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Selective Expression of Dopamine D_3 Receptor mRNA in Proliferative Zones during Embryonic Development of the Rat Brain

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We studied by *in situ* hybridization histochemistry the expression of D_3 receptor (D_3R) mRNA at various stages of rat brain development. The first expression of D_3R mRNA was detected at embryonic day 14 (E14) in the striatal and rhinencephalic neuroepithelia and throughout the tectal neuroepithelium. From E16 to E19 D_3R mRNA expression extended along a rostrocaudal axis to additional proliferative ventricular zones of the basal forebrain, including the neuroepithelia of the olfactory bulb, nucleus accumbens, septum, and amygdala, whereas D_1 and D_2 receptor (D_1R and D_2R) mRNAs were expressed predominantly by migrating neuroblasts and/or differentiating striatal neurons. Only a few neuroblasts, migrating in the lateral cortical stream or developing as cerebellar Purkinje cells, expressed D_3R mRNA from E18. At birth D_3R expression mRNA appeared in differentiating neuronal fields of the nucleus accumbens and medial mamillary body primordia and on P5 reached a distribution similar to that found in adult. In addition, a transient upregulation was detected on P5 in the medial mamillary bodies, parietofrontal cortex, and olfactory tubercle. In the adult brain D_3R gene expression continued in the striatal proliferative subventricular zone. The late expression D_3R mRNA in neurons, after achievement of dopamine innervation, supports the existence of a regulating factor released from dopamine neurons, as suggested by denervation studies in the adult. The sustained and abundant D_3R gene expression, predominantly in germinative neuroepithelial zones actively involved in neurogenesis of most basal forebrain structures, supports the hypothesis of a neurogenetic but minor morphogenetic modulatory role for the D_3R during CNS development.

Key words: in situ *hybridization; neuroepithelium; neurogenesis; forebrain development; dopamines; D_3R mRNA*

Catecholamines seem to affect a number of developmental processes in primitive organisms, including growth, regeneration, and morphogenesis (Lauder, 1993). The dopaminergic system appears early in brain development of higher species (Voorn et al., 1988), and a neurodevelopmental role for dopamine has been suggested (Rosengarten and Friedhoff, 1979; Miller and Friedhoff, 1986).

Dopamine interacts with five dopamine receptor subtypes (D_1R–D_5R) having distinct localization and intracellular signaling, allowing this neurotransmitter to exert pleiotropic influences on target cells (Sibley and Monsma, 1992; Gingrich and Caron, 1993; Sokoloff and Schwartz, 1995). The D_3R (Sokoloff et al., 1990) has a density in the adult rat brain two orders of magnitude lower than that of the D_2R and is expressed in discrete brain regions, including the ventral striatum, mamillary bodies, and archicerebellum (Bouthenet et al., 1991; Lévesque et al., 1992; Diaz et al., 1995).

D_1R and D_3R both occur in the islands of Calleja and nucleus accumbens (Le Moine and Bloch, 1996), whereas colocalization of D_2R and D_3R seems exceptional (Bouthenet et al., 1991). It is not known whether these respective coexpression and segregation originate early in development. Previous works have detailed ontogeny of D_1R and D_2R in the rat brain (Guennoun and Bloch, 1992; Schambra et al., 1994). D_1R and D_2R messages are present from gestational day 14 in low amounts and increase slowly during development. These receptors are expressed mainly by migrating and maturing neurons, similar in location to those found in the adult brain, suggesting that respective receptor expression is an intrinsic property of these neurons (Schambra et al., 1994).

The regulation of D_3R gene expression in adult is highly dependent on dopamine innervation. Thus, a lesion of dopamine neurons, impairment of axonal transport, or reduction of dopamine neuron firing, but not removal of dopamine or its known cotransmitter transmission, decreases D_3R gene expression (Lévesque et al., 1995). This has led to the proposal that D_3R gene regulation is under the positive influence of a yet unidentified anterograde factor released from dopamine neurons.

The prenatal development of D_3R expression is documented very poorly. With the use of the highly sensitive reverse PCR, D_3R transcripts were detected faintly in rodent embryos from gestational days 10–11 and clearly on day 14 (Cadoret et al., 1993; Fishburn et al., 1996). These studies, however, did not reveal the localization of D_3R transcripts in embryonic brain. Autoradiographic analysis of rat brain during prenatal ontogeny with the use of the D_2R/D_3R ligand [^{125}I]iodosulpride (Sales et al., 1988) revealed transient expressions of binding sites in the ventral part of the spinal cord and in the lateral ventricle lining that did not match D_2R mRNA distribution and, therefore, possibly that were related to the D_3R. In addition, a transient expression of D_3R protein was observed in the parietal cortex during postnatal development of mouse brain (Demotes-Mainard et al., 1996).

Using *in situ* hybridization, we mainly show here that D_3R mRNA expression is restricted almost entirely to the ventricular

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Table 1. Abbreviations used in figures

4V	Fourth ventricle
IX	Cerebellum, lobule 9
X	Cerebellum, lobule 10
Acc	Nucleus accumbens
AccSh	Nucleus accumbens, shell part
AccC	Nucleus accumbens, core part
Amy	Amygdala
CC	Corpus callosum
Ci	Cingulate cortex
Cx	Cortex
Fr	Frontal cortex
ICj	Islands of Calleja
ICjM	Island of Calleja, major
In	Insulate cortex
Lcs	Lateral cortical stream
LV	Lateral ventricle
M	Mitotic zone of the neuroepithelium
MB	Medial mamillary bodies
Ne	Neuroepithelium
OB	Olfactory bulb
Pa	Pallidum
PFCx	Parietofrontal cortex
Pu	Purkinje cell layer
Re	Reservoir of lateral cortical stream
Rh	Rhinencephalon
S	Synthetic zone of the neuroepithelium
Sp	Septum
St	Striatum
SVZ	Subventricular zone
TNe	Tectal neuroepithelium
Tu	Pyramidal layer of olfactory tubercle

neuropithelium during the whole prenatal ontogeny and that the neuronal expression of the D_3R appears later, after the settling of dopamine innervation.

MATERIALS AND METHODS

Animals and dissection. Timed pregnant female Wistar rats (Iffa-Credo) were used. Gestational age was determined from the mating time, and embryonic day 1 (E1) was designated as the day after insemination. After a 21 d gestation the day of birth was designed as postnatal day 0 (P0), and subsequent age was defined relative to this. For studies involving prenatal series, pregnant rats were decapitated, and embryos and fetuses were removed by cesarean section on E12, E14, E16, E17, E18, and E19. Postnatal studies were made with animals at ages P0 and P5.

Tissue preparation. Embryos and fetuses (E12–E19) removed free from the amniotic membranes were whole-frozen in liquid monochlorodifluoromethane (−30°C) and stored at −70°C. The postnatal animals (P0 and P5 pups) were decapitated, and their heads were collected and frozen. Tissue sections (8 and 10 μm for pre- and postnatal, respectively) made in coronal and sagittal planes on a cryostat were thaw-mounted at room temperature on SuperFrost Plus slides (Menzel-Glasser), fixed for 40 min at 4°C in 4% paraformaldehyde made up in 0.1 M PBS, and rinsed twice (5 min each) in PBS at 4°C and then for 5 min in PBS at room temperature. The sections were rinsed briefly in distilled water, dehydrated via graded alcohols, dried under a stream of cold air, and stored at −70°C until they were processed for *in situ* hybridization.

Probes. The probes used were ^{33}P-labeled riboprobes. Probes for rat D_2R and D_3R mRNAs corresponded to respective sequences of the third intracellular loop of the receptor and are described elsewhere (Sokoloff et al., 1990; Bouthenet et al., 1991). The probe for rat D_1R corresponded to a C-terminal fragment (nucleotides 1382–1708) obtained by PCR and subcloned into pGEM-4Z. Riboprobes were synthesized with the Riboprobe Gemini System (Promega, Madison, WI), treated by RNase-free DNase (Boehringer Mannheim, Mannheim, Germany), and recovered from Chroma Spin −30 columns (Clontech, Cambridge, UK).

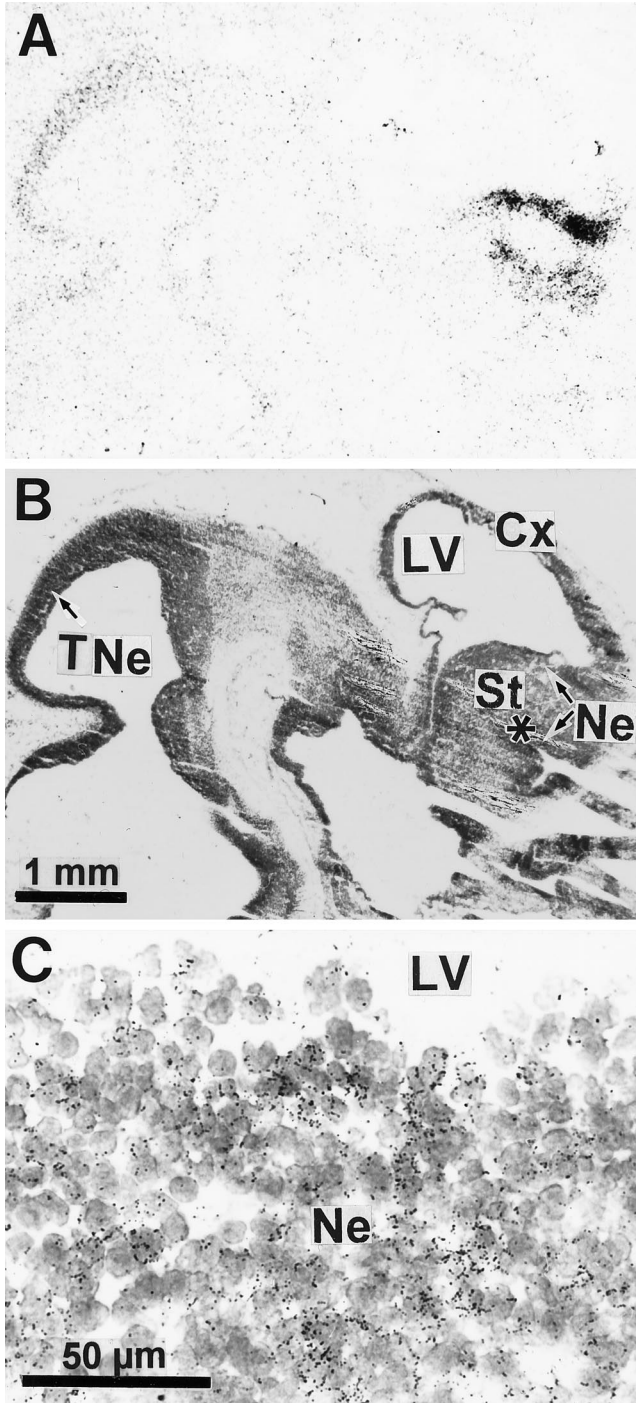

Figure 1. Expression of D_3R mRNA in the brain of the 14 d embryo. *A*, Film autoradiographic hybridization signals from a parasagittal section hybridized with a D_3R mRNA antisense probe. Hybridization signals are present only in neuroepithelia of striatum (*St*), rhinencephalon (indicated by *asterisk*), and tectum (*TNe*). *Ct*, Cortex. *B*, Structure locations are shown on the same section counterstained with Mayer's hemalum solution and photographed with bright-field illumination. *C*, Photomicrograph of an emulsion-dipped section with bright-field illumination at the level of striatal neuroepithelium lining the lateral ventricle (*LV*), showing groups of labeled cells identified by clusters of dark silver grains overlying individual nuclei in the neuroepithelium (*Ne*).

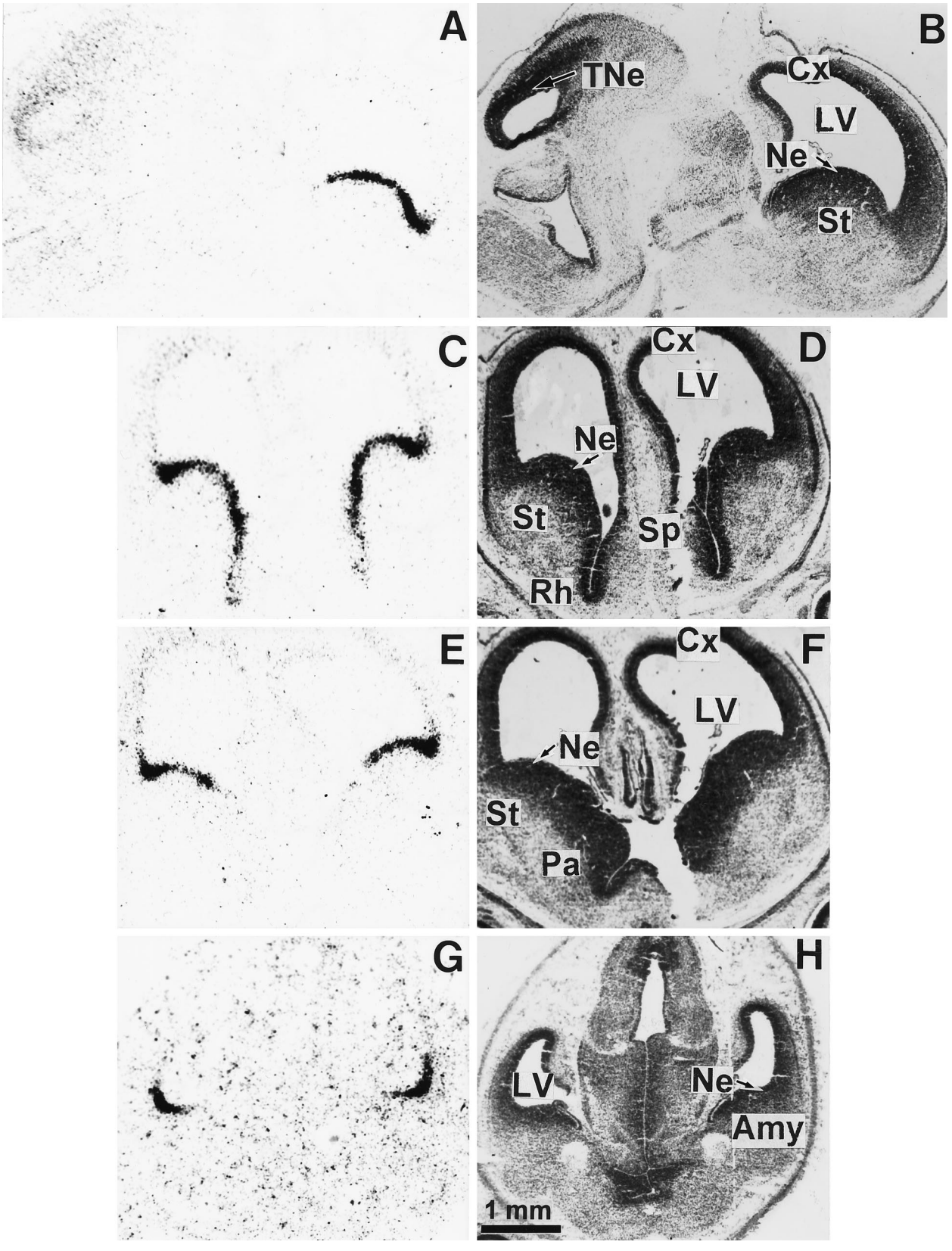

Figure 2. Expression of D_3R mRNA in the brain of the 16 d embryo. The *left panel* shows film autoradiograms from a parasagittal section (*A*) and three coronal serial sections (*C*, *E*, *G*) hybridized with the D_3R mRNA probe. The *C*, *E*, and *G* sections are ordered from rostral to caudal forebrain levels. The *right panel* shows the same sections counterstained with Mayer's hemalum and photographed in bright-field illumination. The expression of D_3R gene transcripts in the basal forebrain neuroepithelium, continuous from rostral striatum (*St*) to amygdala (*Amy*), is interrupted remarkably at the pallidum level (*Pa*). *Cx*, Cortex; *LV*, lateral ventricle; *Ne*, neuroepithelium; *Rh*, rhinencephalon; *Sp*, septum; *TNe*, tectal neuroepithelium.

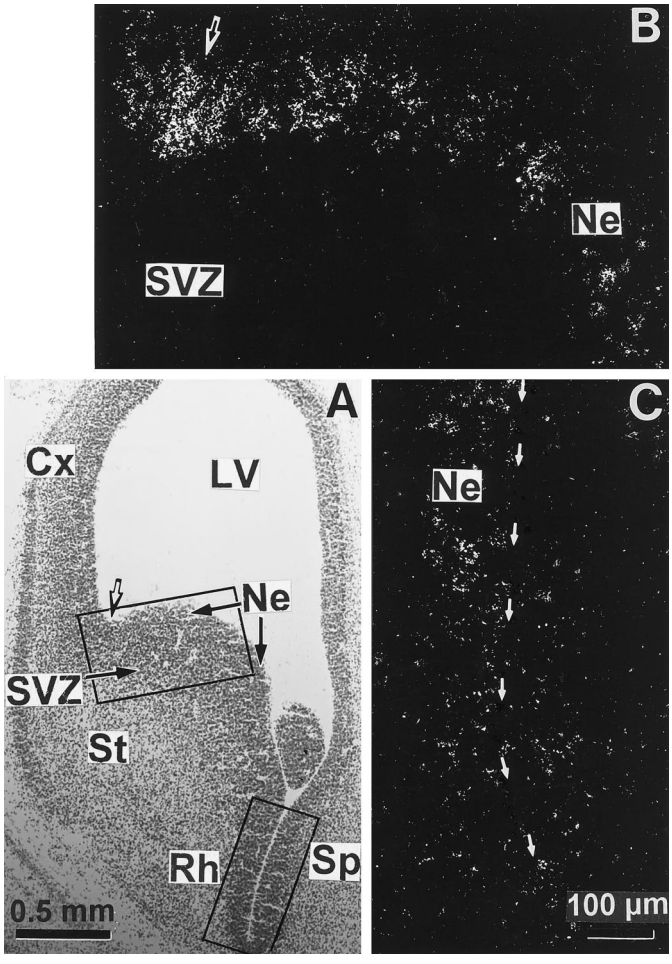

Figure 3. High magnifications of D_3R mRNA hybridization signals in basal forebrain neuroepithelium of the 16 d embryo. The *top* and *bottom* rectangles in a bright-field photomicrograph from a coronal section counterstained with Mayer's hemalum (*A*) delimit the fields shown in *B* and *C*, respectively. *B*, *C*, Dark-field photomicrographs taken from an emulsion-dipped section through the rostral striatal anlage that show the labeling in the striatal (*St*), rhinencephalic (*Rh*), and septal (*Sp*) neuroepithelia bordering the ventral horn of the lateral ventricle (*LV*), indicated by *thin arrows* in *C*, whereas the subventricular zone (*SVZ*) and the differentiating field of striatum are labeled only scarcely. The strongest signal is present approximately at the limit between striatal and cortical neuroepithelia in *A*, as determined by the dorsolateral corner of the lateral ventricle (indicated by *thick arrows* in *A* and *B*). *Cx*, Cortex; *Ne*, neuroepithelium.

In situ *hybridization.* Thawed sections were treated with proteinase K (1 mg/ml) for 10 min at 37°C, acetylated in 0.1 M triethanolamine, pH 8, and 0.25% acetic anhydride for 10 min, rinsed in 2× SSC (20× SSC is 0.3 M Tris-citrate and 3 M NaCl), dehydrated in increased concentrations of graded ethanol, and air-dried. Sections were covered with 50 μl of a hybridization buffer containing 70% formamide (for D_2R and D_3R) or 50% formamide (for D_1R), 10% dextran sulfate, 1× Denhardt's solution, 4× SSC (for D_2R and D_3R) or 2× SSC (for D_1R), 0.1% Na-pyrophosphate, 100 μg/ml yeast-tRNA, 100 μg/ml denatured salmon sperm-DNA, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 2×10^6 dpm of a ^{33}P-labeled antisense or sense probe. Slides were covered with squares of Nescofilm (Roth) and incubated overnight (12–14 hr) at 55°C. Then sections were cooled at room temperature in 2× SSC, treated with RNase A (200 μg/ml) for 1 hr at 37°C, and rinsed twice in 2× SSC for 15 min at room temperature, in 0.5× SSC for 30 min at 55°C, and in 0.1× SSC for 30 min at 60°C. Sections were dehydrated in graded alcohols containing 300 mM ammonium acetate and were dried under a stream of cold air. Autoradiograms were generated by apposing the radiolabeled tissue sections to β-Max Hyperfilms (Amersham, Braunschweig, Germany) for 1–2 weeks for D_1R and D_2R and 3–4 weeks for D_3R.

Some sections from animals at each stage of development studied were dipped in a photographic emulsion (LM-1, Amersham) and exposed for 30–40 d. Dipped sections were counterstained with Mayer's hemalum solution and examined with a photomicroscope Axiophot (Zeiss, Oberkochen, Germany).

RESULTS

We performed *In situ* hybridization using a ^{33}P-labeled D_3R cRNA probe on brain sections of embryos, heads of fetuses and pups, from the 12th gestation day (13 d after insemination, E12) until the 5th day after birth (P5). Two to four animals from at least two different offspring were used at each stage. Structure identification, name, and abbreviations (Table 1) are in agreement with the recommendations of the Boulder Committee (1970) and the atlas of the rat developing brain by Bayer and Altman (1995). The regional and cellular distribution of D_3R mRNA expression was determined with both film autoradiograms and emulsion-coated microautoradiographies on coronal and sagittal sections taken at various levels throughout the whole developing brain, but only relevant levels are illustrated on Figures 1–9. In addition, cRNA probes for D_1R and D_2R subtypes were used in some experiments for comparison. Control hybridization experiments with sense probes resulted in autoradiograms devoid of signal (data not shown).

Midgestational development (E12–E16)

No hybridization signal was seen in any part of the developing brain at E12 (data not shown). Specific hybridization signal for D_3R message was detected on both autoradiograms and emulsion-coated sections from E14 (Fig. 1). At this stage discrete germinative ventricular zones of the basal forebrain and dorsal midbrain exhibited distinct autoradiographic signals. In the forebrain, hybridization labeling was detected at high levels in the neuroepithelium of striatum (also referred to as lateral ganglionic eminence) and at moderate levels in rhinencephalic and septal neuroepithelia (Fig. 1*A,B*). D_3R mRNA expression in the forebrain neuroepithelium abruptly ended at the rostral tip of the lateral ventricle, approximately corresponding to the limit between striatal and cortical neuroepithelia or dorsal and basal forebrain (Fig. 1*A,B*). In the midbrain, hybridization signals were detected at moderate levels in the tectal neuroepithelium. Examination of microautoradiographic hybridization labeling showed that D_3R mRNA-labeled cells appeared as clusters of neuroepithelial cells at the vicinity of the lateral ventricle (Fig. 1*C*). Within the clusters a large majority of cells were labeled.

At E16 high levels of hybridization signals appeared as a narrow band all along the basal forebrain neuroepithelium from the striatum up to the amygdala. However, this continuity of D_3R mRNA expression was interrupted remarkably at the level of the neuroepithelium of pallidum, which did not express transcripts at all (Fig. 2*E*). The hybridization signals were more pronounced at the limit between the striatal and cortical neuroepithelia (Figs. 2*A,C,E*, 3*A*). Labeling at a moderate level was still apparent in the tectal neuroepithelium, whereas faint signals were present in the cortical neuroepithelium.

Photomicrographs (Fig. 3) showed that at E16 D_3R mRNA was expressed by cells lining the lateral ventricle at the level of ventricular zones of striatum, rhinencephalon, septum, and amygdala and appeared mostly in clustering cells rather than in a homogenous layer.

Late gestational development (E17–E19)

The distribution of D_3R mRNA in brain embryo at E17 did not differ greatly from that at E16 (data not shown). At E18 most of

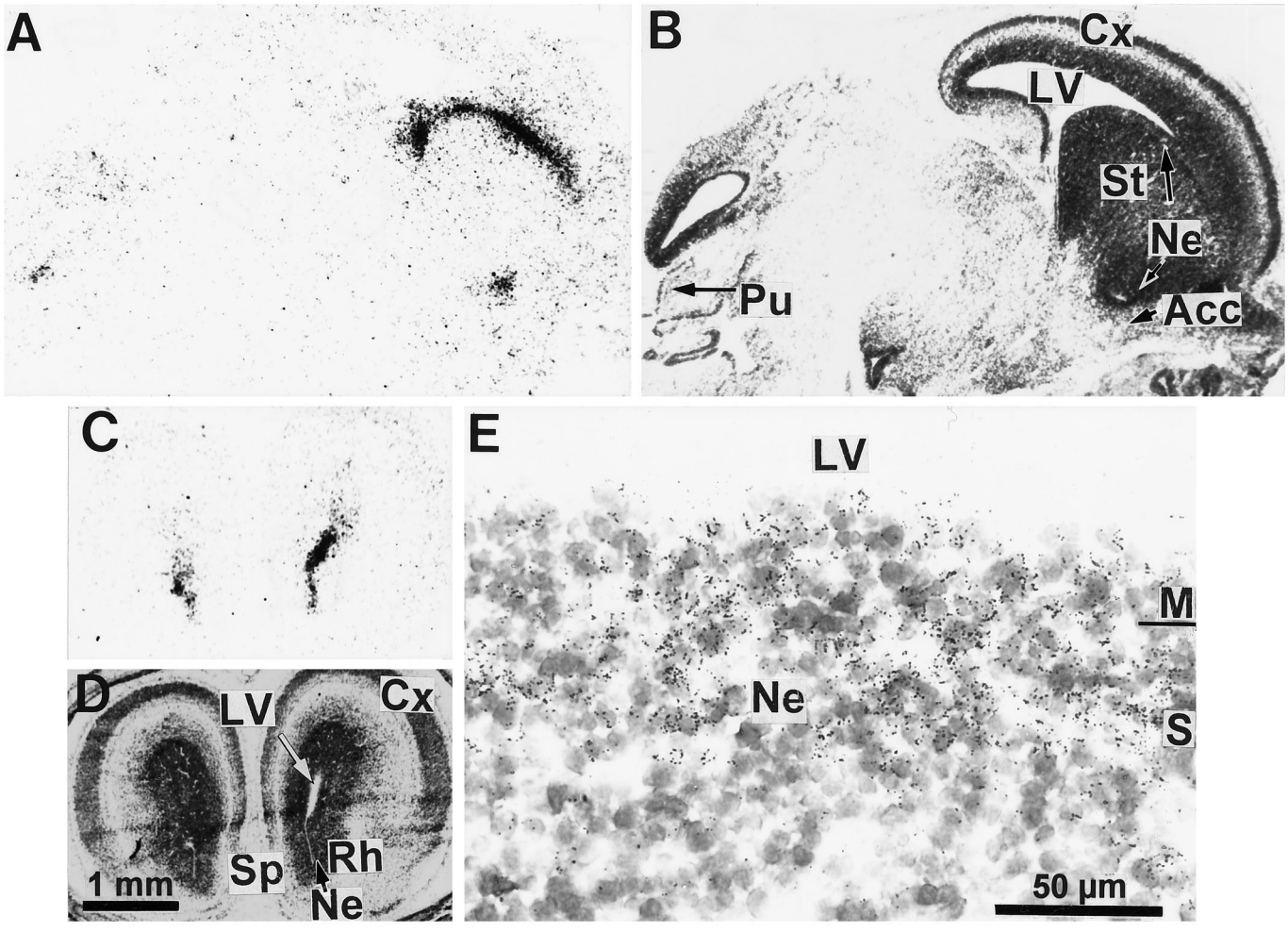

Figure 4. Expression of D_3R mRNA in the basal forebrain and cerebellar primordia of the 18 d embryo. Autoradiograms of parasagittal (*A*) and coronal (*C*) sections hybridized with the D_3R mRNA probe show signals in neuroepithelia (*Ne*) of striatum (*St*), nucleus accumbens (*Acc*), rhinencephalon (*Rh*), septum (*Sp*), and in the more caudal Purkinje cell layer (*Pu*) of cerebellar primordium. *B*, *D*, Corresponding sections counterstained with Mayer's hemalum. High magnification of the striatal ventricular zone under bright-field illumination (*E*) illustrates that labeling is restricted to clusters of labeled cells present through the synthetic (*S*) and mitotic (*M*) neuroepithelial zones, delimiting a continuous periventricular band 100 μm thick. *Cx*, Cortex; *LV*, lateral ventricle; *SVZ*, subventricular zone.

the D_3R mRNA still was expressed in the basal forebrain neuroepithelium, spanning the entire striatal border of the lateral ventricle and extending ventrally up to the ventral tip surrounded by primordia of nucleus accumbens and septum. In addition, hybridization signals were present in tectal neuroepithelium but at much lower levels, as compared with previous stages of gestation and in cortical neuroepithelium (Figs. 4*A,C*, 5*A*). Labeled cells appeared as clusters in a 100-μm-thick band lining the ventricle (Fig. 4*E*), corresponding to the synthetic and mitotic zones of the neuroepithelium, as evidenced by Bayer and Altman (1991) with the use of [^{3}H]thymidine labeling, whereas the differentiating field was labeled only very scarcely. This shows that D_3R-expressing cells are proliferative and not postmitotic differentiating cells.

The first D_3R mRNA-expressing neurons appeared at E18. Scattered labeled migrating neuronblasts were found to leave the junction between striatal and cortical ventricular zones, join the lateral cortical stream, and accumulate in the reservoir (Fig. 5*A,E*), a region in which differentiated neurons sojourn before migrating to piriform and primary olfactory cortice and as yet unidentified areas of the basal telencephalon (Bayer and Altman, 1991, 1995). A labeled cluster of settling Purkinje cells also was seen in the caudal part of the cerebellar anlage (Fig. 4*A*). These migrating Purkinje cells probably correspond to those destined to settle in cerebellar cortex in lobule 10 rather than in lobule 9, in agreement with the caudorostral cytogenetic gradient observed in the neurogenesis of Purkinje cells (Altman and Bayer, 1985).

The distribution of D_3R mRNA was compared with that of D_1R and D_2R mRNAs on adjacent sections of the forebrain. At E18 (Fig. 5) the distributions of the three receptor subtypes were overlapping partially, but distinct. The three receptor mRNAs were found in the reservoir of the lateral cortical stream; this is the unique area in which we found coexpression of D_1R, D_2R, and D_3R mRNAs. D_1R and, to a lesser, extent D_3R, but not D_2R, mRNAs were expressed in the cortical neuroepithelium. In the striatal primordium D_1R, D_2R, and D_3R mRNAs were expressed in distinct compartments: the neuroepithelium expressed exclusively D_3R mRNA; the medial subventricular zone expressed almost exclusively the D_2R mRNA (very scarce D_3R-expressing cells were, however, found in this area); differentiating fields of striatum, nucleus accumbens, and rhinencephalon expressed D_1R and D_2R, but not D_3R, mRNAs (Fig. 5).

At E19 (Fig. 6) the D_3R mRNA was still prominent in the

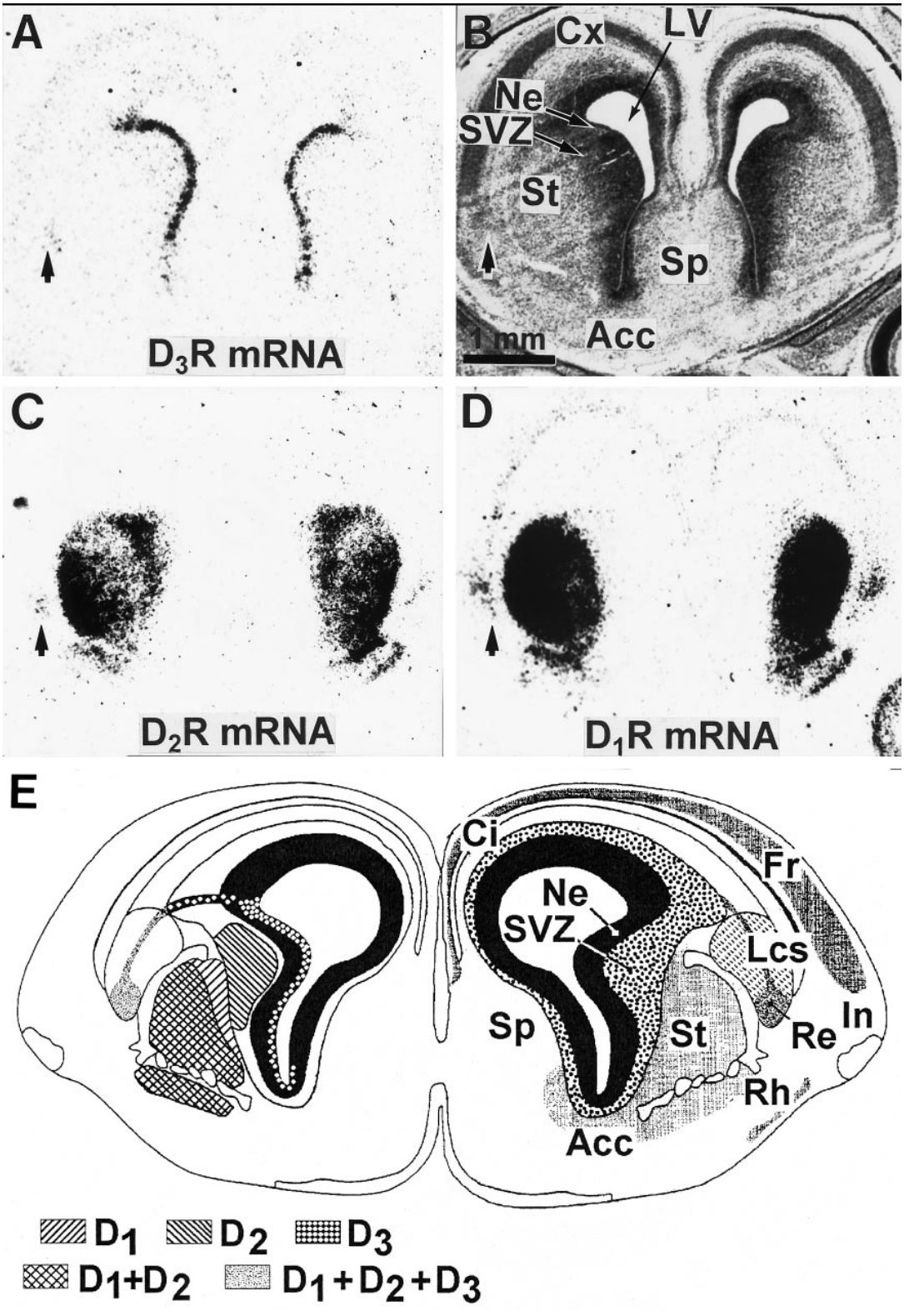

Figure 5. Compared expressions of D_1R, D_2R, and D_3R mRNAs in the forebrain of the 18 d embryo. Shown are film autoradiograms of adjacent coronal sections at a rostral level of the developing striatum, hybridized with probes specific for either D_3R (*A*), D_2R (*C*), or D_1R (*D*) mRNAs. *B*, Bright-field photomicrograph of section *A*, counterstained with the Mayer's hemalum. Cells in the reservoir of lateral cortical stream (indicated by *arrows*) express the three receptor subtype mRNAs (*A, C*, *D*). The cortical neuroepithelium and cortical plate (*Cx*) are labeled by the D_1R and D_3R, but not D_2R, mRNA probes. D_1R, D_2R, and D_3R mRNAs are expressed in different striatal compartments, as shown in *E*, which represents a schematic coronal section at the same level and developmental stage, taken from the atlas by Bayer and Altman (1995) in which the right cerebral hemisphere displays the structures (*Acc*, nucleus accumbens; *Ci*, cingulate cortex; *Fr*, frontal cortex; *In*, insulate cortex; *Lcs*, lateral cortical stream; *Re*, reservoir; *Rh*, rhinencephalon; *Sp*, septum; *St*, striatum) and the left hemisphere displays the respective distributions of D_1R, D_2R, and D_3R mRNAs. In the striatum D_1R mRNA is present in the differentiation field (*St*), D_2R mRNA in the subventricular zone (*SVZ*) and in the differentiation field, and D_3R mRNA in the neuroepithelium (*Ne*).

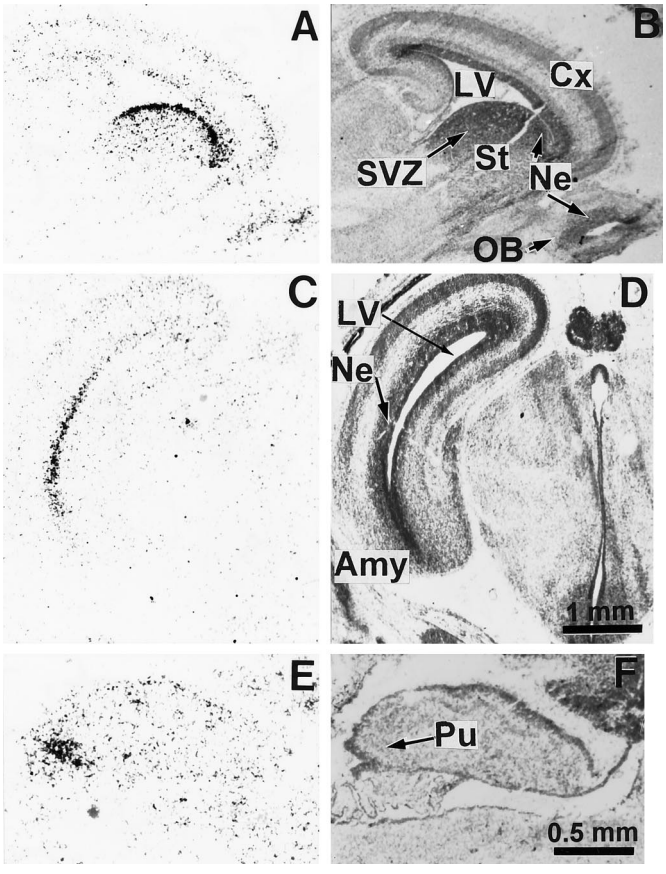

Figure 6. Expression of D_3R mRNA in the brain of the 19 d embryo. The *left panel* shows film autoradiograms from parasagittal (*A*, *E*) and coronal (*C*) sections hybridized with the D_3R mRNA probe. The *right panels* show corresponding sections counterstained with Mayer's hemalum and photographed in bright-field illumination. Hybridization signals are detected at high level in several periventricular neuroepithelial regions (*Ne*) of the basal forebrain, including the striatum (*St* in *A*), amygdala (*Amy* in *C*), and in a group of Purkinje cells (*Pu* in *E*). Low expression of D_3R is seen in the ventricular zone of the cortex and cortical plate (*Cx*), olfactory bulb (*OB*), and subventricular zone (*SVZ*) of the striatum in *A*. *LV*, Lateral ventricle.

neuroepithelium, extending from rostral striatum to amygdala. The neuroepithelium of olfactory bulb also was labeled. At this stage labeling appeared as a band becoming thinner than previously, because the cortical ventricular zone of the neuroepithelium also had become thinner (Bayer and Altman, 1991). Labeling in Purkinje cell plate also appeared stronger and more extended.

Postnatal development (P0–P5)

At birth the D_3R mRNA distribution still resembled that of late embryos, with only a few distinct characteristics found in adults (Fig. 7). The striatal neuroepithelium still was labeled heavily, although to a lesser level than in embryos. Labeling of subventricular zones of striatum and nucleus accumbens became stronger but did not appear in differentiating fields (Fig. 7*A,C*). Strong labeling also appeared in medial mamillary body (Fig. 7*A*), a region of high D_3R expression in adults (Bouthenet et al., 1991). Also as in adults, labeled Purkinje cells were found within the cerebellum, in the entire lobule 10, and as patches in lobule 9 (Fig. 7*A,E–H*).

More prominent changes occurred between P0 and P5. At this latter stage the distribution was already almost identical to that in the adult brain, with a few exceptions. Labeling along the striatal subventricular zone got thinner and was distributed as patches (Figs. 8*D*, 9*A*). In adults D_3R-expressing cells also were found in the striatal subventricular (also referred to as subependymal) zone (Fig. 9*C,D*); a labeling of ependymal cells could not be excluded. At P5 major localizations found in adults were settled, as in lobules 9 and 10 of cerebellum, medial mamillary body, and ventral striatum (Fig. 8*A*). In the ventral striatum, although labeling was identical at P5 and in adults in the shell part of nucleus accumbens and in the islands of Calleja, there was D_3R mRNA in the core of nucleus accumbens and in the olfactory tubercle (Fig. 8*C,D*), which were strongly reduced in adults (Diaz et al., 1995). A moderate labeling was detected at P5 in the parietofrontal cortex (Fig. 8*A,C,D*), which remained in adults as a laminated band of scarce positive cells in layer IV (data not shown).

DISCUSSION

We detected the first appearance of D_3R mRNA at E14, in agreement with results obtained by PCR (Cadoret et al., 1993), and autoradiography by using the highly sensitive D_2R/D_3R radioligand [^{125}I]iodosulpride (Sales et al., 1988). Our results suggest that D_3R mRNA is more abundant during pre- and early postnatal ontogeny than in adult. The striatal neuroepithelium expresses strong signals from E14, culminating at E18, and progressively declines after birth. These signals seem to be of similar amplitude to those generated by a D_2R mRNA probe, although the D_2R is one to two orders of magnitude more abundant than the D_3R in adult brain (Lévesque et al., 1992). The high abundance of D_3R protein in the striatal neuroepithelium also is indicated by the strongest [^{125}I]iodosulpride binding in this region, which exceeds that of D_2R binding in differentiating striatum (Sales et al., 1988). Moreover, labeled neuroepithelium extends through almost the whole basal forebrain, whereas the D_3R mRNA is expressed in the derived structures at a very low level and/or in discrete areas. In addition, transient expressions in neocortical and paleocortical regions, medial mamillary body, and the core part of nucleus accumbens were found in newborns.

Comparisons among the expression patterns of the D_1R, D_2R, and D_3R mRNAs revealed marked differences, suggesting distinct expression regulation and roles (Table 2). Whereas the D_3R mRNA is expressed almost exclusively in the proliferative neuroepithelium during prenatal ontogeny, D_1R and D_2R mRNAs are expressed only transiently and sparingly in this structure and appear mostly in differentiating neurons, before dopamine innervation (Table 2). In addition, there is no overlap of D_1R, D_2R, and D_3R mRNAs, except in the reservoir of the lateral cortical stream. In many instances the distribution of D_3R mRNA markedly differs during prenatal and postnatal developments. According to the fate of D_3R mRNA-expressing cells, three distinct patterns can be distinguished.

The *first and most documented* pattern corresponds to D_3R mRNA-expressing neuroepithelial cells, generating neurons in which this marker thereafter becomes undetectable. Thus, prenatal D_3R mRNA is confined almost entirely in neuroepithelial cells of striatum, amygdala, olfactory bulb, and tectum, most of the progeny of which rapidly lose the capacity to transcribe the D_3R gene during migration and differentiation. D_3R mRNA labeling in the striatal neuroepithelium overlaps the synthetic and mitotic zones, which are restricted to a thin layer close to the ventricle at early stages (E14–E16) but which spread in the subventricular zone at later stages (Bayer and Altman, 1995). At birth and during postnatal development the synthetic zone gets thinner along the lateral ventricle, just as the D_3R mRNA labeling does. Addition-

Table 2. Comparison among appearances of D_1R, D_2R, and D_3R mRNAs, [^{125}I]iodosulpride binding, and dopamine immunoreactivity

Structures	Figure numbers[a]	Dopamine receptor mRNA			[^{125}I]IS binding[b]	DA-IR[c]
		D_1R[d]	D_2R[d]	D_3R		
Olfactory bulb (Ne)	6*A*	E18	0	E16→P0	E17	nd
Rhinencephalon (Ne)	2*C*, 3*C*, 4*C*	0	0	E14	E15	0
Islands of Calleja	8*A,C,D*	nd	nd	[P0–P5]	nd	E19
Septum (Ne)	2*C*, 3*C*, 5*A*, 7*C*	0	0	E16	E15	0
Septum (DF)	8*C*	E18	nd	[P0–P5][h]	[P0–P7]	[P0–P5]
Nucleus accumbens						
Ne	4*A*, 5*A*, 7*C*	0	0	E18	E20	0
SVZ	7*C*, 8*D*, 9*D*	0	0	E19	nd	0
DF (shell)	8*C,D*	E18[e]	E18[e]	Pg5[f]	P0	P2
DF (core)	8*C,D*	E18	E18	[P0–P5][h]	E19	E19[e]
Striatum						
Ne	1–7	E14→E17[g]	E14→E17[g]	E14	E14	E18[g]
SVZ	6*A*, 7*A,B*, 8*D*, 9*A*	E14	E14	E19[g]	E14	E16[g]
DF	8*C*,9*D*	E17	E16	[P0–P5][f]	E15	E14
Pallidum (Ne)	2*E*	0	0	0	0	0
Amygdala (Ne)	2*G*, 6*C*	nd	nd	E16	0	0
Olfactory tubercle	8*C,D*	E18	E18	[P0–P5]	E18	E21
Lateral cortical stream (Re)	5	nd	nd	E18	nd	nd
Cortex (Ne)	2*E*, 6*A,C*, 7*C*	E14→E17	0	E16[g]	E13	0
Cortical plate	6*A,C*	E16	E18	E16[g]	E16	E17
Fronto-parietal cortex (DF)	8*C,D*	E18	E18	[P0–P5][h]	P7	P17
Median mammillary bodies	7*A*, 8*A*	nd	nd	P0	P14	nd
Tectum (Ne)	1*A*, 2*A*	E14	0	E14→E18	P5	0
Cerebellum lobule 9	4*A*, 6*E*, 7*A,E,* 8*A*	0	0	E18	E20	0
lobule 10	7*A*, 8*A*	0	0	[E19–P0]	E20	0

DF, Differentiation field; Ne, neuroepithelium; Re, reservoir; SVZ, subventricular zone; E→E or E→P, transient expression; [E-E or P-P], the exact time of appearance could not be determined; 0, undetectable; nd, not determined from available data. [a]Figure number in the present study illustrating the presence or absence of D_3R mRNA; [b][^{125}I]iodosulpride binding, from Sales et al. (1988); [c]Dopamine immunoreactivity, from Voorn et al. (1988) and Kalsbeek et al. (1992); [d]from Guennoun and Bloch (1992), Schambra et al. (1994), and present study; [e]lateral part; [f]medial part; [g]very sparse labeling; [h]labeling very sparse in adult—the exact date of disappearance is not known.

ally, cells continue to proliferate in the subependymal zone, corresponding to a remnant of neuroepithelium (Privat and Leblond, 1972; Jacobson, 1991), in which D_3R mRNA occurs in adults.

The restricted expression of D_3R mRNA in the neuroepithelium may be linked to the cell proliferative activity via other negative regulation by factors arresting cell division and triggering cell differentiation or may be linked to positive regulation by transcription factors (Arenander and De Vellis, 1994). In this latter respect, it is noteworthy that expressions of *Dlx-2* and *Mash-1* homeobox genes (Porteus et al., 1991, 1994; Robinson et al., 1991), candidates for regulating patterning and differentiation of the forebrain, match, to some extent, D_3R mRNA distribution. Alternatively, D_3R gene expression may be activated by unknown factors, either released from neuroepithelium or circulating in the lateral ventricle and poorly diffusing through the parenchyma.

Both microheterogeneity and compartmentation are observed in the distribution of D_3R gene transcripts within the neuroepithelium. Instead of forming a continuous sheet of neuroepithelial cells, D_3R mRNA-expressing cells, rather, are organized in clusters evident throughout the entire development. Similar alternation of [^{3}H]thymidine-labeled neuroepithelial cells has suggested different migration kinetic properties among these cells (Altman and Bayer, 1990). Distinct properties among neuroepithelial cells also may be related to their distinct fate. Lineage studies with retroviral markers suggest that certain germinal cells become restricted only to generating neurons (Luskin et al., 1988). Most adult medium-sized striatal neurons are distributed among dynorphin/substance P and D_1R-expressing neurons and enkephalin and D_2R-expressing neurons (Gerfen et al., 1990; Curran and Watson, 1995; Le Moine and Bloch, 1995). Adult striatal neurons also belong to either striosomes or matrix, making different outputs (Graybiel, 1984). Birth-dating studies have shown that striosome cells become postmitotic at a distinctly earlier time than matrix cells (Van der Kooy and Fishell, 1987). In adults, whereas colocalization of D_2R and D_3R seems exceptional if it exists (Bouthenet et al., 1991; Diaz et al., 1994), there are examples of D_1R and D_3R colocalization (Le Moine and Bloch, 1996; Bordet et al., 1997) (S. Ridray, N. Griffon, E. Souil, V. Mignon, S. Carboni, J. Diaz, J.-C. Schwartz, P. Sokoloff, unpublished observation) suggesting that D_3R mRNA-expressing cells in the neuroepithelium may correspond to a distinct subpopulation.

In addition, the continuous distribution of D_3R mRNA within the basal forebrain neuroepithelium is interrupted remarkably at the level of the pallidal neuroepithelium while persisting at more ventral or caudal levels. It is noteworthy that cell fate is distinct in the striatal and pallidal neuroepithelium, the latter presumably giving rise mostly to pallidal cholinergic magnocellular cells in no or only very low levels of D_3R mRNA expressed in adults (Diaz et al., 1995).

The *second and less documented* pattern corresponds to cells that express D_3R mRNA in both the neuroepithelium and its progeny. D_3R mRNA is abundant in the neuroepithelium of nucleus accumbens at its appearance on E18 and in the shell subdivision after the appearance of the secondary germinal matrix

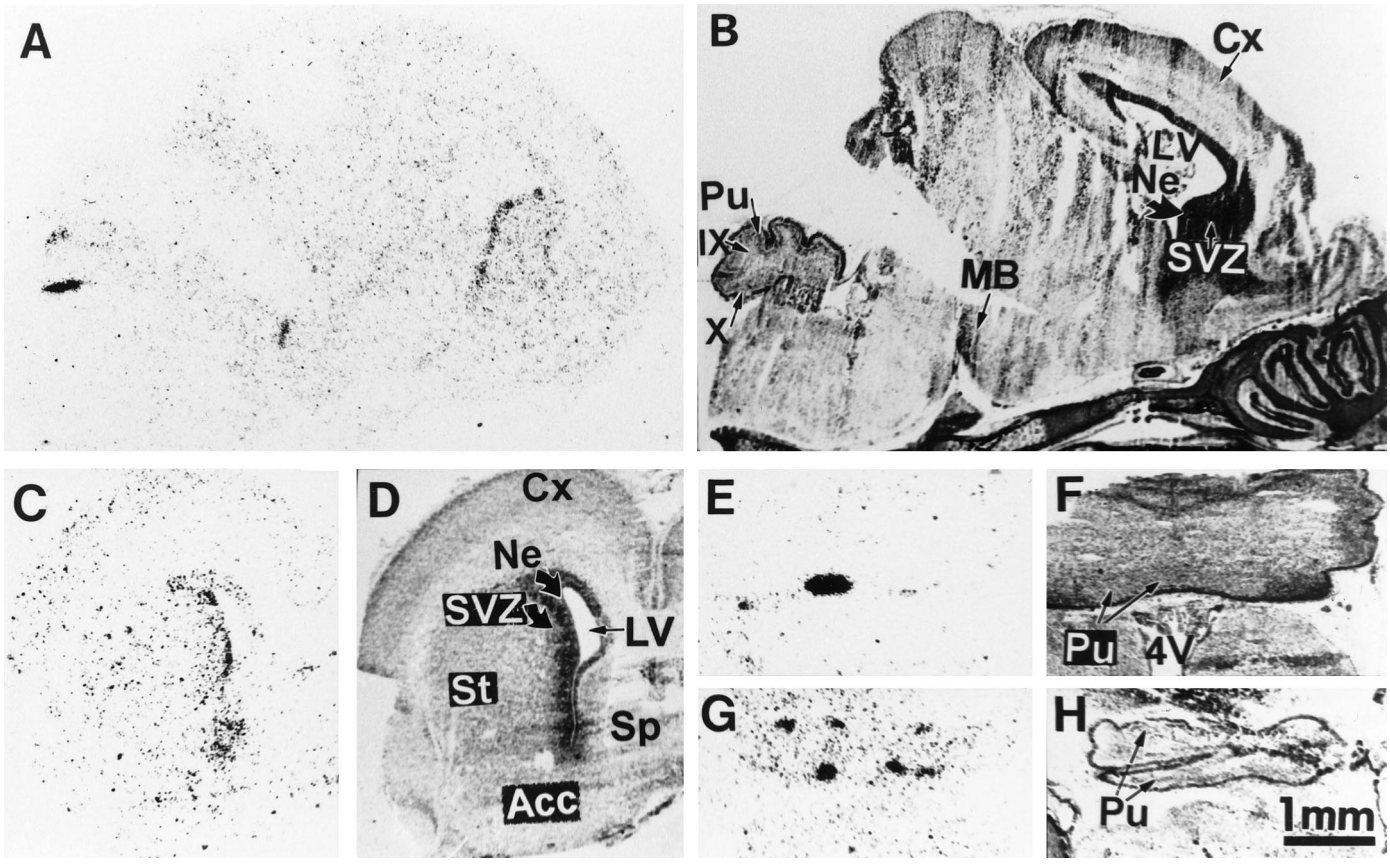

Figure 7. *In situ* hybridization of D_3R mRNA in the brain of a newborn pup. *A*, *C*, *E*, and *G* are autoradiograms of parasagittal (*A*) and coronal (*C*, *E*, *G*) sections hybridized with the D_3R probe; *B*, *D*, *F*, and *H* are corresponding sections counterstained with Mayer's hemalum. D_3R mRNA is seen in the residual neuroepithelium (*Ne*) and the adjacent subventricular zone (*SVZ*) of the striatum (*St*), nucleus accumbens (*Acc*), and septum (*Sp*) as well as in the medial mamillary body (*MB*) and as patches in the Purkinje cell layer (*Pu*) of lobules 9 (*IX*) and 10 (*X*) in the cerebellar cortex. *Cx*, Cerebral cortex; *LV*, lateral ventricle; *4V*, fourth ventricle.

(at P5; see Table 2). Our data indicate that the expression of D_3R mRNA is not continuous during cell differentiation and migration from the neuroepithelium but, rather, is triggered after the settling of dopamine innervation, which might exert a dual trophic influence. This is consistent with our hypothesis that regulation of D_3R gene expression in the shell of nucleus accumbens is under the positive influence of (1) a trophic factor, synthesized by dopamine neurons, distinct from dopamine and its cotransmitters neurotensin and cholecystokinin, and conveyed by axonal transport, the release of which seems dependent on dopamine neuron activity (Lévesque et al., 1995); (2) activation of the D_1R by dopamine or agonists (Bordet et al., 1997).

Nevertheless, a continuous expression of D_3R mRNA occurs in cells originating approximately from the junction between striatal and cortical neuroepithelia, migrating through the lateral cortical stream, and sojourning in the reservoir (Bayer and Altman, 1995). Some cells arising from the cortical neuroepithelium differentiate in the parietofrontal cortex, which transiently expresses high levels of D_3R mRNA (present study) and binding (Demotes-Mainard et al., 1996), and these cells remain in adult rats in layer IV. D_3R mRNA also persists in human parietal cortex, where it could be detected by PCR in brain from normal subjects but not from psychiatric patients who may express an abnormal shortened transcript (Schmauss et al., 1993).

The *third* pattern corresponds to D_3R mRNA-expressing neurons that differentiate from a neuroepithelium in which this marker is undetectable. D_3R mRNA-expressing Purkinje cells, destined to settle in the archicerebellum (lobules 9 and 10) after birth (Bouthenet et al., 1991; Diaz et al., 1995), occur from their differentiation at E18, but not in Purkinje cell progenitors. A strong labeling also appears at P0 in the medial mamillary bodies, which derive from unlabeled hypothalamic neuroepithelium. Thus, in these structures, expression of the D_3R seems to follow a more classical pattern, being triggered after the neuronal differentiation, possibly in relation to a functional specialization.

A specific role of the D_3R in neurogenesis, but not in morphogenesis, can be inferred from its highest level of expression in prenatal ontogeny, as compared with adulthood and its selective localization in the proliferative zone of the neuroepithelium during ontogeny. Such a role is supported by the mitogenic response induced by recombinant D_3R stimulation in transfected cells (Chio et al., 1994; Pilon et al., 1994). However, the origin of dopamine available for D_3R stimulation is unclear in the neuroepithelium, where no dopamine fibers are present. One study (Specht et al., 1978) reported a few cells positive for tyrosine hydroxylase immunoreactivity at E12.5–E15 at the surface of striatal neuroepithelium. Nevertheless, there is no indication that these cells indeed produce dopamine. Another possibility is that the D_3R receives dopamine diffusing through the cerebrospinal fluid from dopamine neurons in diencephalon and mesencephalon. Dopamine has a higher affinity at the D_3R than at any other receptor subtype, allowing this receptor to respond to nanomolar

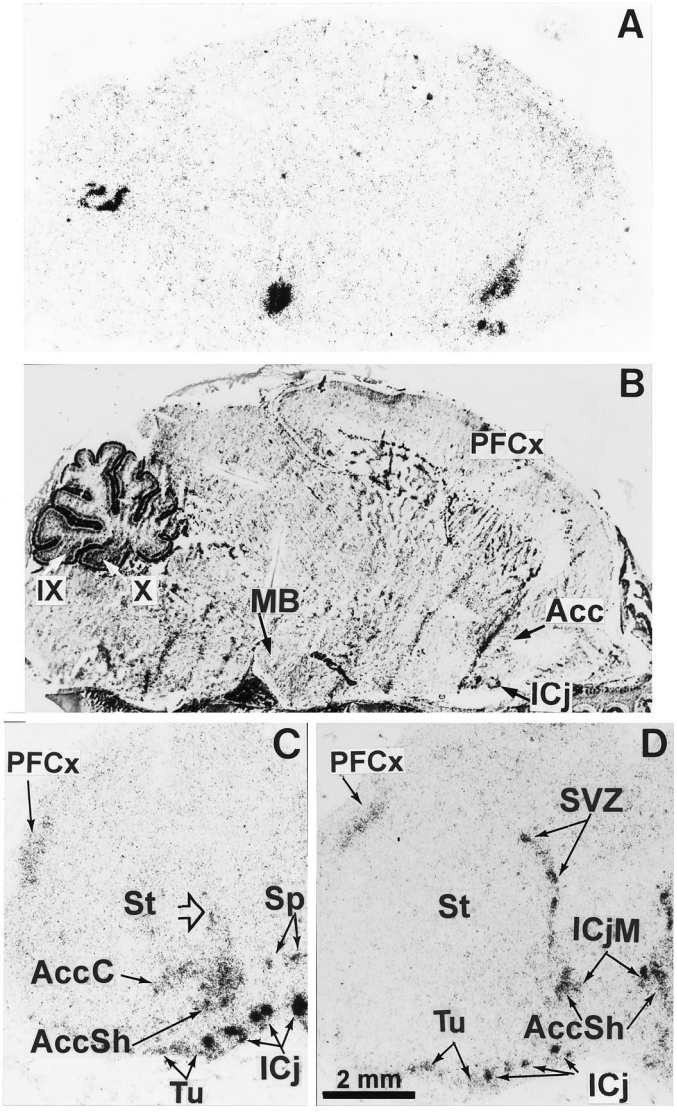

Figure 8. Expression of D_3R mRNA in the brain of the 5 d pup. Shown are autoradiograms of parasagittal (*A*) and coronal [rostral (*C*) and caudal (*D*) level of nucleus accumbens] sections hybridized with the D_3R probe. *B* corresponds to section *A*, counterstained with Mayer's hemalum. High expression of transcripts is observed in the major (*ICjM*) and minor (*ICj*) islands of Calleja, nucleus accumbens shell (*AccSh*), medial mamillary body (*MB*), patches in the subventricular zone (*SVZ*) of striatum (*St*), and Purkinje cell layer of the cerebellar lobules 9 (*IX*) and 10 (*X*), whereas moderate signals are found in the core of nucleus accumbens (*AccC*), ventromedial region of the striatum (*arrowhead*), septum (*Sp*), parietofrontal cortex (*PFCx*), and olfactory tubercle (*Tu*).

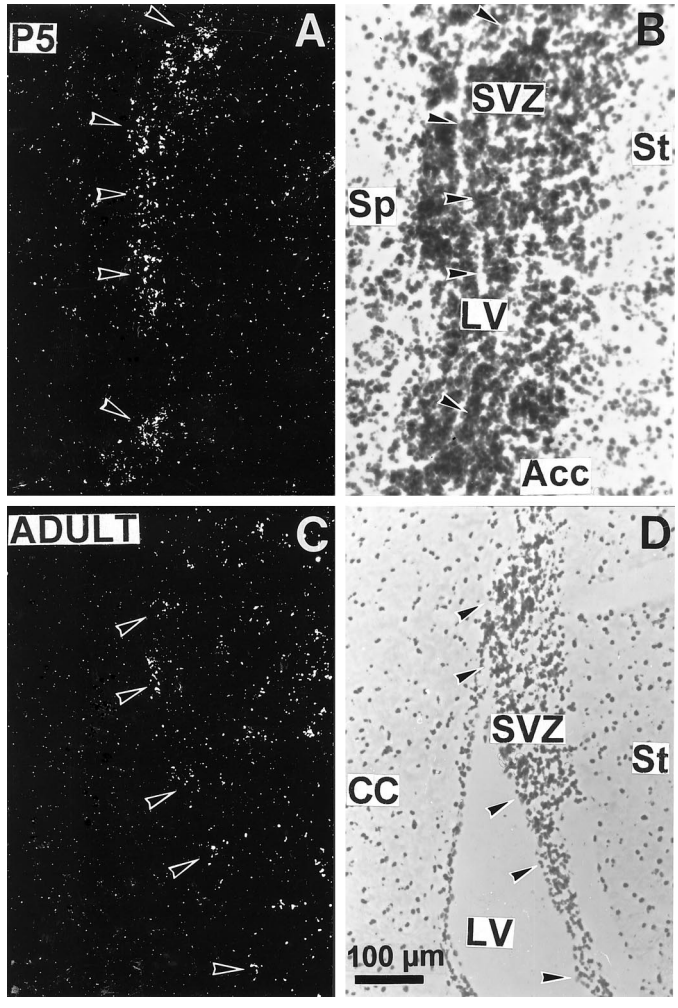

Figure 9. Expression of D_3R gene transcripts in the subventricular zone of striatum in brains from a 5 d pup (*A*, *B*) or adult (*C*, *D*). Photomicrographs under dark-field illumination (*A*, *C*) show labeling in the striatal subventricular zone (*SVZ*) lining the lateral ventricle (*LV*), as indicated by *arrowheads. B*, *D*, Corresponding sections counterstained with Mayer's hemalum. *Acc*, Nucleus accumbens; *CC*, corpus callosum; *Sp*, septum; *St*, striatum.

dopamine concentrations. In adult rat brain examples of D_3R localizations at some distance from dopaminergic fibers have suggested nonsynaptic actions of diffusing dopamine through this receptor (Diaz et al., 1995). Finally, a constitutive, i.e., agonist-independent, activity of the D_3R has been suggested from studies that use transfected cells (Griffon et al., 1996b).

Schizophrenia, sometimes assumed to result from a neurodevelopmental disorder, is marked namely by neuroanatomical abnormalities such as ventricle enlargement (Weinberger, 1987; Waddington, 1993), possibly related to a defect in neuroepithelium proliferation. A role for the D_3R in this pathological process is supported by some (but not all) genetic studies (Crocq et al., 1992; Nimgaonkar et al., 1993; Mant et al., 1994; Griffon et al., 1996a).

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