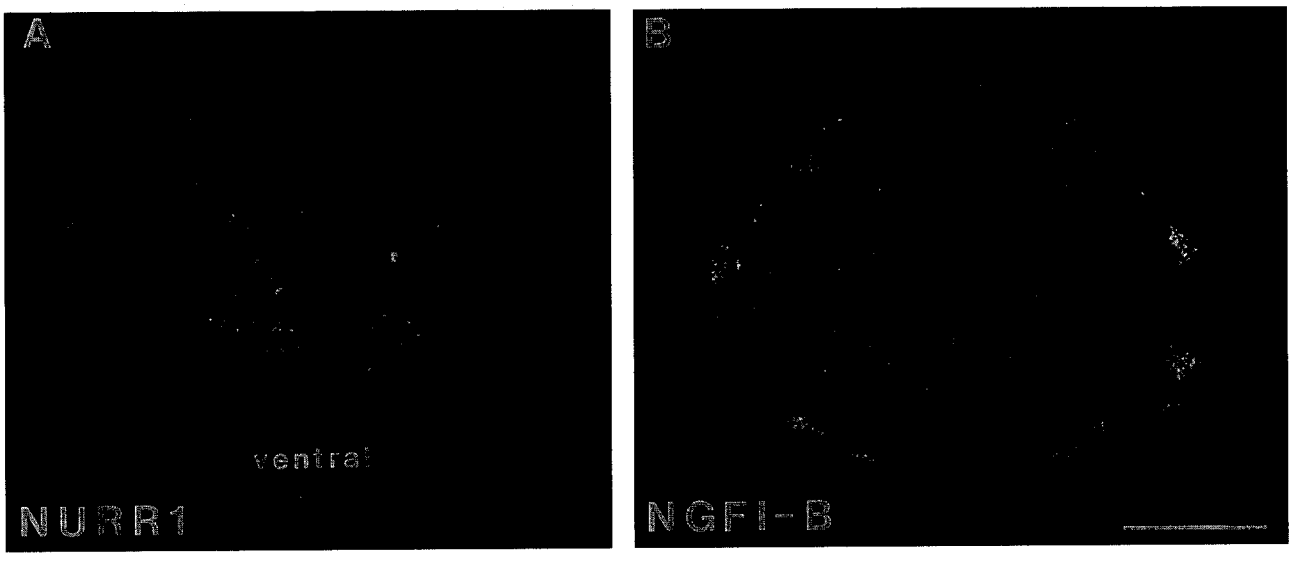


Fig. 6. Expression of Nurr1 (A) and NGFI-B mRNA (B) in the spinal cord of newborn rats. Strongly Nurr1 mRNA-labeled cells are found in small (non-motor) neurons located in central parts of the grey matter. NGFI-B mRNA is found in nerve rootlets and in the dura mater. This expression is probably confined to the Schwann cells. Scale bar = 500 μm.

affected side and this decrease was mainly restricted to the ventral tegmental area. Three days after 6-OHDA only a few TH and Nurr1 mRNA-positive cells were seen on the denervated side. Seven days and 9 months after 6-OHDA treatment, TH and Nurr1 mRNA could no longer be detected on the lesioned side (Fig. 5E,F).

3.3.6. Brainstem

Nurr1 mRNA was found adjacent to locus coeruleus (as identified on adjacent sections with the TH mRNA-specific probe) in what seemed to be Barrington's nucleus. Nurr1 mRNA-expressing cells were also found close to the fourth ventricle, medial to Barrington's nucleus in the area of central gray alpha and nucleus O. In addition, strong Nurr1 expression was seen in the motor nucleus of the vagus nerve.

3.3.7. Spinal cord

In the newborn and P7 rat spinal cord distinct Nurr1 mRNA labeling was found in central parts of the grey matter (Fig. 6A). In P14 and adult animals Nurr1 mRNA could no longer be detected. NGFI-B mRNA-positive cells were found in the dorsal and ventral nerve rootlets in newborn rats (Fig. 6B).

4. Discussion

The present experiments demonstrate that Nurr1 and NGFI-B are expressed in highly specific patterns in the CNS. Nurr1 mRNA is found at many levels along the neuraxis, while NGFI-B mRNA is confined to more rostral areas, being absent from mesencephalon, pons and the spinal cord. The temporal expression of Nurr1 mRNA in the spinal cord differs from that of other Nurr1 mRNA-positive areas by being present during the first weeks of development but not later in life.

The relationship of Nurr1 and NGFI-B to the dopamine system is particularly intriguing. The match between the distribution of Nurr1 and TH mRNAs in substantia nigra pars compacta and the ventral tegmental area of mesencephalon, suggests that Nurr1 mRNA is expressed in dopamine neurons. Strong additional support for such a conclusion was derived from our experiments demonstrating that unilateral stereotaxic injections of a specific neurotoxin for dopaminergic nerve cells, 6-OHDA, leads to rapid, complete and long-lasting disappearance of both TH and Nurr1 mRNA-containing neurons. While Nurr1 mRNA appears to be expressed in the dopamine neurons, NGFI-B mRNA is expressed in the target area of these nerve cells in caudate/putamen. During development of this brain region, NGFI-B mRNA is present in patches and in a marginal zone similar to the characteristic patch/marginal zone distribution of the developing dopamine nerve terminals [17]. As striatum matures, the NGFI-B mRNA becomes more evenly distributed, consistent with the dense and uniform dopamine innervation of the adult structure.

While Nurr1 and NGFI-B mRNA appear to be mutually exclusive in the cell body and target area of the nigrostriatal dopamine system respectively, they appear to coexist in the olfactory bulb. This structure contains a form of local dopamine circuitry in which both cell bodies and terminals are present in the olfactory bulb itself. Therefore, if expression in the olfactory bulb is also related to the dopamine system, both orphan receptors would be expected to be expressed in this area.

Given that both Nurr1 and NGFI-B appear to be related to the dopamine system both in the nigrostriatal system and in the olfactory bulb, it is interesting to note that other members of the steroid/thyroid hormone receptor family, such as the glucocorticoid and progesterone receptors, have been shown to be activated by dopamine in the absence of their ligands [21]. Such a regulatory potential for dopamine could possibly be of physiological relevance for the function of both Nurr1 and NGFI-B in these brain regions.

Nurr1 and NGFI-B are encoded by immediate early genes, and thus a variety of stimuli are able to produce rapid up-regulation of their mRNA levels. Potassium-induced depolarization of PC12 cells is one such stimulus [7]. Focal mechanical lesions of the brain can lead to up-regulation of both receptors [8]. Excitatory epileptogenic treatments with pilocarpine similarly up-regulates Nurr1 and NGFI-B in a specific spatiotemporal pattern (unpublished data). An additional possibility is that Nurr1 and NGFI-B operate by forming heterodimers with RXR [3,20]. This mechanism may be only one of several ways in which Nurr1 and NGFI-B can function and it emphasizes the importance of comparing the distribution of the two orphan receptors to that of the various RXR mRNA species in the brain.

Several patterns of expression of Nurr1 as well as NGFI-B mRNA appear unrelated to dopamine neurons. The striking expression of Nurr1 mRNA in cortex, claustrum, as well as the pyramidal cell layers of hippocampus and subiculum and the presence of NGFI-B mRNA in cortex and hippocampus suggests that the two receptors are also involved in higher CNS functions such as cognition and memory.

We conclude that the two closely related transcription factors Nurr1 and NGFI-B are expressed in the developing and adult brain in patterns suggesting a role both in the nigrostriatal dopamine system and in the cortices. The presence of Nurr1 in the dopaminergic nerve cells, represents a previously unknown constituent of those neurons that degenerate in Parkinson's disease.

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