A. E. BENNETT AWARD PAPER

Expression of the Dopamine D$_2$ Receptor Gene in Brain*

James H. Meador-Woodruff, and Alfred Mansour

The cloning of the dopamine (DA) D$_2$ receptor now permits the characterization and regulation of D$_2$ messenger RNA (mRNA) in the brain. In this article, the authors describe their studies delineating the distribution of D$_2$ receptor mRNA in the rodent and primate brain, and compare the distribution of message to D$_2$ receptor binding sites. The effects of chronic DA agonist and antagonist treatment on D$_2$ receptor mRNA are also presented, and provide insights into receptor regulation. Finally, the autoreceptor role of D$_2$ receptors located in the midbrain is examined with a combination of 6-hydroxydopamine lesions and anatomic colocalization studies with tyrosine hydroxylase. These preclinical results provide a framework for subsequent investigation into the nature of D$_2$ receptor gene expression in postmortem brains from patients with disorders putatively associated with dopaminergic dysfunction, especially schizophrenia. They also lay the groundwork for a more profound understanding of DA neurocircuitry by combining molecular biological and traditional anatomical techniques.

Introduction

Dopamine (DA) is the neurotransmitter most commonly associated with the pathogenesis and symptoms of schizophrenia (Creese et al 1976; Seeman et al 1976; Snyder 1976). Anatomically, the DA system is well suited for the regulation of affect and behavior, with midbrain DA cell groups (the substantia nigra and the ventral tegmental area [VTA]) projecting to a number of forebrain structures, most notably the basal ganglia, many elements of the limbic system, including the septum, hippocampus, amygdala, nucleus accumbens, and limbic cortex, as well as to much of the neocortex (Cooper et al 1986). Dysregulation of these projections, especially the mesocorticolimbic system originating in the VTA, has been implicated in this illness. In its original form, the so-called dopamine hypothesis of schizophrenia postulated that there was excessive dopaminergic tone in these projections, associated with the symptoms seen in the disease (Creese et al 1976; Seeman et al 1976; Snyder 1976). Subsequent refinements have been made to this hypothesis, postulating a diminished tone of these projections, especially to the dorsolateral prefrontal cortex, resulting in relative hyperactivity in subcortical structures secondary to

altered regulation of descending cortical–subcortical pathways (Berman et al 1988; Weinberger et al 1988). Despite the existence of these various permutations of the DA hypothesis, the nature of the dopaminergic lesion in schizophrenia has yet to be identified.

Direct studies of DA functioning in schizophrenia have been inconclusive at best. Early attempts were designed to examine levels of circulating growth hormone and prolactin in patients with this illness, both at rest and following physiological challenge (Meltzer et al 1974; Johnstone et al 1977; Brown and Laughren 1981; Kleinman et al 1982; Rinieris et al 1985; Ettigi et al 1976; Tamminga et al 1977; Rotrosen et al 1979). These hormones are both regulated by DA via the tuberhypophyseal pathway, and the strategy in studying them has been to see if there might be a global dysfunction of all brain DA systems, possibly reflected in the dysregulation of the pituitary system and being manifested in circulating levels of these hormones. The results of these studies have been contradictory, and have never clearly established a dysfunction of this diencephalic DA system in schizophrenia. The major flaw of these studies is that only the mesencephalic systems may be dysfunctional in schizophrenia, with the others remaining intact. Thus, the regulation of prolactin and growth hormone may be normal in schizophrenia, while the mesocorticolimbic and/or nigrostriatal projections may be dysfunctional.

Other indexes of dopaminergic activity have also been measured to further bolster the dopamine hypothesis. These include the measurement of dopamine itself, its metabolites, and DA receptors in postmortem brains from schizophrenics. Here, again, findings have been inconsistent, and may be related to a previous history of neuroleptic exposure. Dopamine itself has been reported to be increased or unchanged in the caudate, putamen, and the nucleus accumbens. Several DA metabolites have also been studied in these structures; dihydroxyphenylacetic acid (DOPAC) is usually reported to be unchanged, but homovanillic acid (HVA) has on occasion been reported to be elevated, perhaps reflecting an increased rate of DA turnover (recently reviewed by Jaskiw and Kleinman 1989).

Much effort has been focused on the DA receptors in schizophrenia, especially the D_2 receptor, because of the good correlation between D_2 receptor binding and the pharmacological efficacy of the neuroleptic drugs (Creese et al 1976; Seeman et al 1976). Although an occasional study reports no changes in D_2 receptor number in the basal ganglia, the overwhelming majority of reports demonstrate an increase in the number of this receptor type in these structures (Seeman et al 1987; Lee and Seeman 1980; Cross et al 1981; Mackay et al 1982; Seeman et al 1984). This finding, however, may be related to previous neuroleptic history, since chronic exposure to antipsychotic medications is likely to cause an upregulation of these receptors (Burt et al 1977; Chipkin et al 1987; MacLennon et al 1988). More recently, efforts have been made to study D_2 receptor density in vivo, in living patients using positron emission tomography. Conflicting results have been reported here as well, with one group (Wong et al 1986) indicating that the number of D_2 receptors is increased in the basal ganglia of drug-free schizophrenics and several other groups (Martinot et al 1990; Farde et al 1990) reporting no change in this marker. These exciting results will need to be replicated, but this technique at least provides one method of circumventing the problem of previous neuroleptic exposure. Unfortunately, the level of resolution possible with these in vivo studies is very poor; hence the continuing need for well-designed postmortem studies.

Recent advances in molecular biology now permit a more detailed examination of the DA systems in brain. The DA receptors have been recently cloned and investigations related to the distribution and regulation of the messenger RNA (mRNA) encoding these

receptors are now possible. The cloning of the rat DA D_2 receptor was originally reported by Bunzow and coworkers (1988). Using the hamster β_2-adrenergic receptor gene as a probe, a partial D_2 receptor clone was isolated from a rat genomic library. This gene fragment was subsequently used as a probe to identify a full-length rat brain cDNA clone. This D_2 receptor cDNA was cloned into a eucaryotic expression vector and the resulting construction used to transfect a mouse fibroblast cell line, and the resulting pharmacological profile of the expressed receptor was characteristic of a D_2 receptor. This, combined with mRNA distribution results from Northern blot analysis, and the conservation of key residues found in all catecholamine receptors for which the sequence is known, strongly supported the identification of the Bunzow clone as encoding the D_2 DA receptor.

Shortly after the original cloning of the rat D_2 receptor, a number of groups reported that instead of one, there are at least two distinct forms of D_2 receptor mRNA, and presumably at least two different translation products (Monsma et al 1989; Dal Toso et al 1989; Giros et al 1989; Selbie et al 1989). The D_2 receptor gene was found to be considerably different from other catecholamine receptor genes that had been identified to date. The D_2 receptor gene contains a number of introns and a particularly long third cytosolic loop (Dal Toso et al 1989; Grandy et al 1989), quite unlike the β_2-adrenergic and other similar seven transmembrane domain receptor genes, which contain no introns in their coding regions, and have a much shorter third cytosolic loop (Emorine et al 1987; O'Dowd et al 1989). The D_1 receptor gene, cloned more recently, is similar to the β_2-adrenergic receptor gene in these respects (Dearry et al 1990; Zhou et al 1990; Sunahara et al 1990; Monsma et al 1990).

The presence of introns within the D_2 receptor gene suggests the possibility of variants of the receptor due to alternate splicing of the gene during transcription. The two different forms of the D_2 receptor that have been identified thus far are in fact the result of different message processing. The original clone that was reported by Bunzow et al (1988) was 87 bases (29 amino acids) shorter than the second form that was subsequently identified; the difference between these two forms is due to the inclusion or exclusion of exon 5 in the final transcripts. The current convention is that the originally cloned, shorter cDNA, is identified as the $D_{2\alpha}$ receptor, and the longer message, which includes the 87 base pair region encoded by exon 5, is termed the $D_{2\beta}$ receptor.

We have been actively involved in the characterization of the mRNA encoding the DA D_2 receptor(s) within the anatomic context of the rodent and primate brain using in situ hybridization techniques. In this article, we describe some of our findings in this area, including the mapping of the distributions of D_2 receptor mRNAs in brain, the comparison of the distribution of message with D_2 receptor binding, and selected regulatory studies. These results lay the framework for subsequent investigations of the nature of D_2 receptor gene expression in postmortem brains derived from individuals who had suffered from schizophrenia. Although much of the data contained in this report is unpublished, in the interest of chronological completeness, we have reviewed some of our previously reported data (Meador-Woodruff et al 1989; Mansour et al 1990a).

Materials and Methods

Animal Preparation

For mapping studies (in situ hybridization and receptor autoradiography), brains of male Sprague-Dawley rats (250 g) that had been killed by decapitation were rapidly removed. Brains of old-world monkeys (*Macaca mulatta*) were similarly removed following ke-

tamine anesthesia and cut into 1–2-cm coronal slabs. Whole rat brain and monkey tissue were frozen in isopentane (– 30°C) for 30 or 120 sec, respectively. Frozen tissue was cryostat-sectioned (15–20 μm) and thaw-mounted onto polylysine-subbed microscope slides. These sections were maintained at – 80°C until the time of hybridization or receptor autoradiography.

For studies of pharmacological regulation of D_2 receptor mRNA, male Sprague–Dawley rats (250–350 g) were treated with either haloperidol or apomorphine (6 animals per group). Haloperidol was administered subcutaneously at a dose of 2.0 mg/kg/day for a total of 14 days. Apomorphine was given subcutaneously twice daily at a dose of 5.0 mg/kg/dose for 7 days. For each drug, a vehicle-treated control group ($n = 6$) was employed. The brains of these animals were removed and processed as described above.

For studies involving immunohistochemistry in conjunction with in situ hybridization, male Sprague–Dawley rats (250–350 g) were anesthetized with sodium pentobarbitol (50 mg/kg, intraperitoneally), and their cardiovascular systems were flushed with 50 ml of ice-cold 0.9% saline. Following the removal of red blood cells, animals were perfused via the aorta with 0.1 mol/L phosphate-buffered 4% formaldehyde (pH 7.4) for 10 min. The brains were then removed and blocked, and refrigerated overnight in phosphate-buffered 15% sucrose, and then frozen in liquid nitrogen (– 70°C). Five-micron brain sections containing the substantia nigra and VTA were thaw-mounted on subbed microscope slides and further processed for immunohistochemistry and in situ hybridization.

For animals given 6-hydroxydopamine (6-OHDA) lesions, male Sprague–Dawley rats ($n = 9$) were given a unilateral infusion of 6-OHDA into the medial forebrain bundle (8 μg in 4 μl) after pretreatment with 25 mg/kg desmethylimipramine (Mansour et al 1990b). These animals were killed 14 days later, and their brains removed and processed as described above for the mapping studies.

Tissue samples for RNA protection assays were conservatively dissected from the brains of male Sprague–Dawley rats that had been killed by decapitation. Pituitaries and dissected striatum were removed on wet ice, and then immediately frozen on dry ice and stored at – 80°C until further processing.

In Situ Hybridization

In situ hybridization was performed using [^{35}S]-labeled riboprobes, or [^{32}P]- or [^{35}S]-labeled oligonucleotides. Specific assay details have been previously described (Meador-Woodruff et al 1989; Mansour et al 1990a). Brief details of each assay are given below.

The determination of total D_2 receptor mRNA was made using [^{35}S]-labeled riboprobes synthesized from a Sac I–Bgl II fragment of a rat D_2 receptor cDNA (Bunzow et al 1988). This probe is a 495 base region corresponding to the third cytosolic loop and the sixth and seventh transmembrane domains of the rat D_2 receptor. This region was selected because the greatest degree of dissimilarity between the catecholamine receptors occurs in the third cytosolic loop, thus providing a greater degree of probe specificity. This probe is 3' to the exon encoding the 29 amino acids that distinguish $D_{2\alpha}$ from $D_{2\beta}$ mRNAs, hence recognizing both forms, or total D_2 receptor mRNA. For rat studies, hybridization was in 75% formamide buffer at 55°C, with the final posthybridization wash at 55°C in water (60 min), as we have previously described (Meador-Woodruff et al 1989). For monkey experiments, the same in situ protocol was used, except hybridization took place in 50% formamide buffer, and the final posthybridization wash (60 min) was at 55°C in 0.5× SSC (300 mmol/L NaCl, 30 mmol/L sodium citrate, pH 7.2).

Messenger RNA encoding the longer isoform of the DA D$_2$ receptor (D$_{2\beta}$) was visualized using a fragment of a rat D$_2$ receptor cDNA, which had been subcloned into pGEM4. This plasmid generates a 78 base riboprobe that is completely contained within the 87 bases comprising exon 5 of the rat D$_2$ receptor gene, thus providing a probe that is D$_{2\beta}$-specific. Hybridization was performed in 75% formamide buffer at 55°C, with a final 60-min posthybridization wash in 0.5× SSC at 55°C.

The mRNA encoding the short isoform of the D$_2$ receptor (D$_{2\alpha}$) was visualized using a [^{35}S]-labeled oligonucleotide (AGCTCGGCGGGCAGCATCCTTGAGTGGTGTCTT-CAG). This 30 base probe is complementary to the 15 bases on either side of the 87 base insert of the longer isoform of the D$_2$ receptor. In the case of the longer form, this oligonucleotide will hybridize to 15 bases, but in the case of the shorter isoform, it will form a 30 base pair RNA–DNA hybrid. The hybridization conditions were designed to not allow visualization of a 15 base pair hybrid, but the longer 30 base hybrids would be retained (Wood et al 1984). Prehybridization of sections was performed as previously described (Meador-Woodruff et al 1989; Mansour et al 1990a), and hybridization was performed in 50% formamide buffer at 42°C overnight. Posthybridization washes were in 4× SSC (three washes of 10 min each, at 22°C), a 10-min, 22°C rinse in 3 mol/L TMACl buffer (3 mol/L tetramethylammonium chloride, 50 mmol/L Tris (pH 8.0), 2 mmol/L EDTA, 0.1% sodium dodecyl sulfate), followed by a 60-min wash at 65°C in the 3-mol/L TMACl buffer. After a final 10-min rinse in 4× SSC (22°C), slides were dried and processed as other in situ hybridization sections. The specificity of this assay was confirmed by generating RNAs either containing or not containing the 87 base insert, electrophoresing these RNAs on standard Northern gels, and transferring the RNA bands to Nytran membranes. These membranes were then treated with the above protocol, and at posthybridization washes above 50°–55°C, only D$_{2\alpha}$ (short isoform) mRNA was detectable.

Visualization of tyrosine hydroxylase (TH) mRNA was accomplished by hybridizing with a [^{32}P]-labeled oligonucleotide (CGGACGGGTCTCCGGGTGACCGTCCTTC). Hybridization was performed in a 50% formamide buffer at 45°C, and posthybridization washes were in 4× SSC (22°C) and 2× SSC (45°C, 60 min).

D$_2$ Receptor Autoradiography

D$_2$ receptor binding was visualized as previously reported (Mansour et al 1990a). Briefly, receptor autoradiography was performed using [^{3}H] raclopride (5.7 nmol/L), a selective D$_2$ antagonist, and labeled slides were apposed to tritium-sensitive Hyperfilm (Amersham) for 1–2 months to produce the images in this report.

RNase Protection Assay

Total RNA from frozen samples of striatum and pituitary was extracted using a modified guanidinium–isothiocyanate (GITC) method first described by Chirgwin et al (1979). Varying amounts of total RNA were hybridized with a 174 base pair [^{32}P]-labeled cRNA probe spanning the 87 base pair insert (exon 5) of the D$_{2\beta}$ isoform and extending an additional 87 base pairs in the 5' direction. Each hybridization reaction consisted of 30 μl: 6 μl 5× hybridization buffer (0.2 mol/L PIPES, pH 6.4, 2.0 mol/L NaCl, 5.0 mmol/L EDTA), 15 μl deionized formamide containing 25 fmol of cRNA probe, and 9 μl total RNA (3–48 μg) resuspended in water. Samples were denatured (85°C for 5 min)

and incubated overnight (60°C). Hybridization reactions were then cooled to room temperature and RNase digested (22°C, 30 min) with 200 μl buffer (10 mmol/L Tris–HCl, pH 7.5, 5 mmol/L EDTA, 0.2 mol/L NaCl, 0.1 mol/L LiCl) containing RNase A (10 μg/ml). Digestion was terminated with the addition of 10 μl 20% (w/v) sodium dodecyl sulfate and 10 μl proteinase K (10 mg/ml) at 37°C for 30 min. Samples were then phenol-chloroform extracted, and electrophoresed on a 8% polyacrylamide denaturing gel.

Tyrosine Hydroxylase Immunohistochemistry

Frozen sections of 4% formaldehyde-fixed brain were allowed to air dry (22°C) and incubated with normal goat serum (NGS, from GIBCO) at a dilution of 1/30 at 37°C for 10 min. NGS was then removed and the slides were incubated with a rabbit TH antibody diluted 1/1000 in 0.02-mol/L phosphate-buffered saline (PBS) and 0.3% Triton X-100 for 1 hr at 37°C, and overnight at 4°C. The following day, brain sections were washed in PBS and incubated with a goat-antirabbit serum (Arnel) at 1/1000 dilution for 30 min at 37°C, and overnight at 4°C. On the third day the slides were washed in PBS and incubated with anti–horseradish peroxidase serum diluted 1/1000 (37°C, 40 min). The brain sections were again rinsed in PBS and incubated at 37°C with horseradish peroxidase (HRP, 4 μg/ml) for 40 min. The HRP reaction product was visualized by treating the slides with 0.03% H_2O_2 and 0.125 mg/ml diaminobenzidine for 15 min at 22°C with constant stirring. The slides were washed in water, osmicated (2% OsO_4), dehydrated through graded alcohols and coverslipped. Sections were analyzed and photographed with a Leitz Orthoplan microscope.

Image Analysis

Quantitation of X-ray images was performed using a computer-based image analysis system, as previously described (Herman et al 1989). Total optical densities for regions of interest were determined. Optical density values were then converted to disintegrations per minute by interpolating values from a standard curve of [35S]-containing brain paste standards of known radioactivity.

Results

Localization of D_2 Receptor mRNA in Rat Brain

Using [35S]-labeled riboprobes corresponding to the third cytosolic loop and the sixth and seventh transmembrane domains of the rat D_2 receptor, we examined the distribution of this message using in situ hybridization (Meador-Woodruff et al 1989). This mRNA was found in all of the traditional dopaminoceptive regions of the brain, with particularly dense labeling in the caudate-putamen, nucleus accumbens, olfactory tubercle, and piriform cortex (right panels, Figures 1 and 2). Moderate labeling was observed in the hypothalamus and much of cortex, as well as a number of other limbic structures, including the lateral septum, hippocampus, and amygdala. The labeling in some brain regions was heterogeneous, and showed rostral–caudal and medial–lateral differences (Figure 1E).

In addition to these dopaminoceptive regions, high levels of this mRNA were observed in several of the DA-containing cell groups (Figures 1F and 2). Specifically, high levels were observed in the substantia nigra (pars compacta), VTA, and zona incerta. The presence of this mRNA in these structures strongly suggests autoreceptor synthesis.

Comparison of the Distributions of D$_2$ Receptor mRNA and Binding Sites

Using sequential adjacent sections through the rat brain, we next compared the distribution of D$_2$ receptor mRNA and D$_2$ binding sites (Figures 1 and 2). A high degree of concordance was observed between these distributions in a number of structures, including the caudate-putamen, globus pallidus, nucleus accumbens, olfactory tubercle, and the midbrain DA-containing cell groups, the substantia nigra and VTA. Several areas manifested dissimilar distributions; these included the olfactory bulb, hippocampus, and many regions of cortex (Mansour et al 1990a).

Localization of D$_2$ Receptor mRNA in Primate Brain

Following these studies in the rat, we began efforts to map this mRNA in the monkey brain. As our ultimate goal is to examine the expression of the DA receptor genes in postmortem schizophrenic brains, we felt that it was important to be able to visualize this particular message in primate neural tissue. Using the in situ hybridization protocol and the rat D$_2$ receptor probe described above, we mapped the distribution of this mRNA in the brain of the old world monkey, *Macaca mulatta* (Figure 3). As in the case of the rodent, high levels of expression were observed in the traditional dopaminoceptive regions, including the caudate and putamen, as well as in a number of limbic regions such as the amygdala, hippocampus, various regions of cortex, and the lateral septum. As in the case of the rat, the globus pallidus had considerably less message than the caudate or the putamen. D$_2$ receptor mRNA was also observed in the substantia nigra, as was seen in the rat, again suggesting autoreceptor synthesis.

Multiple Forms of the DA D$_2$ Receptor

With the identification of the D$_{2\alpha}$ and D$_{2\beta}$ variants, we next turned our attention to the possibility of mapping these two forms in the brain using in situ hybridization. Our original mapping efforts described above used riboprobes corresponding to exons 6 and 7, all of which is 3' to the exon responsible for the variability. Thus, our initial maps visualized *both* D$_{2\alpha}$ and D$_{2\beta}$ mRNAs.

To examine the relative concentrations and distribution of the D$_{2\alpha}$ and D$_{2\beta}$ forms, we first performed solution phase protection assays to determine if we could identify mRNA bands corresponding to these two forms (Figure 4). A probe capable of distinguishing both isoforms identified both D$_{2\alpha}$ and D$_{2\beta}$ in a number of brain regions. The longer isoform, D$_{2\beta}$, was considerably more abundant in all regions studied, consistent with the results of other groups (Monsma et al 1989; Dal Toso et al 1989; Giros et al 1989; Chio et al 1990; Rao et al 1990). Although present at a considerably lower abundance, D$_{2\alpha}$ was identifiable in every region as well, including dopaminergic and dopaminoceptive brain areas.

Following the demonstration of these two forms in tissue homogenates, we next attempted to demonstrate the presence of each form in brain using in situ hybridization. We first compared the distribution of total D$_2$ mRNA with that of the more abundant (longer) form, D$_{2\beta}$. Total D$_2$ mRNA was visualized using the 495 base riboprobe corresponding to exons 6 and 7, which code for the third cytosolic loop and the sixth and seventh transmembrane domains. The distribution of D$_{2\beta}$ mRNA was determined using the same in situ hybridization protocol, with a 78 base riboprobe encoded exclusively by exon 5; this probe recognizes only D$_{2\beta}$, not the short form, D$_{2\alpha}$. Representative images

of this comparison are shown in Figures 5 and 6. In the forebrain (Figure 5), the distribution of total D_2 receptor mRNA and exclusively $D_{2\beta}$ mRNA appear identical, with dense labeling in the caudate-putamen, nucleus accumbens, and olfactory tubercle. In addition, in several DA-containing cell groups, identical labeling was observed: high levels of total D_2 receptor mRNA as well as $D_{2\beta}$ receptor message were observed in the substantia nigra (pars compacta), VTA, and zona incerta (Figure 6), probably encoding DA autoreceptors. The demonstration of similar patterns of total D_2 receptor and $D_{2\beta}$ receptor messages is not unexpected, given that we and others (Monsma et al 1989; Dal Toso et al 1989; Giros et al 1989; Chio et al 1990; Rao et al 1990) have demonstrated that both $D_{2\alpha}$ and $D_{2\beta}$ receptor messages coexist in all brain regions studied, and that $D_{2\beta}$ receptor message is the most abundant in each region studied. Given this, one would expect total D_2 receptor and $D_{2\beta}$ receptor mRNAs to have similar distributions.

More recently, we have developed an assay specific for $D_{2\alpha}$ receptor mRNA (Figure 7). The basis of this particular assay is a 30 base long, [^{35}S]-labeled oligonucleotide and a wash stringency that is calibrated to dissociate 15 base pair hybrids formed with $D_{2\beta}$ but not the 30 base pair hybrids formed with $D_{2\alpha}$, thus allowing the visualization of exclusively $D_{2\alpha}$ receptor mRNA. Using the nigrostriatal system as a model, a comparison of the distributions of $D_{2\alpha}$ and $D_{2\beta}$ receptor mRNAs are presented in Figure 7. Both the nigra and the caudate-putamen can be seen to contain both isoforms of this message, supporting the finding of codistribution based on gel analyses.

Pharmacological Regulation

After these mapping studies, we next attempted to pharmacologically regulate these messages, using classical DA agonist–antagonist treatment. Chronic treatment with apomorphine or haloperidol resulted in no significant change in total D_2 receptor mRNA levels in either the caudate–putamen or in the substantia nigra, as shown in Figure 8.

Demonstration of Midbrain Autoreceptors

Several experiments were undertaken to explore whether the D_2 receptor could have an autoreceptor function in the brain. Thin sequential sections of the rat brain, taken at the level of the midbrain, were probed for either D_2 receptor mRNA or TH immunostaining (Figure 9). Under both low- and high-power views, the concordance between these two markers was very high, thus suggesting that most DA-producing cells (i.e., positive for TH) also are capable of encoding D_2 receptors.

To further confirm this finding, we performed unilateral 6-OHDA lesions in the medial forebrain bundle. As can be appreciated in Figure 10, these lesions produced a total loss of both D_2 receptor binding and D_2 receptor mRNA, as well as TH mRNA in the midbrain DA cell groups. This again strongly supports the notion that the D_2 receptor mRNA seen in the midbrain encodes autoreceptors.

Discussion

These findings demonstrate that D_2 receptor mRNA is distributed in both traditional dopaminoceptive as well as DA-containing regions of the rodent and primate brain, that this distribution is explainable based on the known distribution of D_2 receptor binding sites and DA circuitry in the brain, that multiple isoforms of D_2 receptor mRNA exist in
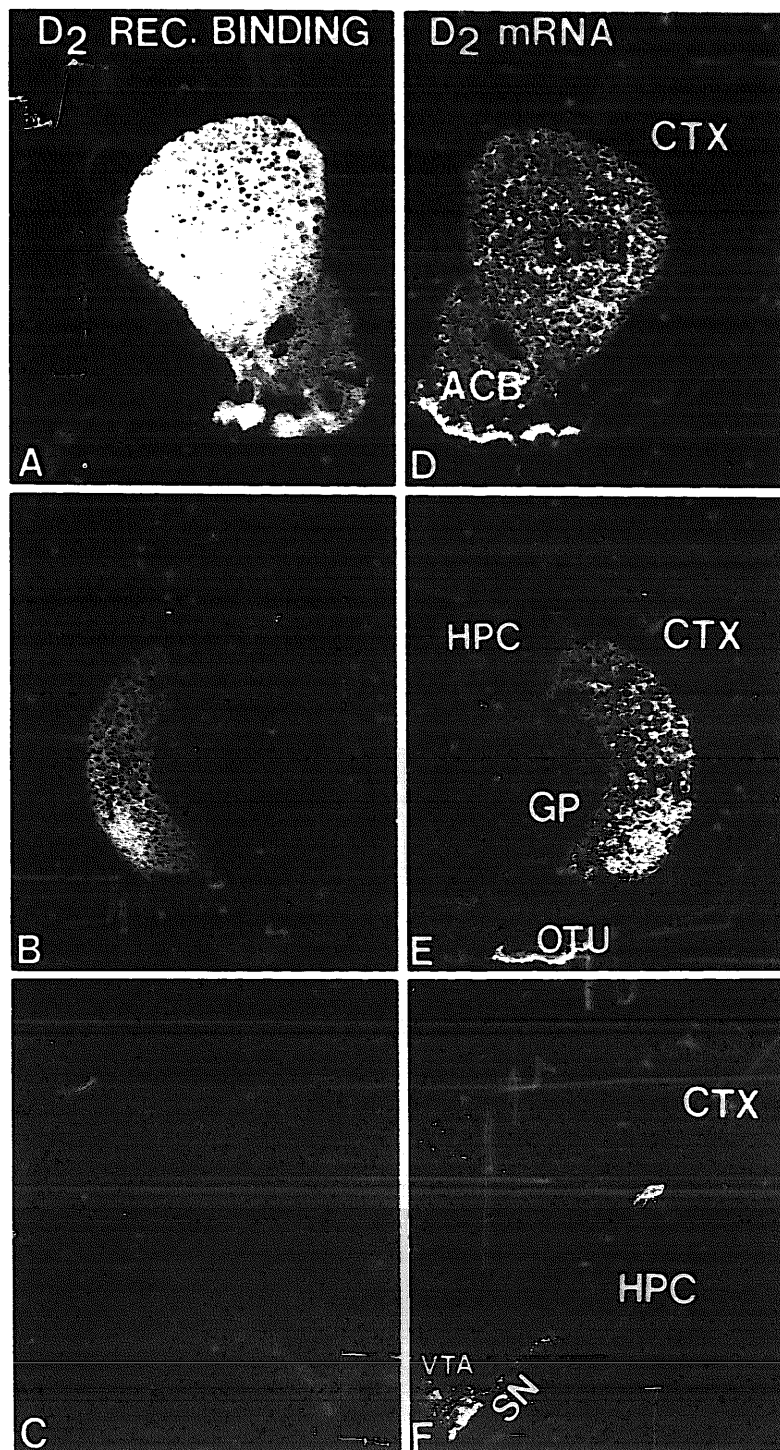
Figure 1. Comparison of the distribution of D_2 receptor binding and messenger RNA (mRNA) in the rat brain. A, D: coronal sections through the rostral forebrain. Note similar patterns of D_2 binding and message in the caudate-putamen (CPU), nucleus accumbens (ACB), and the olfactory tubercle (OTU). There is faint labeling of D_2 receptor mRNA in the cortex (CTX) that is not appreciated by receptor autoradiography. B, E: coronal sections at the level of the globus pallidus (GP). Note similar distributions of message and binding in the CPU, GP, and OTU, but distinct differences in the hippocampus (HPC) and cortex. C, F: coronal sections at the level of the midbrain. Note similar distributions of D_2 receptor binding and mRNA in the pars compacta of the substantia nigra (SN) and ventral tegmental area (VTA). This labeling probably reflects synthesis of DA autoreceptors in these two structures. As in the panel above, distinct differences can be seen in the hippocampus.
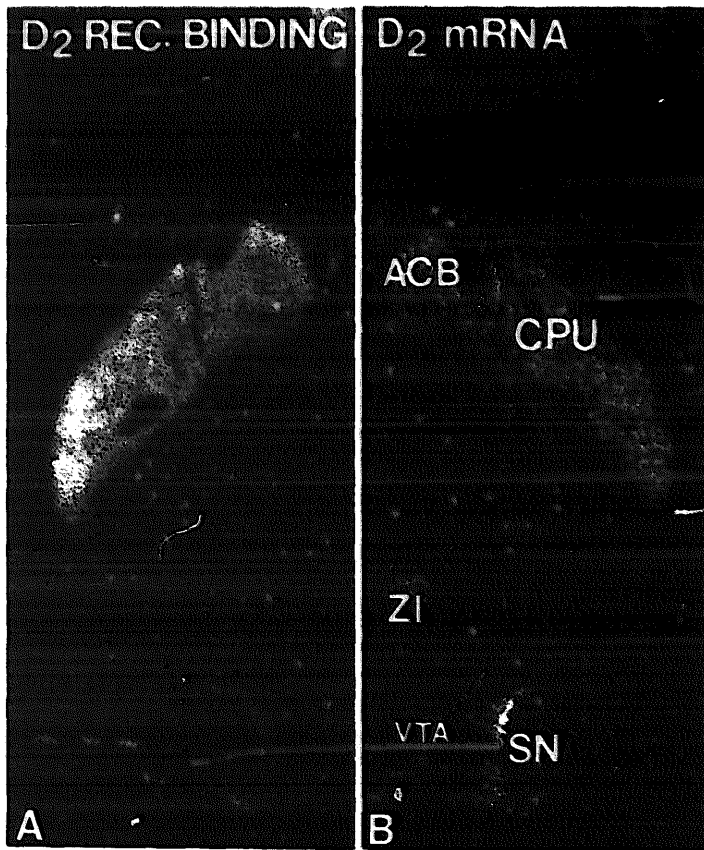
Figure 2. Horizontal sections through the rat brain comparing the distributions of (A) D₂ receptor binding and (B) D₂ receptor mRNA. In addition to the structures visualized in coronal sections in Figure 1, note the presence of D₂ receptor mRNA in the zona incerta (ZI). As in the case of the SN and VTA, this message is probably encoding autoreceptors.
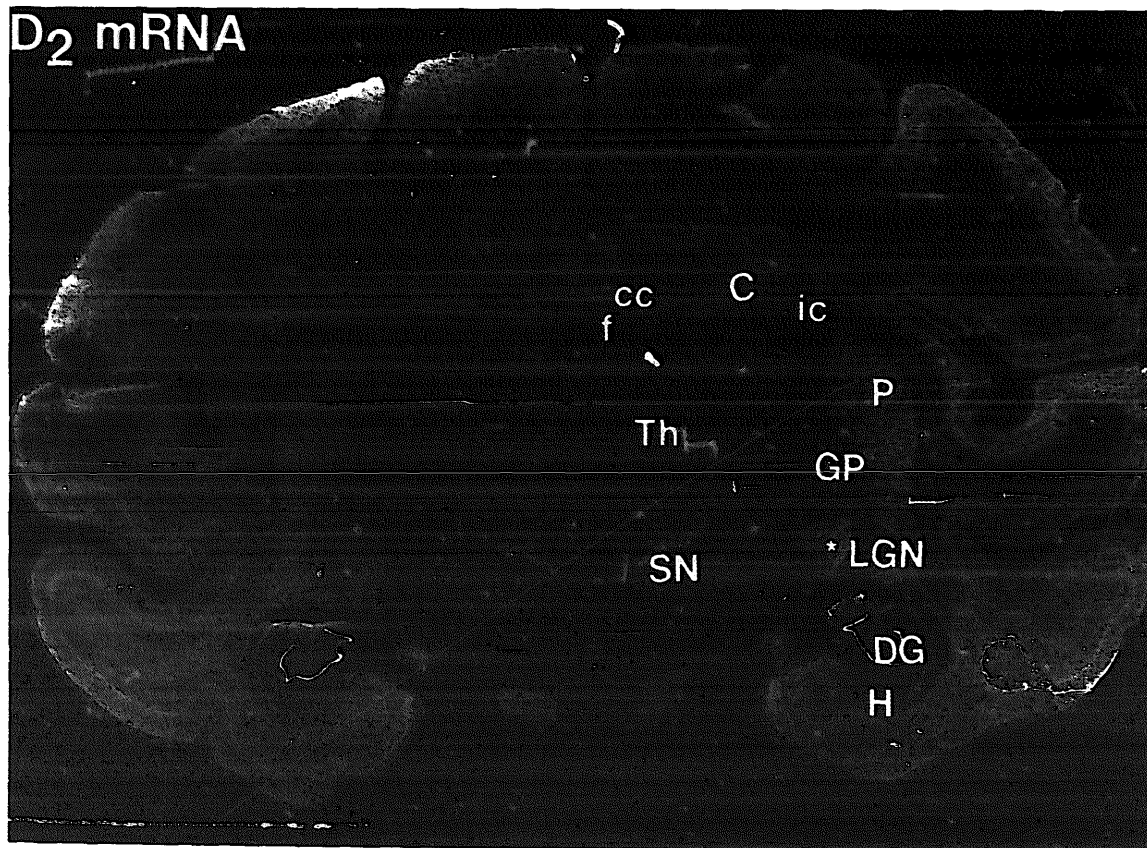
Figure 3. Distribution of D₂ receptor mRNA in a coronal section from the old world monkey. Note the similarity of the distribution of this message with that found in the rat brain, especially in elements of the nigrostriatal system. Both dopaminoceptive and DA-containing regions are visualized, reflecting primarily postsynaptic receptors and autoreceptors, respectively. Abbreviations: C, caudate nucleus; P, putamen; GP, globus pallidus; SN, substantia nigra; Th, thalamus; LGN, lateral geniculate nucleus; DG, dentate gyrus; H, hippocampus. Non-D₂ mRNA-labeled structures are also identified for anatomic orientation: cc, corpus callosum; f, fornix; ic, internal capsule.

PITUITARY   STRIATUM
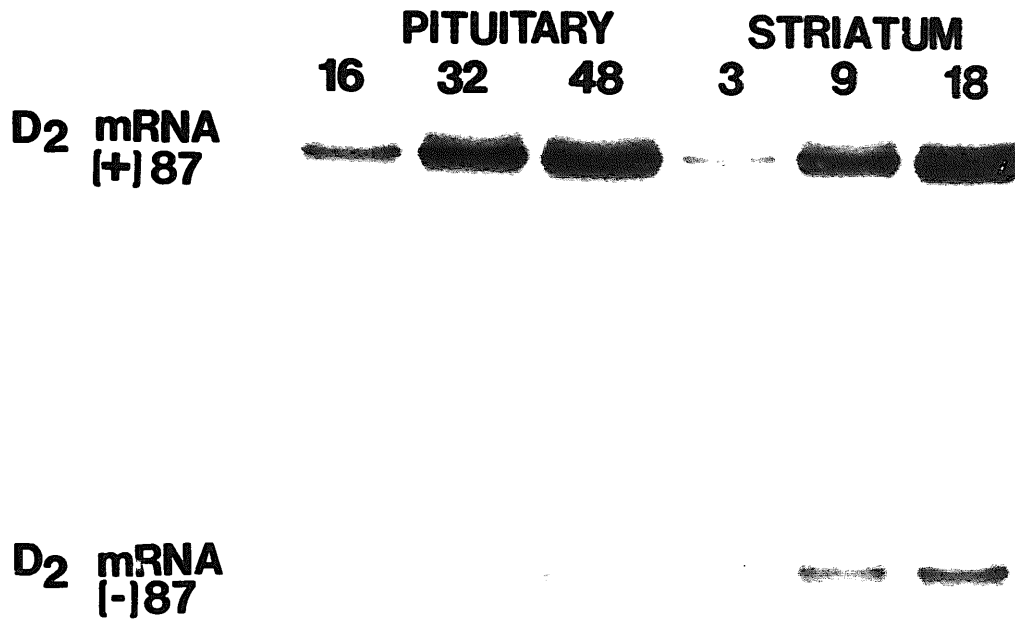16   32   48   3   9   18

D2 mRNA
[+] 87

D2 mRNA
[-] 87

Figure 4. RNase protection assay demonstrating the presence of both $D_{2\alpha}$ and $D_{2\beta}$ receptor mRNAs in rat brain. Extracts of pituitary and striatum were assayed; total RNA (in μg) for each lane is shown at the top of the gel. Note that $D_{2\beta}$ ("+87") is the predominant form of D_2 receptor mRNA, although a faint band corresponding to $D_{2\alpha}$ ("−87") receptor mRNA can also be appreciated.
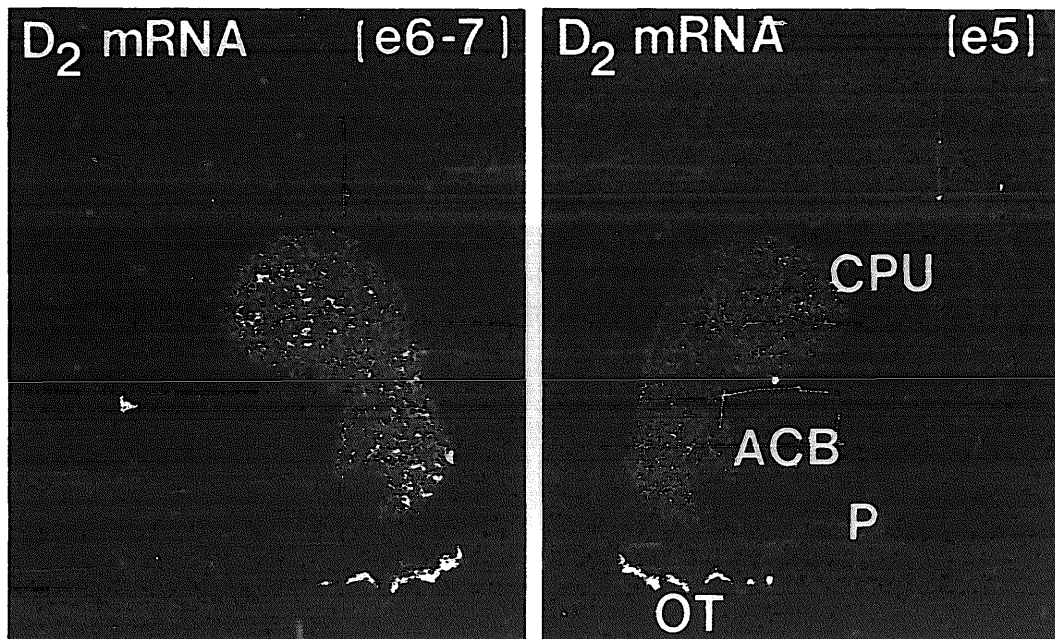

D2 mRNA   [e6-7]   D2 mRNA   [e5]

CPU

ACB

P

OT

Figure 5. Comparison of the distributions of the messenger RNAs encoding both total D_2 receptor mRNA (left panel) and $D_{2\beta}$ receptor mRNA (right panel) in the forebrain of the rat. The probe used for identifying total D_2 receptor mRNA was generated from cDNA corresponding to exons 6 and 7, and the probe used for visualizing $D_{2\beta}$ receptor mRNA corresponded to exon 5; see text for further details. Note the apparent identical distributions of these messages, which is expected, as $D_{2\beta}$ mRNA is the predominant form of the total D_2 receptor mRNA in these structures. Abbreviations: CPU, caudate-putamen; ACB, nucleus accumbens; OT, olfactory tubercle; P, piriform cortex. Although faint in the left panel (total message), piriform cortex is identifiable with both cRNA probes.
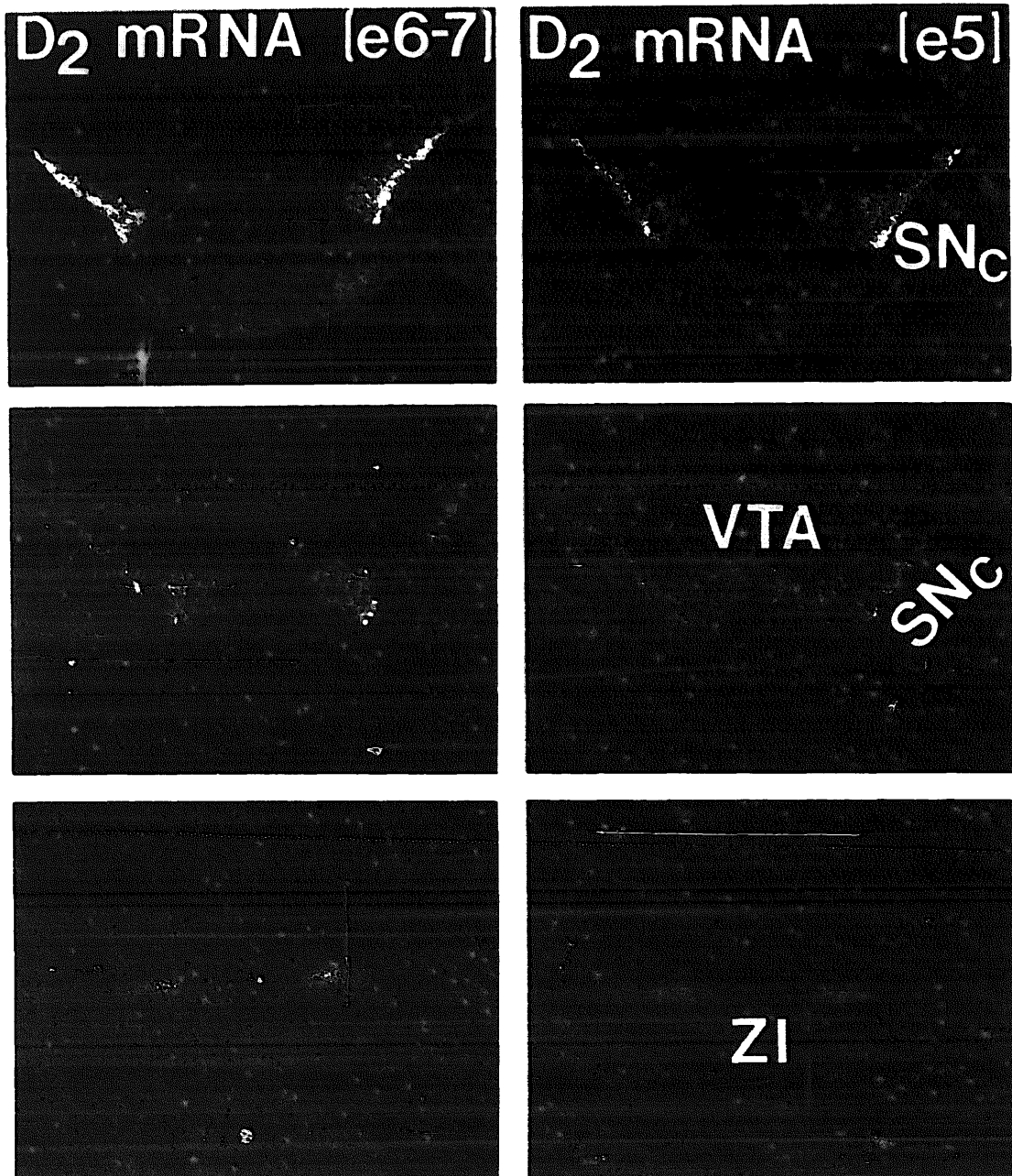
Figure 6. Comparison of the distributions of total D_2 and D_{2B} receptor mRNAs in DA-containing cell groups. The probes described for Figure 5 were used to generate these images. As in the case of the forebrain, note the apparent identical distributions of these messages in these DA-containing regions. It is likely that at least some of the DA autoreceptors are D_{2B} receptors. Abbreviations: SNc, substantia nigra pars compacta; VTA, ventral tegmental area; ZI, zona incerta.
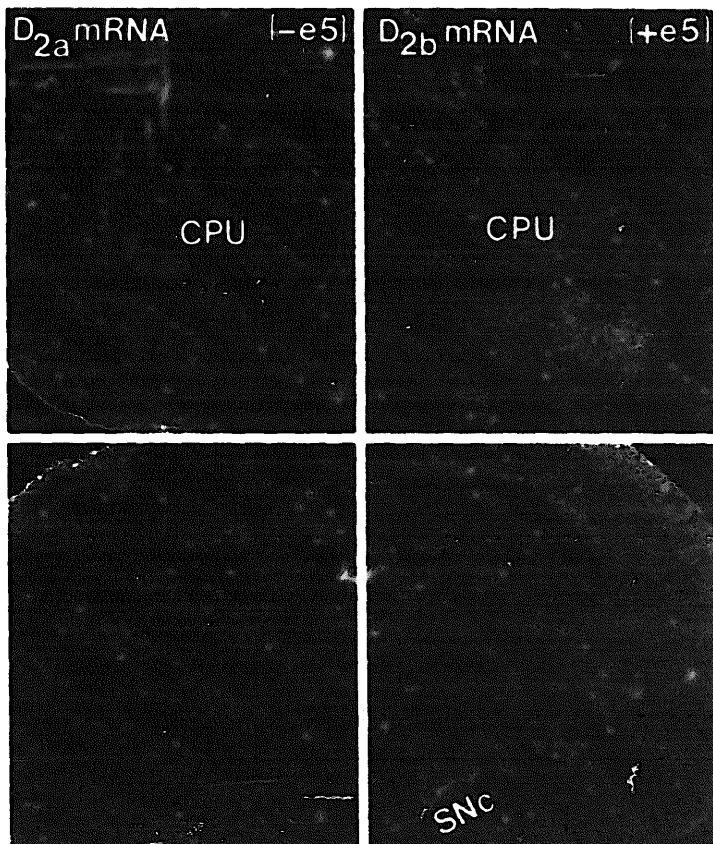
Figure 7. Comparison of the distributions of the two isoforms of the D_2 receptor, $D_{2\alpha}$ ($-e5$) and $D_{2\beta}$ ($+e5$), in the nigrostriatal system of the rat. Note similar distributions in both the caudate-putamen and in the substantia nigra; faint labeling of the hippocampus can also be appreciated in the bottom two panels. Postsynaptic as well as autoreceptors in the nigrostriatal system probably exist as both $D_{2\alpha}$ and $D_{2\beta}$ receptors.
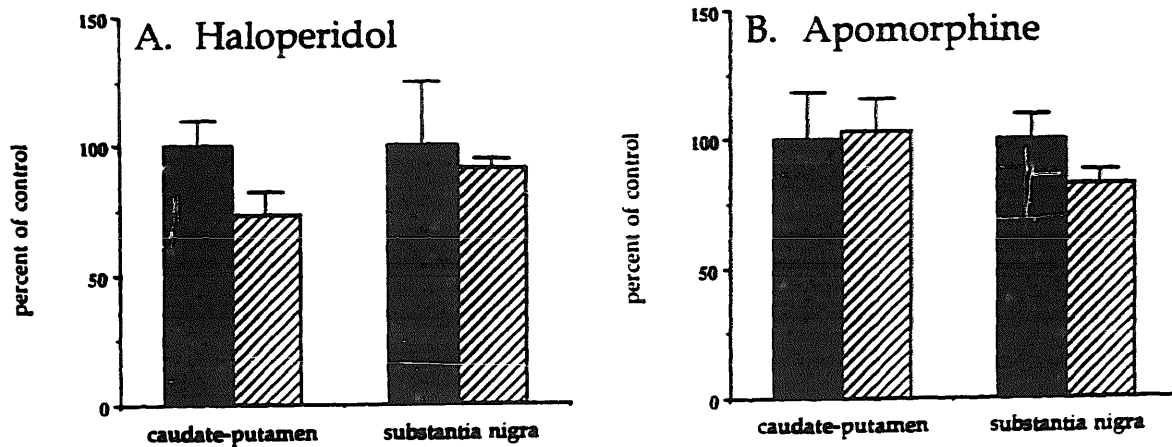

Figure 8. Effect of chronic haloperidol (A) and apomorphine (B) treatment on D_2 receptor mRNA in the caudate-putamen and the substantia nigra. Results are expressed as means ± SEM, and are percentages of control values, representing $n = 6$ rats per group. None of the results are significantly different from control values.
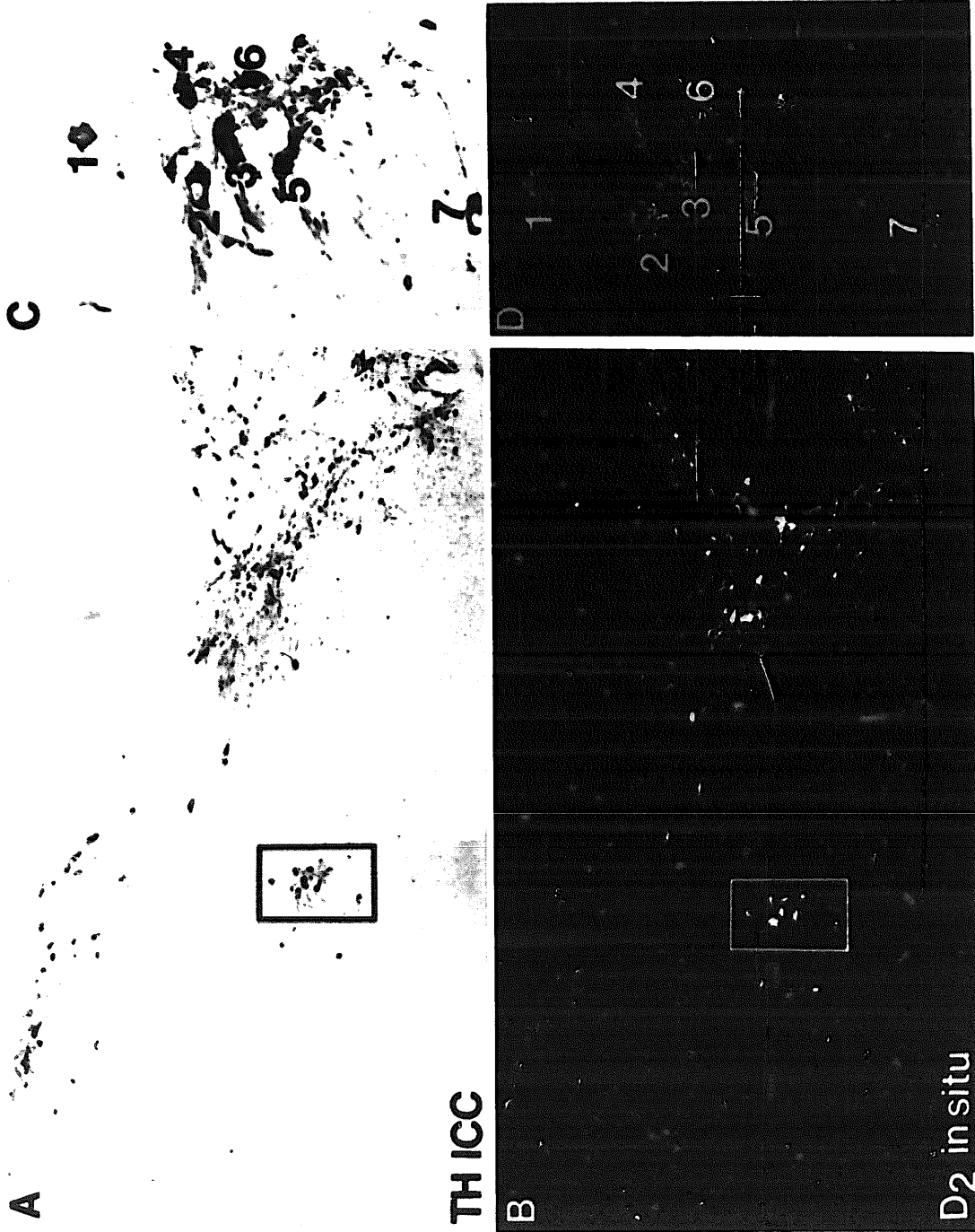
Figure 9. Colocalization of total D_2 receptor mRNA and tyrosine hydroxylase (TH) in the midbrain of the rat. D_2 receptor mRNA was visualized with in situ hybridization, and TH was identified with immunocytochemistry, in adjacent sections through the substantia nigra. A, B: low-power view through the midbrain. C, D: high-power view corresponding to the small box shown in the left panels. Individual cells are labeled by number in both panels C and D. Every nigral cell that is DA-containing (i.e., TH-positive) also appears to contain D_2 receptor mRNA. Thus, most if not all DA-containing nigral cells are capable of expressing D_2 receptors.
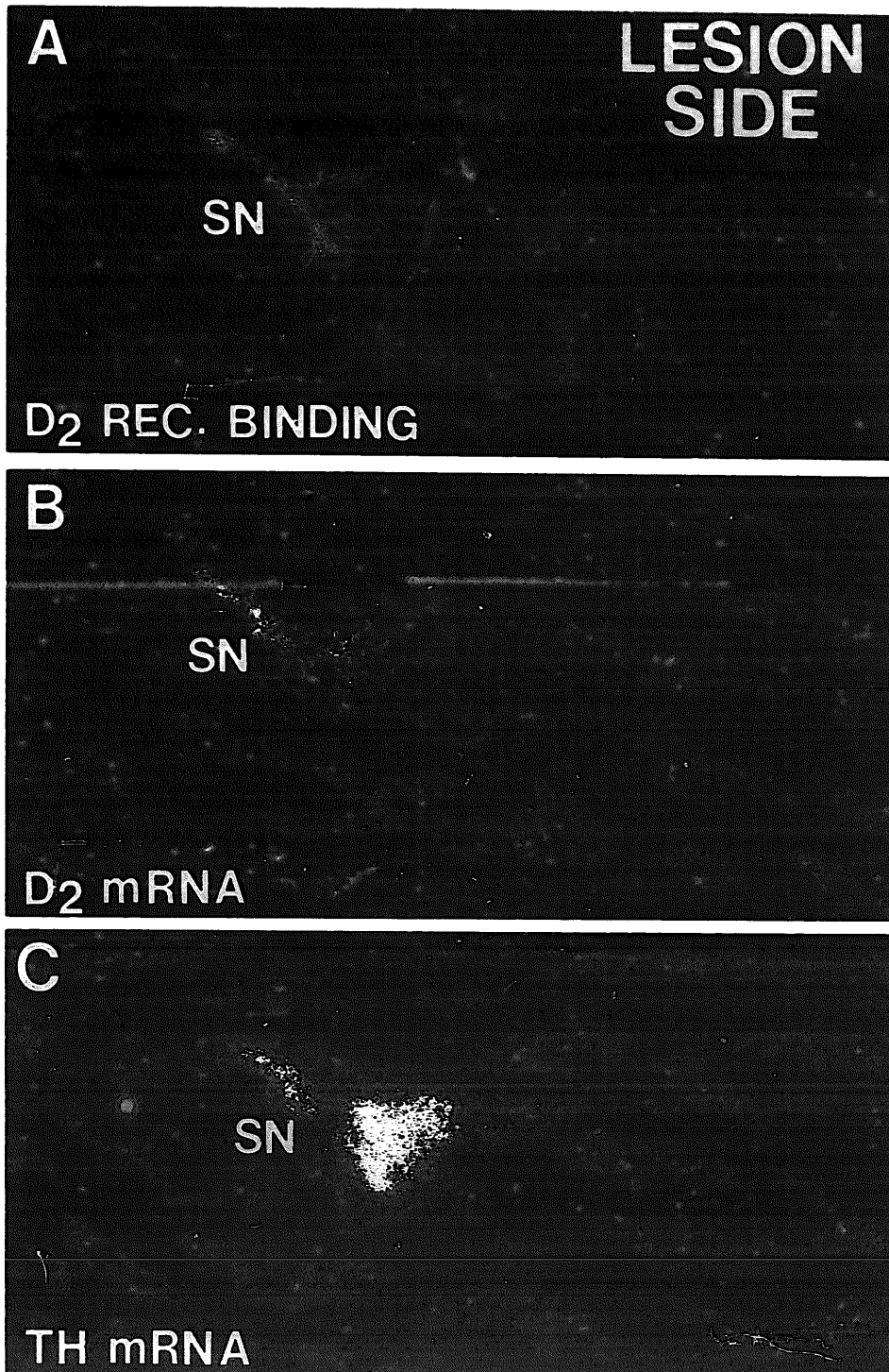
Figure 10. Effect of 6-hydroxydopamine (6-OHDA) lesions on the substantia nigra of the rat. Rats received unilateral 6-OHDA injections into the medial forebrain bundle. Note the disappearance of D_2 receptor binding (A) and mRNA (B), as well as tyrosine hydroxylase (TH) mRNA (C) on the side ipsilateral to the lesion. This supports the autoreceptor role of the D_2 receptor in the midbrain of the rat.

brain, and appear to be codistributed, and that chronic agonist or antagonist treatments fail to regulate D$_2$ receptor mRNA; finally, anatomic evidence is provided for an auto-receptor role of D$_2$ receptors in the midbrain.

The localization of total D$_2$ receptor mRNA parallels that would be predicted based on past autoradiographic and immunohistochemical research identifying dopaminergic circuits in the brain (Boyson et al 1986; Wamsley et al 1989; Bouthenet et al 1987; Charuchinda et al 1987; Dawson et al 1988; Richfield et al 1987). In fact, we and others (Meador-Woodruff et al 1989; Mengod et al 1989; Weiner and Brann 1989; Najlerahim et al 1989) have demonstrated D$_2$ receptor mRNA in every traditional region of the brain implicated as a DA receptive field, reflecting postsynaptic receptor synthesis. These studies provide direct anatomic confirmation of the dopaminoceptive nature of these various limbic and striatal regions, and in conjunction with the findings in the midbrain provide a more comprehensive anatomic understanding of these receptors.

The parallel distributions of D$_2$ receptor mRNA and binding sites provides some confirmation of previously defined DA circuits in the brain. In situ hybridization, however, is only capable of resolving the somata synthesizing a given mRNA, whereas receptor autoradiography can visualize distant axonal and dendritic receptor binding sites in addition to receptors on the cell bodies themselves. The high degree of concordance of D$_2$ binding and message distributions in a number of structures is not unexpected, as the receptor itself may be expressed in close proximity to the cell body likely to synthesize it. Areas of striking dissimilarity between these distributions have been observed, especially in the hippocampus, regions of cortex, and the olfactory bulb. We have previously speculated on the reasons for this observation (Mansour et al 1990a), and have proposed the differential localization of binding sites (on processes as well as cell bodies) and message (cell bodies only) and receptor transport as explanations of this phenomenon.

The finding of D$_2$ receptor mRNA in some DA-containing cell groups (i.e., the substantia nigra, VTA, and zona incerta) by a number of groups (Mengod et al 1989; Weiner and Brann 1989; Najlerahim et al 1989), in addition to our own work, suggests that D$_2$ receptors may have an autoreceptor function. To confirm that the D$_2$ receptor mRNA found in the midbrain could encode autoreceptors, we performed several additional studies. We have been able to demonstrate that cells in the substantia nigra and VTA expressing D$_2$ receptor mRNA also are positive for the presence of TH, a biochemical marker for catecholamine synthesis. This extensive colocalization of D$_2$ receptor message and TH immunostaining strongly support the contention that this receptor in the midbrain has an autoreceptor function. To further confirm this, we performed 6-OHDA lesions. By infusing 6-OHDA into the medial forebrain bundle, the dopaminergic axons projecting from the midbrain to the forebrain are specifically destroyed. The coincident disappearance of all TH and D$_2$ receptor mRNA, as well as all D$_2$ receptor binding, in the substantia nigra and VTA supports the hypothesis that the D$_2$ receptor mRNA in the midbrain is encoding autoreceptor synthesis. These findings are consistent with previous studies indicating that the DA autoreceptor is D$_2$ in nature (Reisine et al 1979; White and Wang 1983; Stoof et al 1982; Brown et al 1985), and further suggest that most, if not all, midbrain DA cells are capable of synthesizing D$_2$ autoreceptors.

A number of DA-containing cell groups do not manifest D$_2$ receptor mRNA, most notably the medullary periventricular groups corresponding to A14 and A15. Whether this suggests that these systems do not contain autoreceptors is not clear. These are very small neurons (relative to nigral neurons), and a message of low abundance in such small cells might escape detection. Thus it is unclear if these groups lack autoreceptors, or

contain them but are undetected due to sensitivity problems of this technique. It is clear, however, that if autoreceptors are produced in A14 and A15, the message would be present at a considerably lower concentration than found in the substantia nigra, VTA, or the zona incerta.

The discovery of at least two isoforms of D_2 receptor mRNA originally caused considerable excitement, suggesting the possibility that the postsynaptic receptor was slightly different from the autoreceptor, or that the postsynaptic receptors found in the motor (nigrostriatal) system were distinct from those in the more limbic structures. Such simple and elegant solutions are not the case, however, since both $D_{2\alpha}$ and $D_{2\beta}$ mRNAs appear to be codistributed in every region of the brain examined. Certainly, these two forms both exist as autoreceptors and postsynaptic receptors, and appear to be distributed in all of the DA receptor fields, including the motor, limbic, and hypothalamic systems. It remains to be determined what significance these two forms represent. They have similar distributions, albeit the longer isoform is more abundant, and have similar ligand binding characteristics (Giros et al 1989; Grandy et al 1989; Monsma et al 1989; Chio et al 1990). The region of difference (exon 5) falls in the third cytosolic loop of the translated protein, and this is the locus of G-protein coupling in the seven-transmembrane domain receptors. Perhaps these two isoforms have subtle differences in G-protein binding and hence second messenger coupling. The exact nature of the difference between these forms awaits elucidation.

Neither haloperidol nor apomorphine treatment caused significant changes in the levels of D_2 receptor mRNA in the basal ganglia or in the substantia nigra, as determined by in situ hybridization. This is in agreement with at least two other reports (van Tol et al 1990; Srivastava et al 1990), although a study has been published in which chronic haloperidol promoted a slight increase in D_2 receptor mRNA in the striatum and other brain regions (Coirini et al 1990). The reasons for these discrepancies are not yet clear, but it seems that any regulation of D_2 receptor mRNA by haloperidol is not particularly reproducible nor robust. The previously reported upregulation of D_2 receptor binding following chronic haloperidol treatment (Burt et al 1977; Chipkin et al 1987; MacLennon et al 1988) may be the result of posttranslational as opposed to transcriptional events, but this remains to be clarified. Nonetheless, if the finding of a lack of regulation of this mRNA by chronic antagonist treatment is confirmed, it may be quite useful in the study of this marker in postmortem tissue from patients who had received chronic antipsychotic treatment.

The data presented in this article tend to be consistent with our previous understanding of DA systems based on past receptor binding, immunohistochemical, and lesion studies. The studies described in this report do more than merely confirm these previous results, however, and offer important additional information relevant to the understanding of brain DA circuitry. For example, when a gene is first cloned, the technique of in situ hybridization identifies the anatomic distribution of the corresponding mRNA, which helps to confirm the identity of a given clone. Our results (Meador-Woodruff et al 1989) helped to solidify the identification of these particular clones as in fact encoding D_2 receptors. As has been mentioned previously, in situ hybridization only labels the cell bodies of origin of the receptors, in contrast to receptor autoradiography, which reveals a different anatomic pattern that includes distant processes where receptor insertion into the cell membrane has occurred. This distinction allows the comparison of the locations of biosynthetic origin of a receptor versus its final destination, which will ultimately allow us to characterize the distinct cellular events of receptor synthesis, transport, and subsequent insertion into the cell membrane. Finally, the ability to measure mRNA levels in an anatomic context now allows the study of the regulation of the DA receptors at both the level of gene transcription (mRNA) and transla-

tion (final receptor protein). In fact, in situ hybridization has sufficient resolution to allow the determination of regulatory changes within a single cell.

In addition to this extension of our understanding of the brain DA systems, these studies have demonstrated the feasibility of examining the expression of the D_2 receptor gene in an anatomic context, thus setting the stage for postmortem investigations in schizophrenia. The study of mRNA levels in postmortem brains may have certain advantages over the determination of neurotransmitters and other proteins. Messenger RNA levels tend to be quite stable in brain, requiring hours to days to change appreciably in concentration (Birnberg et al 1983). Thus, mRNA levels tend not to be altered rapidly at the time of death, unlike neurotransmitters, which may be acutely released, causing changes in receptor occupancy. In addition, the level of a given mRNA in the brain probably reflects the cumulative history of the particular system rather than the more recent terminal events, thus providing a glimpse of the underlying pathology or functioning of a given system. There is of course degradation of RNA after death, as there is degradation of enzymes and other proteins. Unlike proteins, however, mRNA degradation occurs in a somewhat predictable fashion, due to the ubiquitous distribution of the RNases, as opposed to the more heterogeneous distribution of various proteolytic enzymes in brain. Whether the study of D_2 receptor mRNA in postmortem brain will reveal any differences of the expression of this gene in schizophrenia remains to be seen, but the studies themselves are feasible, and this should be an exciting area of research for the next few years.

This series of studies has begun to characterize the mRNA encoding the D- DA receptors in the central nervous system, and sets the stage for our next series of experiments aimed at examining these receptors directly in postmortem brains of patients who had schizophrenia. In addition to the D_2 receptors, a DA D_1 receptor (Dearry et al 1990; Zhou et al 1990; Sunahara et al 1990; Monsma et al 1990), and a so-called D_3 receptor (Sokoloff et al 1990) have recently been cloned. We are aggressively pursuing the characterization of these and other dopamine receptor clones in brain as well. It is only with this fundamental understanding of these multiple DA receptor types and forms, and the combination of molecular biological and traditional anatomic techniques, that we may ultimately be able to clarify the role of these receptors in the pathogenesis of schizophrenia. The cloning of the D_2 receptors has provided a new set of tools with which we can test the dopamine hypothesis of schizophrenia, and heralds advances in our understanding of the genetic basis of this disease.

References

Berman KF, Iliowsky BP, Weinberger DR (1988): Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia. IV. Further evidence for regional and behavioral specificity. *Arch Gen Psychiatry* 45:616–622.

Birnberg N, Lissitsky J, Hinman M (1983): Glucocorticoids regulate pro-opiomelanocortin gene expression at the level of transcription and secretion. *Proc Natl Acad Sci USA* 80:6982–6986.

Bouthenet M-L, Martres MP, Sales N, Schwartz JC (1987): A detailed mapping of dopamine D₂ receptors in rat central nervous system by autoradiography with [^{125}I] iodosulpride. *Neuroscience* 20:117–155.

Boyson SJ, McGonigle P, Molinoff PB (1986): Quantitative autoradiographic localization of the D₁ and D₂ subtypes of dopamine receptors in rat brain. *J Neurosci* 6:3177–3188.

Brown F, Campbell W, Mitchell PJ, Randall K (1985): Dopamine autoreceptors and the effects of drugs on locomotion and dopamine synthesis. *Br J Pharmacol* 84:853–860.

Brown WA, Laughren T (1981): Low serum prolactin and early relapse following neuroleptic withdrawal. *Am J Psychiatry* 138:237–239.

Bunzow JR, van Tol HHM, Grandy DK, et al (1988): Cloning and expression of a rat D₂ dopamine receptor cDNA. *Nature* 336:783–787.

Burt DR, Creese I, Snyder SH (1977): Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. *Science* 196:326–328.

Charuchinda CP, Supavilai P, Karobath M, Palacios JM (1987): Dopamine D₂ receptors in the rat brain: Autoradiographic visualization using a high affinity selective agonist ligand. *J Neurosci* 7:1352–1360.

Chio CL, Hess GF, Graham RS, Huff RM (1990): A second molecular form of D₂ dopamine receptor in rat and bovine caudate nucleus. *Nature* 343:266–269.

Chipkin RE, McQuade RD, Iorio LC (1987): D₁ and D₂ dopamine binding site up regulation and apomorphine-induced stereotypy. *Pharmacol Biochem Behav* 28:477–482.

Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979): Isolation of biologically active ribonucleic acids from sources enriched for ribonucleases. *Biochemistry* 18:5294–5299.

Coirini H, Schumacher M, Angulo JA, McEwen BS (1990): Increase in striatal dopamine D₂ receptor mRNA after lesions of haloperidol treatment. *Eur J Pharmacol* 186:369–371.

Cooper JR, Bloom FE, Roth RH (1986): *The Biochemical Basis of Neuropharmacology*, 5th ed. New York: Oxford University Press.

Creese I, Burt IR, Snyder SH (1976): Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192:481–483.

Cross AJ, Crow TJ, Owen F (1981): ³H-Flupenthixol binding in postmortem brains of schizophrenics: Evidence for a selective increase in dopamine D₂ receptors. *Psychopharmacology* 74:122–124.

Dal Toso R, Sommer B, Ewert M, et al (1989): The dopamine D₂ receptor: two molecular forms generated by alternative splicing. *EMBO J* 8:4025–4034.

Dawson TM, Barone P, Sidhu A, Wamsley JK, Chase TN (1988): The D₁ dopamine receptor in the rat brain: Quantitative autoradiographic localization using an iodinated ligand. *Neuroscience* 26:83–100.

Dearry A, Grinrich JA, Falardeau P, Fremeau RT, Bates MD, Caron MG (1990): Molecular cloning and expression of the gene for a human D₁ dopamine receptor. *Nature* 347:72–76.

Emorine LJ, Marullo S, Delavier-Klutchko C, et al (1987): Structure of the gene for human β₂-adrenergic receptor: Expression and promoter characterization. *Proc Natl Acad Sci USA* 84:6995–6999.

Ettigi P, Nair NPV, Lal S, Cervantes P, Guyda H (1976): Effect of apormorphine on growth hormone and prolactin secretion in schizophrenic patients, with and without oral dyskinesia, withdrawn from chronic neuroleptic therapy. *J Neurol Neurosurg Psychiatry* 39:870–876.

Farde L, Wiesel F-A, Stone-Elander S, et al (1990): D₂ dopamine receptors in neuroleptic-naive schizophrenic patients. A position emission tomography study with [^{11}C] raclopride. *Arch Gen Psychiatry* 47:213–219.

Giros B, Sokoloff P, Martres M-P, Riou J-F, Emorine LJ, Schwartz J-C (1989): Alternative splicing directs the expression of two D₂ dopamine receptor isoforms. *Nature* 342:923–926.

Grandy DK, Marchionni MA, Makam H, et al (1989): Cloning of the cDNA and gene for a human D₂ dopamine receptor. *Proc Natl Acad Sci USA* 86:9762–9766.

Herman JP, Shafer MK-H, Young EA, et al (1989): Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. *J Neurosci* 9:3072–3082.

Jaskiw G, Kleinman J (1989): Postmortem neurochemistry studies in schizophrenia. In Schulz SC, Tamminga CA (eds), *Schizophrenia: Scientific Progress*. New York: Oxford University Press, pp 264–273.

Johnstone EC, Crow TJ, Mashiter K (1977): Anterior pituitary hormone secretion in chronic schizophrenia—An approach to neurohumoral mechanisms. *Psychol Med* 7:223–228.

Kleinman JE, Weinberger DR, Rogol AD, et al (1982): Plasma prolactin concentrations and psychopathology in chronic schizophrenia. *Arch Gen Psychiatry* 39:655–657.

Lee T, Seeman P (1980): Elevation of brain neuroleptic/dopamine receptors in schizophrenia. *Am J Psychiatry* 137:191–197.

Mackay AVP, Iverson LL, Rossor M, et al (1982): Increased brain dopamine and dopamine receptors in schizophrenia. *Arch Gen Psychiatry* 39:991–997.

MacLennon AJ, Atmadja S, Lee N, Fibiger HC (1988): Chronic haloperidol administration increases the density of D₂ dopamine receptors in the medial prefrontal cortex of the rat. *Psychopharmacology* 95:255–257.

Mansour A, Meador-Woodruff JH, Bunzow JR, Civelli O, Akil H, Watson SL (1990a): Localization of dopamine D₂ receptor mRNA and D₁ and D₂ receptor binding in the rat brain and pituitary: An in situ hybridization-receptor autoradiographic analysis. *J Neurosci* 10:2587–2600.

Mansour A, Meador-Woodruff JH, Camp DM, et al (1990b): The effects of nigrostriatal 6-hydroxydopamine lesions on dopamine D₂ receptor mRNA and opioid systems. In Quirion R, Jhamandas K, Gianoulakis C (eds), *The International Narcotics Research Conference (INRC) '89*. New York: Liss, pp 227–230.

Martinot J-L, Peron-Megnan P, Huret J-D, et al (1990): Striatal D₂ dopaminergic receptors assessed with positron emission tomography and [⁷⁶Br] bromospiperone in untreated schizophrenic patients. *Am J Psychiatry* 147:44–50.

Meador-Woodruff JH, Mansour A, Bunzow JR, van Tol HHM, Watson SJ, Civelli O (1989): Distribution of D₂ dopamine receptor mRNA in rat brain. *Proc Natl Acad Sci USA* 86:7625–7628.

Meltzer HY, Sachar EJ, Frantz AG (1974): Serum prolactin levels in unmedicated schizophrenic patients. *Arch Gen Psychiatry* 31:564–569.

Mengod G, Martinez-Mir MI, Vilaro MT, Palacios JM (1989): Localization of the mRNA for the dopamine D₂ receptor in the rat brain by in situ hybridization histochemistry. *Proc Natl Acad Sci USA* 86:8560–8564.

Monsma FJ, McVittie LD, Gerfen CR, Mahan LC, Sibley DR (1989): Multiple D₂ dopamine receptors produced by alternative RNA splicing. *Nature* 342:926–929

Monsma FJ, Mahan LC, McVittie LD, Gerfen CR, Sibley DR (1990): Molecular cloning and expression of a D₁ dopamine receptor linked to adenlyl cyclase activation. *Proc Natl Acad Sci USA* 87:6723–6727.

Najlerahim A, Barton AJL, Harrison PJ, Heffernan J, Pearson RCA (1989): Messenger RNA encoding the D₂ dopaminergic receptor detected by in situ hybridization histochemistry in rat brain. *FEBS Lett* 255:335–339.

O'Dowd BF, Lefkowitz RJ, Caron MG (1989): Structure of the adrenergic and related receptors. *Annu Rev Neurosci* 12:67–83.

Rao DD, McKelvy J, Kebabian J, MacKenzie RG (1990): Two forms of the rat D₂ dopamine receptor as revealed by the polymerase chain reaction. *FEBS Lett* 263:18–22.

Reisine TD, Nagy JI, Beaumont K, Fibiger HC, Yamamura HI (1979): The localization of receptor binding sites in the substantia nigra and striatum of the rat. *Brain Res* 177:241–252.

Richfield EK, Young AB, Penney JB (1987): Comparative distribution of dopamine D₁ and D₂ receptors in the basal ganglia of turtles, pigeons, rats, cats, and monkeys. *J Comp Neurol* 262:446–463.

Rinieris P, Markianos M, Hatzimanouis J, Stefanis C (1985): A psychoendocrine study in male paranoid schizophrenics with delusional ideas of homosexual content. *Acta Psychiatr Scand* 72:309–314.

Rotrosen J, Angrist B, Gershan S, et al (1979): Neuroendocrine effects of apomorphine: Characterization of response patterns and application to schizophrenia research. *Br J Psychiatry* 135:444–456.

Seeman P, Lee T, Chau-Wong M, Wong K (1976): Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* 261:717–719.

Seeman P, Ulpian C, Bergeron C, et al (1984): Bimodal distribution of dopamine receptor densities in brains of schizophrenics. *Science* 225:728–731.

Seeman P, Bzowej NH, Guan HC, et al (1987): Human brain D₁ and D₂ dopamine receptors in schizophrenia, Alzheimer's, Parkinson's and Huntington's diseases. *Neuropsychopharmacology* 1:5–15.

Selbie LA, Hayes G, Shine J (1989): The major dopamine D₂ receptor: Molecular analysis of the human D₂A subtype. *DNA* 8:683–689.

Snyder SH (1976): The dopamine hypothesis in schizophrenia: Focus on the dopamine receptor. *Am J Psychiatry* 133:197–202.

Sokoloff P, Giros B, Martres M-P, Bouthenet M-L, Schwartz J-C (1990): Molecular cloning and characterization of a novel dopamine receptor (D₃) as a target for neuroleptics. *Nature* 347:146–151.

Srivastava LK, Morency MA, Bajwa SB, Mishra RK (1990): Effect of haloperidol on expression of dopamine D₂ receptor mRNAs in rat brain. *J Mol Neurosci* 2:155–161.

Stoof JC, De Boer T, Sminia P, Mulder AH (1982): Stimulation of D₂ dopamine receptors in rat neostriatum inhibits the release of acetylcholine and dopamine but does not affect the release of gama-aminobutyric acid, glutamate, or serotonin. *Eur J Pharmacol* 84:211–214.

Sunahara RK, Niznik HB, Weiner DM, et al (1990): Human dopamine D₁ receptor encoded by an intronless gene on chromosome 5. *Nature* 347:80–83.

Tamminga CA, Smith RC, Pandey G, Frohman LA, Davis JM (1977): A neuroendocrine study of supersensitivity in tardive dyskinesia. *Arch Gen Psychiatry* 34:1199–1203.

van Tol HHM, Riva M, Civelli O, Creese I (1990): Lack of effect of chronic dopamine receptor blockade on D₂ dopamine receptor mRNA level. *Neurosci Lett* 111:303–308.

Wamsley JK, Gehlert DR, Filloux FM, Dawson TM (1989): Comparison of the distribution of D-1 and D-2 dopamine receptors in the rat brain. *J Chem Neuroanat* 2:119–137.

Weinberger DR, Berman KF, Illowsky BP (1988): Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia. III. A new cohort and evidence for a monoaminergic mechanism. *Arch Gen Psychiatry* 45:609–615.

Weiner DM, Brann MR (1989): The distribution of a dopamine D₂ receptor mRNA in rat brain. *FEBS Lett* 253:207–213.

White FJ, Wang RJ (1983): Differential effects of classical and atypical antipsychotic drugs on A9 and A10 dopamine neurons. *Science* 221:1054–1057.

Wong DF, Wagner HN, Tune LE, et al (1986): Position emission tomography reveals elevated D₂ dopamine receptors in drug-naive schizophrenics. *Science* 234:1558–1563.

Wood WI, Gitschier J, Lasky LA, Lawn RM (1984): Base composition-independent hybridization in tetramethylammonium chloride: A method for oligonucleotide screening of highly complex gene libraries. *Biochemistry* 82:1585–1588.

Zhou Q-Y, Grandy DK, Thambi L, et al (1990): Cloning and expression of human and rat D$_1$ dopamine receptors. *Nature* 347:76–80.