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Dopamine Receptor Expression in the Central Nervous System

Alfred Mansour and Stanley J. Watson, Jr.

INTRODUCTION

The cloning of the D_2 dopamine receptor (6) in 1988 and the subsequent identification of multiple dopamine receptors referred to as D_1, D_3, D_4, and D_5 (10, 35, 46, 48, 49, 52, 54, 59) has profoundly changed our understanding of dopamine receptor anatomy and pharmacology. Prior to the isolation of these dopamine receptor subtypes, the dopamine field distinguished two subtypes of dopamine receptors (referred to as D_1 and D_2) that differed in their coupling to G-proteins, their distribution in the central nervous system (CNS), and their pharmacology (5, 47, 56). The cloning of these receptors and their genes has given us a better appreciation of a larger number of dopamine receptors present in the nervous system and how they may be organized in specific neuronal circuits. Given the multiple introns present in the D_2, D_3, and D_4 receptor genes, alternative splicing can yield several forms of these receptors, adding further to this complexity, and may be the basis for more subtle pharmacological differences.

This chapter will focus on the anatomical distribution of the dopamine receptors and will primarily examine the mRNA expression of the D_1, D_2, D_3, and D_5 receptors in the rat CNS. The D_4 receptor, despite its clinical importance as the site where clozapine and other atypical antipsychotics are thought to mediate their therapeutic effects (43, 54), will not be discussed because its level is so low in the rat CNS that it has thus far been difficult to reliably detect. We have chosen to concentrate our efforts on the rat brain, because with the exception of a few publications in the human and primate brain (20, 21, 31), the vast majority of anatomical information concerning the localization and circuitry of the dopamine receptor messenger ribonucleic acids (mRNAs) has been derived from the rat CNS. The chapter begins with a description of the receptor mRNA distributions in the brain, followed by a comparative analysis of dopamine receptor binding sites defined by selective ligands and receptor autoradiographic techniques. Next we focus on the basal ganglia, where on the basis of lesion and colocalization studies, the dopamine receptors have been suggested to be localized in different circuits and perhaps mediate distinct physiological effects. The chapter concludes with a discussion of the possible directions anatomical studies will take in the future to elucidate the role of the multiple dopamine receptors in the CNS. For further information concerning the molecular biology of the dopamine receptor subtypes, readers should refer to Molecular Biology of the Dopamine Receptor Subtypes, Dopamine Autoreceptor Signal Transduction and Regulation, and Signal Transduction Pathways for Catecholamine Receptors).

ANATOMICAL LOCALIZATION OF DOPAMINE RECEPTOR mRNAs

The cloned dopamine receptors (D_1–D_5) can be divided into two groups of receptors that correspond to the D_1 and D_2 receptor classification that had been previously identified pharmacologically. The D_1 and D_5 receptors have a D_1-like pharmacology, whereas the D_2, D_3, and D_4 receptors have a D_2-like pharmacological profile. In general, the D_1 and D_2 receptor mRNAs have a wider distribution and are more abundant in the CNS as compared to their pharmacologically related counterparts. The D_5 receptor mRNA, for example, is restricted to specific thalamic and hypothalamic nuclei and to the cells of the hippocampus, whereas the D_1 receptor mRNA is detected in numerous regions of the CNS. Similarly, cells expressing D_3 receptor mRNA are detected in far fewer nuclei than those expressing D_2 receptor mRNA. The wider distributions of cells expressing D_1 and D_2 receptor mRNA may be reflective of the broader number of functions mediated by these receptors in the CNS, including the modulation of cognitive, sensorimotor, and neuroendocrine effects, as compared to more limited functions that may be mediated by the other dopamine receptor types.

Several laboratories have described the mRNA distributions of the dopamine receptors in the CNS (4, 14, 34, 57, 58), and while in large part there is agreement, differences do exist. These differences may be methodological or in some cases reflect technical differences such as the use of radiolabeled oligonucleotides in some studies and cRNA probes in others. The anatomical description that follows is based on findings largely generated from this laboratory (26, 27, 28, 29, 30, 32).

TELENCEPHALON

The dopamine receptor mRNAs vary in their cortical distributions. Cells expressing D_1 are widely distributed in both neocortical and palleocortical areas, with the highest levels of expression in the anterior cingulate, orbital, insular, piriform, and entorhinal cortex Fig 1 and Fig 2( ) and). Neocortical areas, such as the frontal, parietal, temporal, and occipital cortex, also express D_1 receptor mRNA, with cells localized predominantly in layers V and VI. In contrast, cells expressing high levels of D_2 receptor mRNA are observed only in the entorhinal cortex, with moderate levels of expression in the anterior cingulate, orbital, and insular cortex. Scattered cells in layers IV–VI of the frontal, parietal, temporal, and occipital cortex also express D_2 mRNA Fig 1 and 2 (and). Cells expressing D_3 and D_5 receptor mRNAs are not detected in either neocortical or palleocortical areas.

The olfactory nuclei similarly demonstrate a heterogeneity of dopamine receptor mRNA expression. Cells expressing D_1 receptor mRNA are localized in all the divisions of the anterior olfactory nuclei, including the dorsal, lateral, ventral, and medial divisions, whereas cells expressing D_2 receptor mRNA are primarily in the dorsal and lateral divisions. Cellular expression of D_2 in the dorsal and lateral olfactory nuclei is comparatively low. In contrast, no cells expressing D_3 receptor mRNA are detected in any division of the anterior olfactory nucleus.

More caudally, D_1, D_2, and D_3 receptor mRNA expression is high within the rat striatum figure 1 and 3 ( ) and). Cells expressing high levels of D_1 and D_2 receptor mRNA are found in all levels of the caudate-putamen and extend ventrally into the nucleus accumbens. Medial–lateral differences are observed with higher levels of cellular expression of both D_1 and D_2 in the lateral caudate-putamen fig 1( ). In contrast, cells expressing D_3 receptor mRNA are predominantly in the nucleus accumbens, with fewer scattered cells expressing comparatively lower levels of D_3 mRNA in the medial caudate-putamen fig 3( ). The cellular expression within the nucleus accumbens is also heterogeneous with high levels of expression and more cells expressing D_3 mRNA in the accumbens shell and septal pole. Cellular expression of D_1 and D_2 receptor mRNAs is also higher in the accumbens shell and septal pole, but the precise distribution of cells expressing the three mRNAs differ fig 1 and 3(and). In addition, there are higher levels of expression of D_1 and D_2 mRNA in the accumbens core relative to accumbens shell than observed with D_3. More ventrally, cells in the islands of Calleja express high levels of D_3 mRNA and no D_1 and D_2 receptor mRNA, whereas the cells of the olfactory tubercle express high levels of D_1 and D_2 receptor mRNA and no D_3 receptor mRNA fig 1 and 3 (and). The expression of D_3 receptor mRNA in the islands of Calleja is the highest observed in the CNS and appears to be selective for D_3.

The globus pallidus, a major efferent pathway of the striatum, shows a heterogeneity in dopamine receptor mRNA expression. Of the dopamine receptor mRNAs examined, only D_2 is present in the large cells of the globus pallidus fig 1 and 2 (and). Levels of D_2 receptor mRNA expression are lower compared to the striatum, with cells scattered throughout the globus pallidus and extending into the ventral pallidum. The number of cells expressing D_2 receptor mRNA are comparatively lower in the ventral pallidum. Interestingly, in the ventral pallidum, which receives direct projections from the shell portion of the nucleus accumbens, few scattered D_3 receptor expressing cells are detected.

In the septal nuclei, cells expressing D_1 receptor mRNA are primarily localized in the dorsal division of the lateral septum, whereas those cells expressing D_2 mRNA extend more medially and ventrally from the dorsal lateral septum to the intermediate lateral septum fig 1 ( ). Scattered cells expressing D_2 receptor mRNA are also observed in the medial septum and extend into the diagonal band of Broca, where D_2 receptor expression is prominent in the horizontal limb. Cells expressing D_3 receptor mRNA are localized in the medial portion of the lateral septum, with scattered cells in the medial septum and diagonal band of Broca.

Rostral–caudal differences are observed in the dopamine receptor expression in the hippocampal formation. While few, if any, D_1 expressing cells can be detected in the dorsal hippocampus, in the ventral hippocampus numerous cells express D_1 in the CA1–CA3 fields fig1 and 2 (versus). D_1 mRNA expression levels in these cells are low compared to the high levels of expression observed in the cells of the dentate gyrus fig 1 and 2 (and). Scattered cells expressing low levels of D_2 and D_5 receptor mRNA are found in the dorsal and ventral hippocampus, and as can

be seen in fig 3 , D_3 expressing cells are detected in the hippocampus and dentate gyrus.

Cells expressing D_1 mRNA are extensively distributed throughout the amygdaloid complex. Highest levels of D_1 receptor mRNA expression are found in the intercalated nuclei of the basolateral amygdala fig 2 ( ). D_1 expressing cells are also localized in the basolateral, medial, central, and cortical amygdala. In contrast, D_2 expressing cells are primarily localized in the lateral division of the central nucleus, with scattered cells in the basomedial amygdala. Only a few scattered cells expressing D_3 mRNA are detected in the medial amygdala.

Other regions in the telencephalon, where distribution of the dopamine receptors differ, include the endopiriform nucleus and claustrum. Cells in these areas express D_1 receptor mRNA fig 1 ( ) and no detectable D_3 or D_5 mRNA. Cells in the bed nucleus of the stria terminalis similarly express D_2 receptor mRNA, with no detectable D_1, D_3, or D_5.

DIENCEPHALON

The level of dopamine receptor mRNA expression in the thalamus is low compared to other regions of the CNS. Of the dopamine receptor mRNAs, D_1 is expressed most widely in the thalamus, with D_1 expressing cells in the anterior dorsal, anterior ventral, centromedial, paracentral, ventromedial, ventrolateral, and posterior nuclei, as well as the lateral habenula and dorsolateral geniculate body. The distribution of cells expressing D_2 receptor mRNA is more restricted, with high levels of expression in the cells of the zona incerta fig 2 ( ). Cells expressing D_3 mRNA are prominent in the paraventricular nucleus, with scattered cells in the centromedial, gelatinosus, ventromedial, ventrolateral nuclei, as well as the zona incerta and lateral and medial geniculate bodies. D_5 receptor mRNA expression is limited to the cells of the parafascicular nucleus.

In the hypothalamus, cells expressing D_1 receptor mRNA have a more limited distribution and are localized in the supraoptic, suprachiasmatic fig 1( ), paraventricular, and rostral arcuate nuclei. In contrast, cells expressing the D_2 receptor mRNA are more widely scattered in the hypothalamus and are found in the large cells of the lateral preoptic area, anterior hypothalamic area fig 2( ), and lateral hypothalamus. More caudally, cells in the posterior division of the arcuate nucleus and the ventral and dorsal premammillary nuclei express D_2 receptor mRNA. The distribution of cells expressing D_2 and D_3 mRNAs are clearly differentiated in the mammillary nuclei, where high levels of D_2 receptor mRNA are expressed in the cells of the lateral mammillary nuclei, whereas D_3 expressing cells are localized in the medial and mediolateral mammillary nuclei fig 3( ). In the posterior medial mammillary nucleus, however, both D_2 and D_3 receptor mRNAs are expressed. Tiberi et al. (52) suggest that cells expressing D_5 receptor mRNA are also localized in the lateral mammillary nuclei. Large scattered cells of the lateral hypothalamus also express D_3 receptor mRNA, suggesting that the D_3 receptors may also play a role in hypothalamic regulation.

MESENCEPHALON

Of the cloned dopamine receptors, cells expressing D_2 receptor mRNA are more widely distributed in the midbrain and hindbrain, and may be involved in a host of autonomic functions and in the regulation of dopamine release. Cells expressing D_2 receptor mRNA are prominent, for example, in the dopaminergic cells of the substantia nigra and ventral tegmental area, where their expression levels are high fig 2 ( ). Within the substantia nigra, cells expressing D_2 receptor mRNA are primarily in the pars compacta, with a few cells in the pars reticulata fig 2( ). Higher numbers of cells expressing D_2 receptor mRNA are observed in the caudal portion of the pars reticulata. In addition to the dopaminergic cells of the substantia nigra and ventral tegmental area, D_2 receptor mRNA is also localized in the magnocellular cells of the red nucleus that are part of the rubrospinal pathway. In contrast, while there are high levels of D_1 receptor binding in the substantia nigra, pars reticulata, no cells expressing D_1 receptor mRNA could be detected in the substantia nigra or ventral tegmental area. Similarly, while some reports suggest the localization of D_3 receptor mRNA in the cells of the substantia nigra (4, 46), research from our laboratory has failed to replicate these findings.

More dorsally in the superior colliculus, cells expressing D_2 receptor mRNA are localized in the intermediate and deep layers, with no cells detected in the superficial layer of the superior colliculus, where D_2 receptor binding is localized. Cells in both the central and external cortex of the inferior colliculus express moderate levels of D_2 receptor mRNA. In contrast, cells expressing D_1, D_3, or D_5 are not detected in either the superior or inferior colliculus.

Cells expressing D_2 receptor mRNA are also localized in the periaqueductal gray. D_2 expressing cells are visualized in both the dorsal and ventral central gray; however, there are higher numbers of D_2 cells in the ventral division, where they may be important in modulating analgesic responses. Large scattered cells in the midbrain reticular nuclei and more caudally in the pontine reticular and gigantocellular reticular nuclei of the hindbrain express moderate to high levels of D_2 receptor mRNA. These cells have been implicated in morphine-induced analgesia, and these findings are consistent with the role of D_2 receptors in the modulation of analgesic responses.

Cells in the rostral division of the interpeduncular nucleus express low levels of D_3 receptor mRNA. This represents a relatively selective dopamine receptor expression as D_1, D_2, and D_5 receptor mRNA is not detected in the interpeduncular nucleus.

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D_2 receptor mRNA expression is high in a number of raphe nuclei, where they may serve to regulate serotonin release. Cells expressing the D_2 receptor mRNA are visualized in the dorsal and caudal linear raphe, as well as the large cells of the raphe magnus. Cells expressing D_1 receptor mRNA are also observed in the raphe nuclei, where their primary localization is in the dorsal raphe. D_2 receptor mRNA is moderate to high in a number of brainstem nuclei (including the dorsal tegmental, lateral lemniscus, locus coeruleus,

parabrachial, and trigeminal) and the rostral nucleus of the solitary tract. Within the trigeminal nuclei, it is primarily the cells of the sensory and spinal trigeminal that express D_2 receptor mRNA. Scattered cells, comparatively few in number, also express D_2 receptor mRNA in the medial vestibular, hypoglossal, cuneate, and gracilis nuclei. D_1 receptor mRNA expression is more limited in the hindbrain, with D_1 expressing cells detected in the locus coeruleus, lateral parabrachial, and facial nuclei.

While D_3 receptor mRNA expression is not easily measured in most hindbrain nuclei, low levels of D_3 mRNA are observed in the inferior olivary nucleus. Low levels of D_2 receptor mRNA expression are also seen in the inferior olive.

In the cerebellum, there is a heterogeneity of dopamine receptor mRNA expression. High levels of D_1 mRNA expression are observed in the granular cells of the cerebellum. D_3 receptor mRNA expression, on the other hand, is limited to lobules 9 and 10 and in the parafluculus, where it is localized in large Purkinje cells fig 3( ). In contrast, no cells expressing either D_2 or D_5 receptor mRNA can be detected in the lobules of the cerebellum, but D_2 expressing cells are observed in the lateral cervical nucleus of the cerebellum.

MULTIPLE DOPAMINE RECEPTOR mRNA FORMS

Given the intronic organization of the D_2, D_3, and D_4 genes, multiple mRNA transcripts may be generated by each gene by alternative splicing. While variant and truncated forms of the D_3 and D_4 receptors have been reported (13, 16, 41, 55), two forms of the D_2 receptor that differ by a 29-amino-acid insertion in the third cytosolic loop have been studied most extensively (3, 17, 18, 36, 37, 39, 45, 53). In situ hybridization studies in pituitary and brain suggest that both mRNAs are expressed in the same cells, with the longer D_2 form (444 amino acids) being the more abundant species (29, 45, 53). The relative ratios of $D_{2(444)}$ and $D_{2(415)}$, however, do vary with brain area, and some studies have suggested that the D_2 receptor forms may be differentially regulated with antipsychotics or denervation (3, 39, 45). This is of both clinical and physiological relevance, because it suggests that there may be cellular mechanisms regulating the rate of splicing and the final ratios of receptor products that are inserted into the cell membrane. Several studies, for example, have demonstrated that the shorter form of D_2 [$D_{2(415)}$] is more efficiently coupled to G-proteins (18, 36, 37), suggesting that a change in receptor ratios of $D_{2(415)}/D_{2(444)}$ may result in an enhanced cellular response. A similar observation has been noted with the D_4 receptor, where the least number of insertions in the third cytosolic loop showed the highest affinity for dopamine receptor ligands and coupled more effectively to G-proteins (55).

In localization and regulatory studies, it is imperative, therefore, that multiple forms of the dopamine receptors are considered in interpreting the results. Multiple probes spanning different domains of the dopamine receptors need to be examined in order to evaluate distribution and regulatory effects on several dopamine receptor variants. This is more easily accomplished using cRNA protection assays, but can also be accomplished with in situ hybridization using oligomers that bridge divergent regions of two receptor

forms. The importance of examining the dopamine receptor variants has recently been highlighted by Schmauss et al. (41), who report a differential loss of D_3 receptor mRNA forms in the parietal and motor cortex of schizophrenics (see also New Developments in Dopamine and Schizophrenia).

COMPARISON OF THE DISTRIBUTION OF DOPAMINE RECEPTOR mRNAs AND BINDING SITES

The cloning of the dopamine receptors has allowed the direct comparison of the cells synthesizing the mRNA encoding these receptors to the sites of ligand binding as defined by receptor autoradiographic techniques. While such comparisons are never perfect because binding sites are localized in both cell bodies and terminals, and the mRNAs are predominantly in cell bodies, they do provide several kinds of valuable information concerning the anatomical organization of the receptor systems. First, receptors and other proteins are often cloned from cell lines that express a receptor at high levels. Localization of the mRNA encoding this receptor by in situ hybridization and the subsequent comparison to receptor autoradiographic distributions is important in determining whether the receptor is expressed in the CNS and has any physiological relevance. Second, by examining the anatomical connections in areas of the brain where there is an apparent mismatch between the expression of the mRNA and the binding, one may glean insights into the possible transport of receptors and the cellular origins of a receptor protein (26, 28). Third, a mismatch between mRNA expression and receptor binding may be indicative of the labeling of additional receptors that have not been pharmacologically characterized or identified with molecular biological techniques. Examples of how comparisons of receptor binding and receptor mRNA have been useful in understanding dopamine receptor anatomy follow.

In general, studies examining the distributions of cells expressing the dopamine receptor mRNAs and dopamine receptor binding sites have shown a good agreement between distributions (24, 26, 28). For example, D_2 receptor binding sites and the cells expressing D_2 receptor mRNA are similarly distributed in the caudate-putamen, nucleus accumbens, olfactory tubercle, globus pallidus, substantia nigra, ventral tegmental area, locus coeruleus, lateral parabrachial nucleus, and the nucleus of the solitary tract. Clear differences are seen in the zona incerta, where there are high levels of receptor mRNA but little, if any, receptor binding, which may be indicative of receptor transport. The converse is observed in the superior colliculus, where high levels of D_2 receptor binding are detected in the superficial layer, with no D_2 receptor mRNA expression. Because the superficial layer receives direct projections from retinal ganglia cells, the cell bodies and, therefore, the mRNA encoding these D_2 receptor sites is likely localized in the retina. This has been confirmed by in situ hybridization studies (58).

Clearly, not all mismatches observed in receptor binding and receptor mRNA distributions are due to receptor transport. The choice of receptor ligand and binding conditions are critical to ensure the labeling of a single receptor population. A particularly relevant example of this problem can be demonstrated with the "selective" D_2 ligand sulpiride. Many of the differences noted in the distribution of cells expressing D_2

mRNA and D_2 receptor binding when sulpiride was used as the labeling ligand may have been due to the binding of sulpiride to D_3 receptor sites. For example, the labeling of the islands of Calleja, medial mammillary nuclei, and lobule 9 and 10 of the cerebellum by sulpiride (56) suggest the labeling of D_3 binding sites and would have been interpreted as a mismatch when compared to the mRNA distribution visualized by D_2-selective cRNA probes.

Similar comparisons of the cells expressing D_1 receptor mRNA and D_1 receptor binding defined by [^{3}H]SCH 23390 in the presence of ketanserin show a good correspondence in regions such as the neocortex, caudate-putamen, nucleus accumbens, amygdala, and the suprachiasmatic nucleus, whereas other regions show a lack of correspondence (28). For example, high levels of D_1 receptor binding are observed in the entopeduncular nucleus and the substantia nigra, pars reticulata fig 4( ), whereas no D_1 mRNA can be detected in these areas. This lack of correspondence is suggestive that D_1 receptors are synthesized in the striatum and transported to efferent projections in the entopeduncular nucleus and substantia nigra, with some portion of D_1 binding sites remaining in striatal cell bodies. Ibotenic acid lesions in the striatum are consistent with this conclusion, and they demonstrate a coordinate loss of D_1 receptor mRNA and binding in the caudate-putamen that is accompanied by a degeneration of fibers projecting to the entopeduncular nucleus and substantia nigra (28). Differences in the laminar distribution of D_1 binding and D_1 receptor mRNA in the dentate gyrus and the cerebellum may also be due to receptor transport. Cells expressing D_1 receptor mRNA are localized in the granular cell layer of the dentate gyrus and cerebellum, while D_1 receptor binding is detected in the molecular layer of these brain areas. It is likely, then, that the granular cells in the dentate gyrus and the cerebellum synthesize D_1 receptors that are subsequently transported to either their dendritic or axonal fields, respectively, in the molecular layer.

A good correspondence between the distribution of cells expressing D_3 receptor mRNA and D_3 receptor binding defined by 7-OH-DPAT (24) and 7-*trans*-OH-PIPAT (33) has also been reported. High levels of D_3 receptor mRNA expression and D_3 binding are observed in the islands of Calleja, the rostral portion of the nucleus accumbens and in lobules 9 and 10 of the cerebellum. Lower densities of 7-*trans*-OH-PIPAT binding were also observed in medial caudate-putamen, substantia nigra, inferior olive, interpeduncular nucleus, and selected nuclei of the hypothalamus and thalamus. Interestingly, the D_3 binding observed in the substantia nigra was restricted to the pars reticulata (33), and not the dopaminergic cells of the pars compacta, as would be expected if D_3 receptors were autoreceptors. Given the lack of D_3 receptor mRNA expression detected by this laboratory in the rat substantia nigra, these findings suggest that the D_3 binding observed in the pars reticulata may be on extrinsic fibers projecting to the substantia nigra. Similarly, the localization of D_3 receptor mRNA in the Purkinje cells of lobules 9 and 10 of the cerebellum, along with the presence of D_3 receptor binding in the molecular layer of lobules 9 and 10, again suggests D_3 receptor transport.

The presence of relatively high levels of both D_1 and D_3 receptor binding and mRNA expression in the cells of the cerebellum is somewhat surprising, given the lack of a known dopaminergic projection to this region. This receptor–neurotransmitter mismatch

has been observed in several other neurotransmitter systems and is suggestive that perhaps not all receptors are in direct synaptic contact with their transmitter. In some cases—such as in the hippocampus and dentate gyrus, where a dopamine receptor–neurotransmitter mismatch has been suggested—a small dopaminergic projection has been reported by some investigators (51). Whether this projection to the hippocampus and dentate gyrus from the ventral tegmental area and medial tip of the substantia nigra (51) is functional and results in the formation of specific synaptic contacts with cells expressing dopamine receptors remains to be determined.

LESION AND COLOCALIZATION STUDIES

Selective lesion and dual mRNA localization studies have been very useful in differentiating the neuronal circuits in which the dopamine receptors may be localized. Because of the relative abundance of the D_1, D_2, and D_3 receptors in the basal ganglia and their clinical importance in schizophrenia, Parkinson's disease, and Huntington's chorea, most studies have focused on these brain regions. Both lesion and colocalization studies in the striatum suggest that the dopamine receptors are differentially distributed and organized into distinct neuronal systems.

With regard to the dopamine binding sites found within the caudate-putamen, lesions designed to selectively destroy cell bodies suggest that the vast majority of D_1 binding sites are postsynaptic and localized in intrinsic striatonigral cells (2, 11). In contrast, D_2 binding sites in the striatum are largely on presynaptic terminals originating most likely from cells in the cortex and midbrain (12, 38, 42). Only a small proportion of D_2 binding sites found within the striatum are postsynaptic and localized in striatal neurons. Of the intrinsic striatal neurons expressing D2 receptor mRNA, colocalization and tract-tracing studies suggest that a small proportion are localized in cholinergic neurons (9, 23), whereas the vast majority of cells examined in the dorsal striatum are colocalized with proenkephalin and project to the globus pallidus (15, 23). The vast majority of cells expressing D_1 receptor mRNA, on the other hand, coexpress prodynorphin and substance P mRNAs (15, 22) and project to the substantia nigra and entopeduncular nucleus, with a small proportion (10–20%) of cholinergic cells intrinsic to the striatum also expressing D_1 receptor mRNA (22). Cells expressing D_1 receptors are therefore localized in the dynorphin striatonigral pathway, whereas cells expressing D_2 receptors are part of the enkephalinergic striatopallidal pathway.

As indicated earlier, comparison of D_3 mRNA and D_3 binding distributions suggests that D_3 binding sites are largely synthesized by cells intrinsic to the striatum. Given the presence of D_3 binding and no D_3 receptor mRNA in the substantia nigra, pars reticulata, the D_3 binding sites are likely synthesized in the striatum, with a portion transported to the substantia nigra. This organization is very similar to the D_1 receptor, but lesion and tract-tracing studies need to be performed to confirm this conclusion. Given the lack of D_3 receptor binding reported in the entopeduncular nucleus, D_3 receptors may be localized only in a subpopulation of striatonigral neurons. A complete colocalization of D_3 and D_1 receptors is unlikely because D_3 expressing cells have a more restricted

distribution, being localized in the ventral striatum and medial portion of the dorsal striatum, whereas D_1 expressing cells are seen throughout the dorsal and ventral striatum.

While colocalization and lesion experiments suggest that D_1 and D_2 receptors are present in distinct populations of striatal cells and in different neuroanatomical circuits, electrophysiological studies suggest a high degree of D_1 and D_2 receptor colocalization (for review, see ref. 8). A possible explanation of these discrepant findings is that early electrophysiological studies may have used ligands that did not discriminate between D_2 and D_3 receptors, resulting in an apparent colocalization of D_1 and D_2. More recently, however, using a polymerase chain reaction (PCR) strategy, it has been suggested that D_1, D_2 and D_3 receptors may be colocalized in the same striatonigral neurons. Surmeier et al. (50) demonstrated that they could amplify D_1, D_2, and D_3 mRNAs from individual dissociated striatonigral neurons, and the vast majority of neurons tested showed a coexpression of all three dopaminergic receptors. It is presently unclear whether these mRNAs may have been induced in the process of tissue culturing, or are representative of a high incidence of colocalization of the dopamine receptors. It is certainly possible that striatal neurons may express all three dopamine receptor mRNAs to different extents, so that when a PCR strategy is used, each mRNA would be amplified, but when a colocalization approach is used, mRNAs expressed at low levels would go undetected. Further research is needed to resolve the extent of dopamine receptor colocalization.

DOPAMINE AUTORECEPTORS

Since the pioneering research of Carlsson (7), it has been clear that the activity of dopaminergic neurons in the midbrain can be modulated by the release or the exogenous application of dopamine. These receptors were termed "autoreceptors" and are thought to be important in maintaining dopaminergic activity in the nigrostriatal and mesolimbic dopamine systems (1, 44). With the cloning of the multiple dopamine receptors, the question arose as to which member of this family could serve as an autoreceptor. The available evidence suggests that the cloned D_2 receptor is the most likely candidate for a dopaminergic autoreceptor. Several lines of evidence support this conclusion: (i) D_2 receptor mRNA and binding is localized in the substantia nigra and the ventral tegmental area (30, 34, 57); (ii) colocalization studies demonstrate that D_2 receptor mRNA and tyrosine hydroxylase are expressed in the same dopaminergic neurons of the substantia nigra and the ventral tegmental area (29); and (iii) 6-hydroxydopamine lesions in the medial forebrain bundle result in simultaneous loss of tyrosine hydroxylase and D_2 receptor mRNA in the substantia nigra and the ventral tegmental area (27, 29).

It has been suggested by others that the D_3 may also function as an autoreceptor, but the evidence is not compelling. In situ hybridization studies performed in this laboratory in the rat suggest that the cells of the substantia nigra and ventral tegmental area do not express D_3 receptor mRNA and that D_3 receptor binding is localized in the pars reticulata of the substantia nigra and not the pars compacta, as would be expected for an autoreceptor. High levels of D_3 receptor mRNA have been reported in the lateral division of the substantia nigra pars compacta (4), but we have been unable to replicate these results. We can detect cells expressing D_3 receptor mRNA in the peripeduncular nucleus,

which is in close proximity to the lateral substantia nigra. The expressed D_3 receptor has a somewhat higher affinity for dopamine than does the D_2 receptor (46), but the anatomical evidence suggests it may not function as an autoreceptor in terms of modulating mesencephalic dopaminergic release. Similarly, the lack of a D_1 and D_5 receptor mRNA localization in the substantia nigra and ventral tegmental argues against these receptors serving as autoreceptors.

FUTURE DIRECTIONS

Future anatomical studies are likely to focus on several questions. In situ hybridization procedures need to be developed to specifically label the D_4 dopamine receptor. One report has suggested that the D_4 receptor mRNA is more abundant in the periphery (40), but this finding has not been confirmed by other laboratories. Northern blot analysis suggests that the D_4 receptor mRNA is expressed in the cortex and striatum of primates (54) at one-tenth the levels observed for the D_2 receptor. It is presently unclear whether the difficulty in detecting the D_4 receptor mRNA in the rat reflects a species difference in the level of expression or the lack of D_4 receptor mRNA expression in the rat CNS.

Further colocalization studies are also needed to more specifically define subpopulations of cells expressing dopamine receptors. Thus far, most colocalization studies have concentrated on the striatum, with relatively few neurotransmitters and receptors being explored. Colocalization studies need to be extended to a wider number of neurotransmitters and to other regions of the CNS. In conjunction with tract-tracing studies, such investigations will provide a better appreciation of dopamine receptor anatomy and circuitry, which is imperative in understanding of how dopaminergic drugs may function in the brain. These basic anatomical findings provide the framework for posing more precise questions concerning the regulation of the dopamine receptors and in addressing the neural systems that may be dysfunctional in psychiatric disorders such as schizophrenia (see Dopamine Receptors: Clinical Correlates, Acute Treatment of Schizophrenia, and Maintenance Drug Treatment for Schizophrenia), as well as in neurological diseases such as Huntington's and Parkinson's disease (Parkinson’s Disease). Dysregulation of the dopamine systems has also been implicated with the development of movement disorders or tardive dyskinesia, with chronic neuroleptic treatment (Maintenance Drug Treatment for Schizophrenia) and in opiate and cocaine addiction (Cocaine and Opioids).

The recent development of specific antibodies for D_1 and D_2 receptors (19, 25) has provided a means for examining the cellular distributions of these proteins with immunohistochemical techniques. These antibodies have provided a new means for examining dopamine receptor pathways in the CNS, and will be invaluable in examining the subcellular organization of the dopamine receptors. The development of these antibodies will also allow the study of receptor regulation at a third level, that of protein translation. This complements the ongoing studies examining receptor regulation at the gene transcription and ligand binding levels. Similar efforts are needed to develop selective antibodies for the D_3, D_4, and D_5 dopamine receptors.

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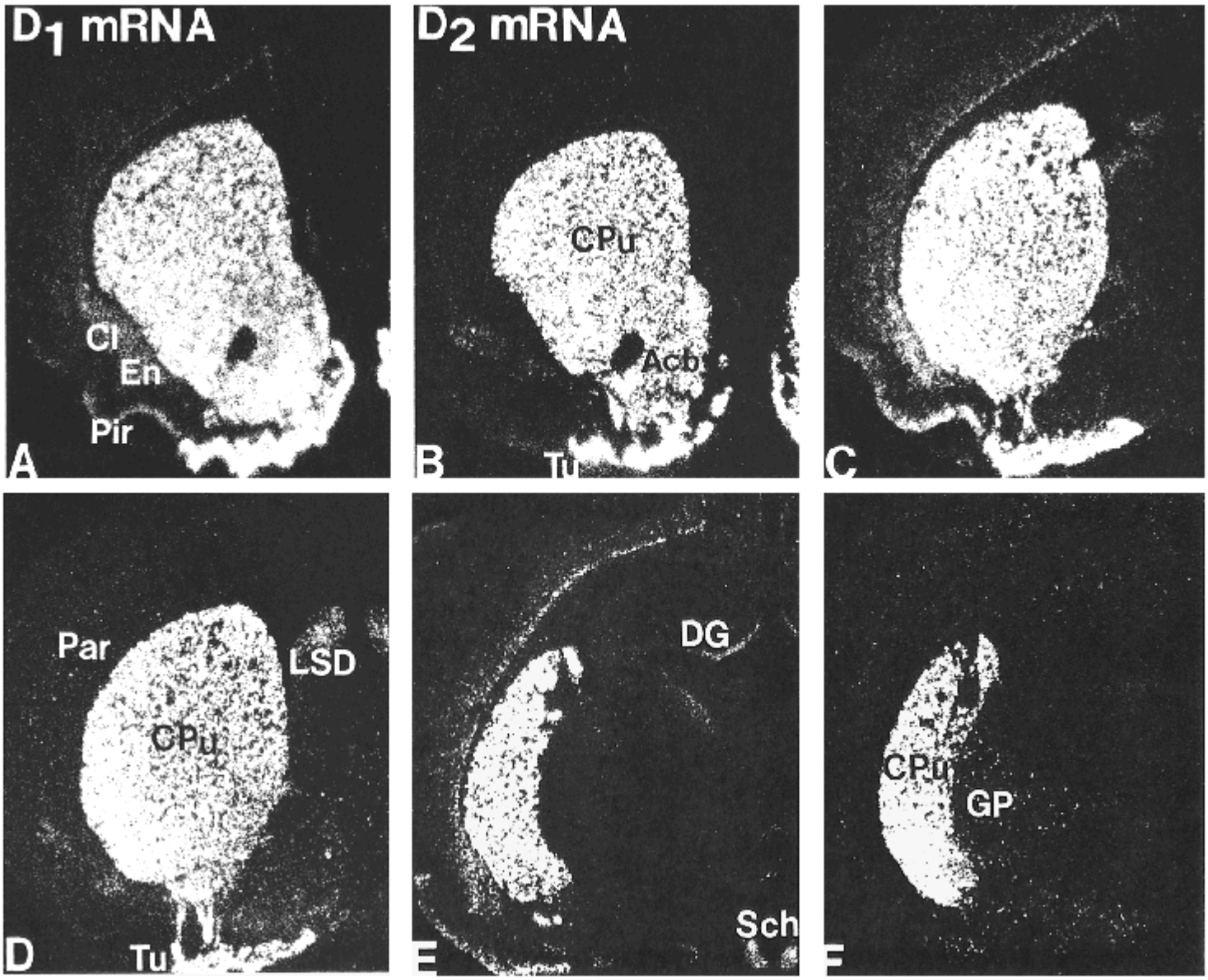
Figure 1.



Dark-field autoradiograms comparing the distributions of D_1 (**A, C, E**) and D_2 (**B, D, F**) receptor mRNAs in the rostral forebrain of the rat. D_1 and D_2 receptor mRNA expression is high in the caudate-putamen (CPu), nucleus accumbens (Acb), and olfactory tubercle (Tu). D_1 expressing cells are extensively distributed in neo- and palleocortical areas with particularly high levels in the piriform cortex (Pir). Other cells expressing D_1 receptor mRNA are localized in the dentate gyrus (DG), claustrum (Cl), endopiriform nucleus (En), and the suprachiasmatic nucleus of the hypothalamus (Sch). Cells expressing D_2 receptor mRNA demonstrate a different distribution, with cells localized in the lateral dorsal septum (LSD), in the globus pallidus (GP), and in scattered cells of the parietal (Par) and frontal cortex.

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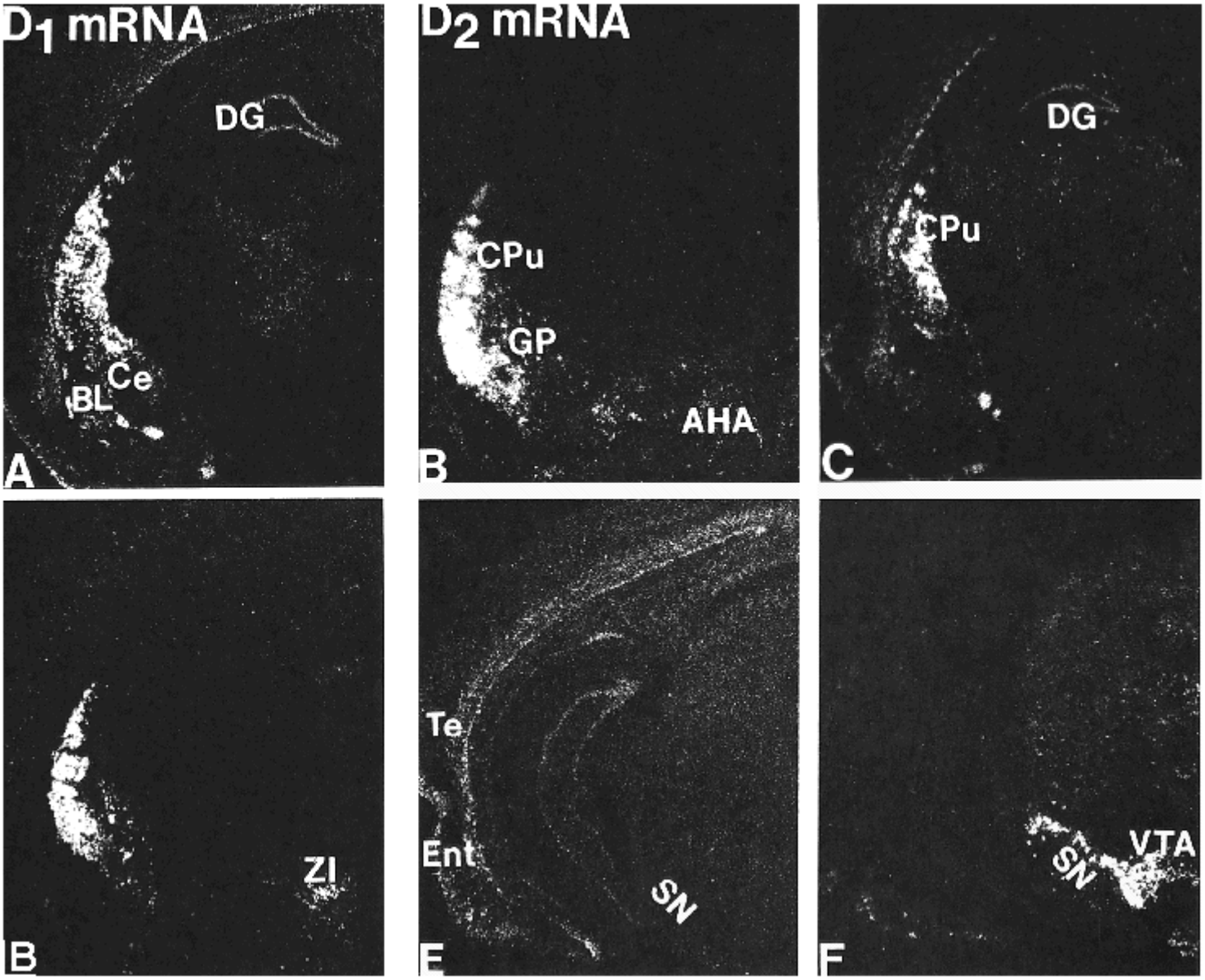
Figure 2.



Dark-field autoradiograms comparing the distribution of D_1 (**A, C, E**) and D_2 (**B, D, E**) receptor mRNAs in the rat di- and mesencephalon. Cells expressing D_1 and D_2 receptor mRNAs are differentially distributed, with cells expressing D_1 in the caudate-putamen (CPu), dentate gyrus (DG), basolateral (BL) amygdala and in the temporal (Te) and entorhinal (Ent) cortex. In contrast, cells expressing D_2 receptor mRNA are localized in the caudate-putamen (CPu), globus pallidus (GP), scattered cells of the anterior hypothalamic area (AHA), zona incerta (ZI), central amygdala (Ce), substantia nigra (SN), and ventral tegmental area (VTA).

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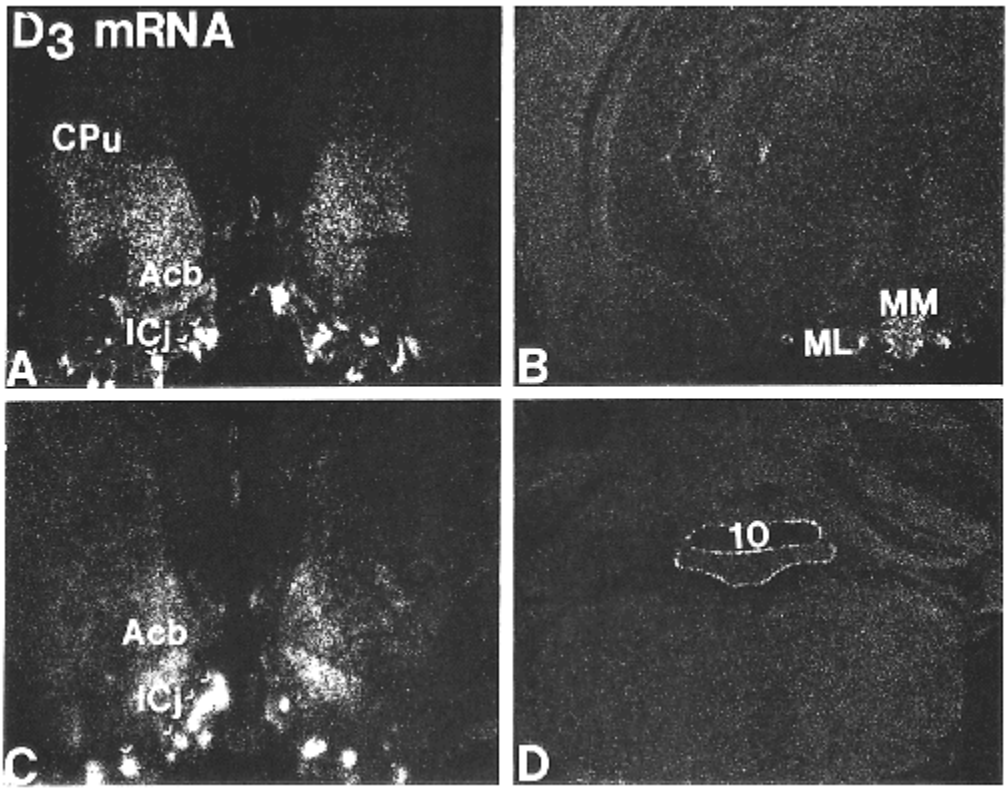
Figure 3.



Dark-field autoradiograms showing the distribution of D_3 receptor mRNA at two levels of the nucleus accumbens (**A** = rostral, **C** = caudal), caudal hypothalamus (**B**), and cerebellum (**D**). Cells expressing D_3 receptor mRNA are localized in the islands of Calleja (ICj), nucleus accumbens (Acb), medial caudate-putamen (CPu), medial (MM) and mediolateral (ML) mammillary nuclei of the hypothalamus, and lobule 10 of the cerebellum (10).

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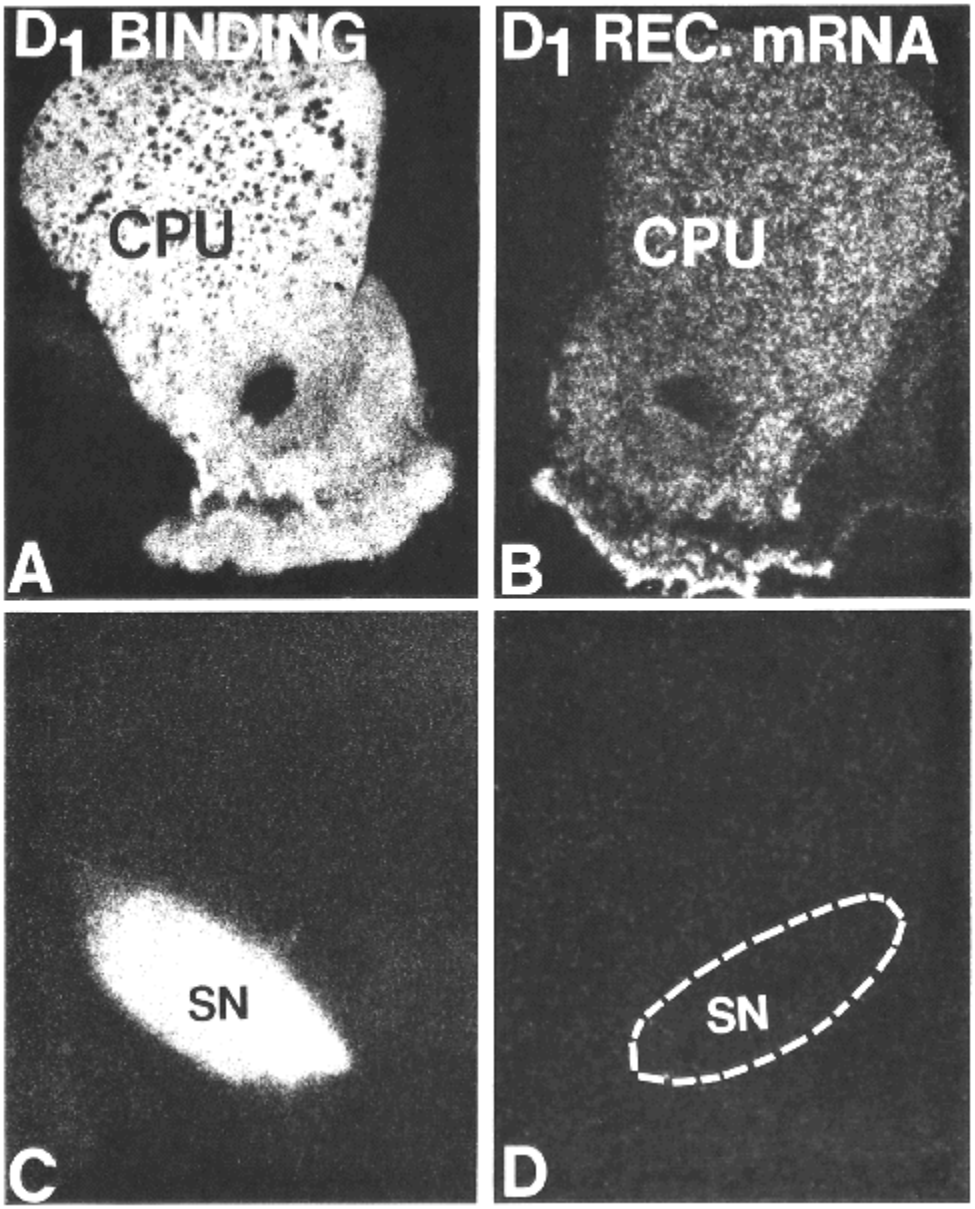
Figure 4.



Dark-field autoradiograms comparing the distribution of D_1 receptor binding (**A, C**) defined by [^{3}H]SCH 23390 in the presence of 1 μM ketanserin to D_1 receptor mRNA (**B, D**) in the rat striatum and substantia nigra. While there is a good correspondence between D_1 receptor mRNA expression and D_1 receptor binding in the caudate-putamen (CPU), nucleus accumbens, and olfactory tubercle, there is a lack of correspondence in the substantia nigra (SN), where high levels of D_1 receptor binding are observed and no D_1 receptor mRNA.

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