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A COMPARISON OF D_1 RECEPTOR BINDING AND mRNA IN RAT BRAIN USING RECEPTOR AUTORADIOGRAPHIC AND *IN SITU* HYBRIDIZATION TECHNIQUES

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Abstract—D_1, a subtype of the dopamine receptors, is widely distributed in the nervous system and has been shown to be positively coupled to adenylate cyclase. Using a combination of *in vitro* receptor autoradiographic and *in situ* hybridization techniques, the present study examines the co-distribution of D_1 receptor binding sites and D_1 receptor messenger RNA in adjacent rat brain sections. D_1 receptor binding sites were labeled using the selective antagonist [^{3}H]SCH23390 (4.6 nM) in the presence of 1 μM ketanserin, while the D_1 receptor messenger RNA was visualized with a ^{35}S-labeled riboprobe corresponding to a region between transmembrane domains III and VI of the rat D_1 receptor (bp 383–843). Analysis of serial sections suggested a good agreement between D_1 receptor binding and messenger RNA in several brain regions, including the paleocortex, caudate–putamen, nucleus accumbens, amygdala and suprachiasmatic nucleus. Marked discrepancies between D_1 receptor binding and messenger RNA were observed in other brain regions including the entopeduncular and subthalamic nuclei, substantia nigra (pars reticulata), hippocampus and cerebellum. While technical considerations may contribute to these results, much of the discordance between the distributions is likely due to the differential localization D_1 receptor messenger RNA in cell bodies and receptor binding sites on fibers and may provide insights into receptor synthesis, transport and membrane insertion. In the basal ganglia, for instance, D_1 receptors are synthesized in the striatum and are either transported to efferent projections in areas such as the substantia nigra, or remain localized in striatal cells bodies. Ibotenic acid lesions in the striatum are consistent with these conclusions and demonstrate a coordinate loss of D_1 receptor binding and messenger RNA in the caudate–putamen that is accompanied by a degeneration of fibers projecting to substantia nigra and a loss of D_1 binding in the pars reticulata. Neurons in the dentate gyrus and in the granular layer of the cerebellum, on the other hand, synthesize D_1 receptors and transport them entirely to either their dendritic or axonal fields, respectively, in the molecular layer.

This analysis provides a better understanding of dopaminergic receptor systems in the CNS and their anatomical organization.

Dopamine receptors in the CNS can be differentiated into two subtypes referred to as D_1 and D_2. Because of their clinical relevance in Parkinson's disease and schizophrenia, a great deal of research has been generated to localize these receptor sites in the CNS,[10,18,39] examine their regulation following drug treatment and lesions[5,13,20,31] and study the second messenger systems to which they are coupled.[36] The D_1 dopamine receptor subtype is widely distributed in the CNS,[2,4,16] can be upregulated by dopamine receptor antagonists, such as (*R*)-(+)-8-chloro-2,3,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol (SCH23390),[7,20] and is positively coupled to adenylate cyclase via a G_s protein.[6,32] D_2 receptors, on the other hand, are abundant in the basal ganglia and pituitary,[3,9,12] are upregulated following 6-hydroxydopamine lesions[13,31] and chronic haloperidol treatment,[25] and are either uncoupled or negatively coupled to adenylate cyclase.[29]

The recent cloning of the D_1 receptor[17,30,37,41] has provided a new means of examining issues of receptor structure, regulation and function. The D_1 receptor is a 446-amino acid protein and has a 40–44% amino acid identity with other cloned catecholamine receptors including the D_2 receptor. Based on hydrophobicity analysis, the receptor appears to have seven transmembrane domains and contains no introns within its protein coding region. It differs from the D_2 receptor which contains multiple introns and a long third cytosolic loop.[11] Transfection of the D_1 clone into COS cells produced the expression of D_1-specific binding ([^{3}H]SCH23390) and a positive coupling to adenylate cyclase.

The goal of this study is to provide a qualitative examination of the D_1 receptor mRNA and binding site distributions in the brain using a combination of *in situ* hybridization and receptor autoradiographic

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Abbreviations: EDTA, ethylenediaminetetra-acetate; 5-HT, 5-hydroxytryptamine; SCH23390, (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol; SCH23982, (5*R*)-8-iodo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol; SSC, sodium chloride–sodium citrate buffer.

techniques. An obvious concern in such an analysis is that receptor binding sites and mRNA may be localized on different neuronal elements. Receptor binding is localized on both cell bodies and fibers, while mRNA is thought to be localized primarily in cell bodies. Therefore, while regions of concordance would be expected since the D_1 receptor mRNA and binding sites are products of the same gene, some anatomical discordance would not be surprising due to differential localization on neuronal fibers. Such a lack of correspondence between receptor binding and mRNA, in fact, suggests receptor transport within a neuron, something with has previously only been effectively demonstrated with lesion studies.[1,38]

Given the complexity of this task, this study focuses on regions of the CNS with better known circuitry, such as the hippocampus, basal ganglia and cerebellum, with a minimal discussion of other regions of the forebrain and midbrain. To aid in determining whether the differences observed in receptor mRNA and binding are due to a differential localization on neuronal fiber, ibotenic acid lesions were performed to selectively destroy cell bodies[24] and trace their degenerating terminals.

EXPERIMENTAL PROCEDURES

Tissue preparation and incubation medium for receptor binding studies

Adult male Sprague–Dawley rats (Charles River, 200–250 g) were killed by decapitation and their brains were quickly removed and frozen in liquid isopentane (−30°C, 30 s). Frozen tissues were sectioned on a Bright cryostat (20 μm), thaw-mounted on precleaned and subbed microscope slides, and stored at −80°C. Immediately prior to using the tissue, the slide-mounted sections were gradually brought to room temperature and incubated (90 min, 22°C) with 200–400 μl of the selective D_1 antagonist [^{3}H]SCH23390[8,22] (71.3 Ci/mmol) in 50 mM Tris buffer (pH 7.5 at 25°C) containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$. The labeling concentration of [^{3}H]SCH23390 (4.6 nM) was chosen to correspond to three times the K_D value of the ligand[27] and results in a 75% receptor occupancy. As SCH23390 has been reported to bind serotonergic sites (5-HT_2), 1 μM ketanserin, a selective 5-HT_2 antagonist, was added to all [^{3}H]SCH23390 binding studies.

Following a 90-min incubation period,the slides were drained, washed in four 4-min consecutive 250 ml, 50 mM Tris (pH 7.6, 4°C) washes containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$. All slides were then quickly dipped in 250 ml distilled water (4°C) and dried with a portable hair dryer set to "cool". Nonspecific binding was evaluated by treating a parallel set of slides with the same concentration of tritiated ligand with a 1 μM final concentration of unlabeled SCH23390. The slides were then apposed to tritium-sensitive Hyperfilm (Amersham) for four weeks. The Hyperfilm was exposed at room temperature, developed in Kodak D-19 (4 min, 19°C), agitated in 2% acetic acid (30 s), fixed in Kodak Rapidfix (5 min), and washed under running water (30 min). Anatomical structures were determined using Nissl-stained sections in conjunction with the atlas of Paxinos and Watson.[33]

In situ *hybridization*

Slides adjacent to those used for autoradiographic mapping of D_1 binding sites were directly removed from storage at −80°C and placed into 4% formaldehyde for 60 min (22°C) prior to being processed for *in situ* hybridization.[28,35,40] Following three 5-min rinses in phosphate-buffered saline (pH 7.4), sections were treated with proteinase K (1 μg/ml in 100 mM Tris, pH 8.0, 50 mM EDTA) for 10 min at 37°C. Slides were then rinsed in water, followed by 0.1 M triethanolamine (pH 8.0), and treated with a mixture of 0.1 M triethanolamine (pH 8.0) and acetic anhydride (400:1, vol:vol) with stirring for 10 min. The sections were then rinsed in 2 × sodium chloride–sodium citrate buffer (SSC) (300 mM NaCl, 30 mM sodium citrate, pH 7.2) for 5 min, dehydrated through graded alcohols, and allowed to air dry.

Brain sections were hybridized with a [^{35}S]UTP-labeled 480-bp riboprobe generated to the region of the rat D_1 receptor spanning transmembranes III–VI (bp 383–843). cRNA probes generated with a pGEM transcriptional vector were diluted in hybridization buffer (75% formamide, 10% dextran sulfate, 3 × SSC, 50 mM Na_2PO_4 (pH 7.4), 1 × Denhardt's, 0.1 mg/ml yeast tRNA, 0.1 mg/ml sonicated, denatured salmon sperm DNA, 10 mM dithiothrietol) to result in a final concentration of 2×10^6 d.p.m./30 μl. Volumes of 30 and 50 μl of diluted probe were applied to coronal and horizontal sections, respectively.

After hybridization (overnight, 55°C) the slides were rinsed in 2 × SSC (5 min) and treated with RNase A (200 μg/ml in 10 mM Tris, pH 8.0, and 0.5 M NaCl) for 60 min at 37°C. Subsequently, sections were rinsed in 2 × SSC for 10 min (22°C), 1 × SSC for 10 min (22°C), 0.5 × SSC at 55°C for 60 min, 0.5 × SSC at room temperature for 10 min, and finally dehydrated in graded alcohols and air dried. Sections were then either exposed to Kodak XAR-5 X-ray film for three to 10 days and developed, or dipped in Kodak NTB-2 emulsion and stored at 4°C prior to development 14 days later.

Ibotenic acid lesions

Ten male Sprague–Dawley rats (200–250 g) were anesthetized and placed in a Kopf stereotaxic apparatus. To ensure that the right rostral caudate–putamen was completely ablated, ibotenic acid (2.0 μg/μl) dissolved in phosphate-buffered saline was infused at three different coordinates in the caudate–putamen using a 30-gauge cannula. With skull flat, the coordinates were B + 2.0/2.5/5.5, B + 1.0/3.0/5.5 and B − 0.4/4.0/5.5 corresponding in millimeters to the anterior–posterior/lateral/and ventral planes as illustrated in the atlas of Paxinos and Watson.[33] An infusion of 1 μl was given over a time interval of 2 min at each site and the cannula was allowed to remain in place for 5 min prior to removal. Fourteen days following the lesion, animals were killed by decapitation and their brains were frozen as described above. Brains were sectioned and processed for *in situ* hybridization and receptor autoradiography as described above. The extent of neuronal cell loss was evaluated with Nissl-stained sections and reference to the atlas of Paxinos and Watson.[33]

"Sense"-strand control

To assess whether the *in situ* signal reflected specific hybridization to the D_1 receptor mRNA, a "sense"-strand control study was performed whereby a series of paired, adjacent sections was divided into two sets. One set of slides was treated according to the *in situ* protocol described above, while the second set was treated identically, except that the cRNA probe used in the hybridization mixture was [^{35}S]UTP labeled sense-strand RNA.

Materials

[^{3}H]SCH23390 was purchased from New England Nuclear and the unlabeled SCH23390 was a kind gift from Dr James Woods, University of Michigan, Ann Arbor, U.S.A. Ibotenic acid was purchased from Vachon Natural Products, Washington.

RESULTS

D_1 receptor binding sites were widely distributed in the CNS with high levels observed in the basal ganglia and amygdaloid nuclei. By comparison, few other regions demonstrate the same high levels of D_1 receptor binding, but moderate amounts of receptor binding are seen in the septum, superior colliculus and the deep layers of cortex. D_1 receptor mRNA is also widely distributed in the CNS and generally shows a good agreement with the distribution of the D_1 binding sites. Given that the caudate–putamen contains high levels of both D_1 receptor mRNA and binding, descriptions of other brain areas are made relative to the binding or mRNA levels within this anatomical region.

Telencephalon

D_1 receptor binding sites are seen in all the cortical divisions and are particularly prominent in the piriform and entorhinal cortex. Within the neocortex, superficial layers (I–IV) of the frontal, parietal, temporal and occipital cortex have low levels of D_1 binding, with deep layers (V and VI) demonstrating moderate amounts. Paleocortical regions, such as the piriform and entorhinal cortex, demonstrate moderate to dense binding in layers I, II, V and VI and low levels in layers III and IV. Anterior cingulate cortex

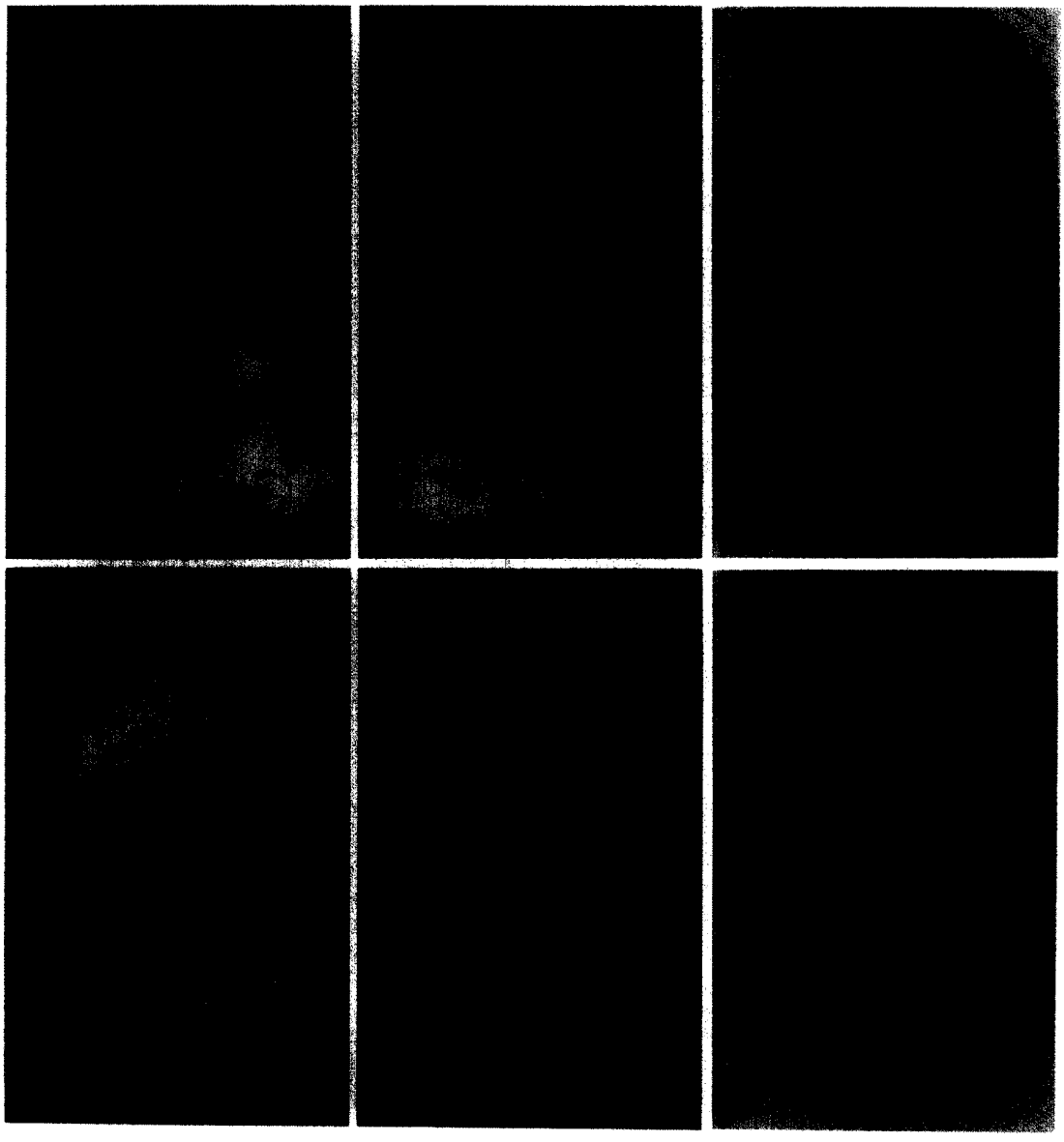

Fig. 1. Dark-field autoradiograms comparing D_1 receptor binding (A, D) and D_1 receptor mRNA (B, E) in the caudate–putamen (CPU), nucleus accumbens (ACB) and olfactory tubercle (OTU). Note the high levels of D_1 receptor binding and mRNA in the striatum and olfactory tubercle. Nissl-stained sections (C, F) are provided for anatomical reference. ac, anterior commissure.

appears more similar to the neocortical regions, with moderate levels of D_1 binding in layers V and VI and low levels in the superficial layers. D_1 receptor mRNA is also predominantly in the piriform and entorhinal cortex, with moderate levels in layers II, V and VI and no detectable mRNA in layer I. Only low levels of D_1 receptor mRNA are observed in the deep layers (VI) of frontal, parietal, temporal, occipital

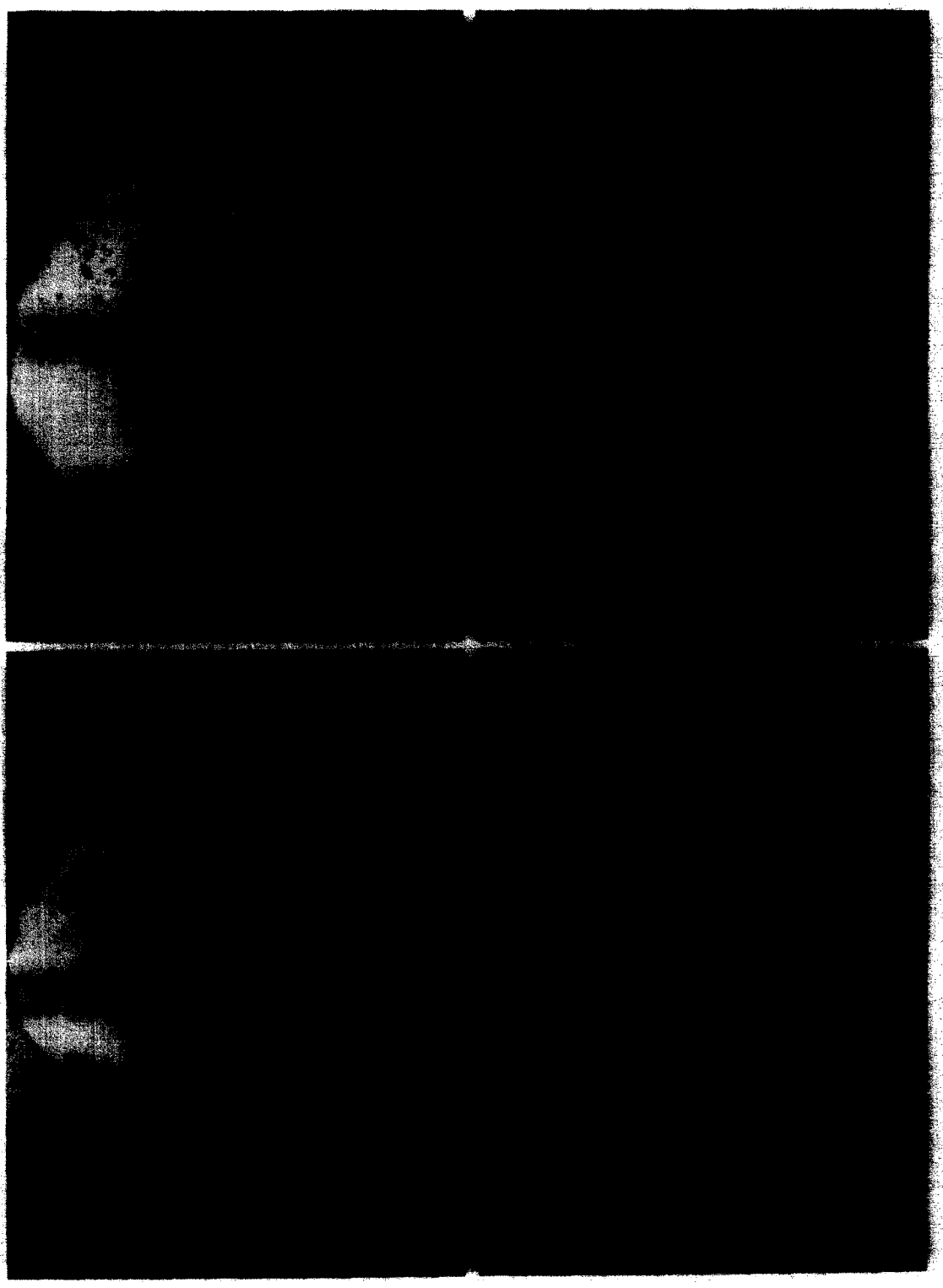

Fig. 2. Dark-field autoradiograms of D_1 receptor binding (A, C) and D_1 receptor mRNA (B, D) in serial sections. A good correspondence between D_1 receptor binding and mRNA is found in the caudate–putamen (CPU), amygdala (AMG) and suprachiasmatic nucleus (SCN), while a discordance is seen in the globus pallidus (GP), hippocampus (HPC) and thalamus (THL)

and cingulate cortex, and none can be detected in the more superficial layers (I–V). Deep layers of the medial, lateral and ventral orbital cortex show moderate D_1 binding and low levels of mRNA.

Within the telencephalon, the highest levels of D_1 receptor binding and mRNA are observed in the basal ganglia. In the caudate–putamen (Fig. 1A,D) D_1 receptor binding is densest in the head of the caudate–putamen with a rostral-to-caudal gradient observed. In addition, there are medial–lateral differences, with the densest binding found in the dorsomedial and ventrolateral portions of this nucleus. D_1 receptor mRNA (Fig. 1B,E) appears more uniform in the caudate–putamen, with somewhat higher levels observed ventrolaterally. Ventrally in the nucleus accumbens, dense levels of D_1 receptor binding and mRNA are observed particularly in the shell and extend into the core (Fig. 1D,E). Both D_1 receptor binding and mRNA show a rostral to caudal gradient in the nucleus accumbens, with the highest level of D_1 receptors observed rostrally. The olfactory tubercle similarly demonstrates high levels of D_1 receptors, with the D_1 receptor mRNA levels (Fig. 1B,C) exceeding those observed in the caudate–putamen and nucleus accumbens.

A region of discordance between D_1 receptor mRNA and D_1 receptor binding is the globus and ventral pallidum (Fig. 2A,B). In the globus pallidus, a region with major projections from the striatum, there are low levels of D_1 receptor binding with moderate levels observed in the ventral pallidum. In contrast, only a few cells of either the globus or ventral pallidum show D_1 receptor mRNA. Similarly in the septum, moderate levels of D_1 receptor binding are observed in the lateral nucleus with dense binding along its outer boundary, yet little D_1 receptor mRNA can be detected. The medial nucleus of the septum also shows a low level of D_1 receptor binding and no D_1 receptor mRNA, suggesting an absence of receptor synthesis in this region.

Within the hippocampal formation and dentate gyrus, D_1 receptor binding is moderate to low, with binding observed in the molecular layer of the dentate gyrus and in the stratum moleculare of the hippocampal formation (Fig. 2C). Interestingly, D_1 receptor mRNA is localized in the granular cell layer of the dentate gyrus and no D_1 receptor mRNA could be detected in the hippocampal formation (Fig. 2D). This suggests both a transport of D_1 receptors within dentate gyrus and an extra-hippocampal projection to the stratum moleculare of the hippocampus.

The amygdaloid complex, a region with varying densities of D_1 receptor binding, shows a good correspondence between D_1 receptor binding and D_1 receptor mRNA (Fig. 3A,B). The basolateral, lateral and central nuclei show low levels D_1 receptor mRNA and a moderate amount of D_1 binding (Fig. 3). Similarly, moderate to dense levels of D_1 binding and mRNA appear co-localized in the cortical and basomedial amygdaloid nuclei (Fig. 3A,B).

Diencephalon

The vast majority of the nuclei of the diencephalon show only low levels of D_1 receptor binding. Within the hypothalamus, low levels of D_1 binding are observed in most nuclei (Fig. 2C) with somewhat higher levels in the arcuate nucleus. Only the suprachiasmatic nucleus of the hypothalamus shows moderate to dense D_1 binding (Fig. 2A). Similarly, D_1 receptor mRNA is undetectable in most of the hypothalamic nuclei with low to moderate levels observed only in the suprachiasmatic nucleus (Fig. 2B). More dorsally, the thalamus shows only low levels of D_1 receptor binding in most nuclei with the habenula demonstrating moderate levels. D_1 receptor mRNA is undetectable in most of the thalamus.

The entopenduncular nucleus, a region receiving projections from the striatum, shows dense levels of D_1 receptor binding and no D_1 receptor mRNA (Fig. 4A,B). Such a marked discordance between D_1 receptor binding and mRNA suggests that the dopaminergic binding observed in this nucleus is localized on striatal projections. There is dense D_1 receptor labeling in the caudate–putamen that extends to the entopeduncular nucleus and substantia nigra, as can be seen from Fig. 4A. In fact, the D_1 containing fibers appear to course through the internal capsule and terminate in the substantia nigra. By comparison, D_1 receptor mRNA (Fig. 4B) is observed only in the caudate–putamen which likely contains the cells of origin.

Similarly, the subthalamic nucleus, a region receiving projections from the globus pallidus, also demonstrates dense D_1 receptor binding and no D_1 mRNA suggesting that the binding sites are localized on fibers.

Mesencephalon

In the midbrain, low levels of D_1 receptor binding are observed in the interpeduncular nucleus, periaqueductal gray, raphe nuclei, inferior colliculus and medial geniculate, with the superior colliculus demonstrating a moderate density of receptor binding (Fig. 5A). Within the dopaminergic cell groups, low levels of D_1 receptor binding can be observed in the ventral tegmental area and the pars compacta of the substantia nigra, while dense D_1 binding is found in the pars reticulata of the substantia nigra (Figs 4A, 5A). Despite the presence of D_1 receptor binding, no D_1 receptor mRNA could be detected in the substantia nigra, ventral tegmental area, superior and inferior colliculi, periaqueductal gray, raphe or medial geniculate nucleus (Figs 4B, 5B).

Cerebellum

Because of its precise lamination, the cerebellum, like the hippocampus, is an excellent tissue for examining receptor localization. D_1 receptor binding is predominantly in the molecular layer of the cerebellum (Fig. 5C), where low to moderate levels are

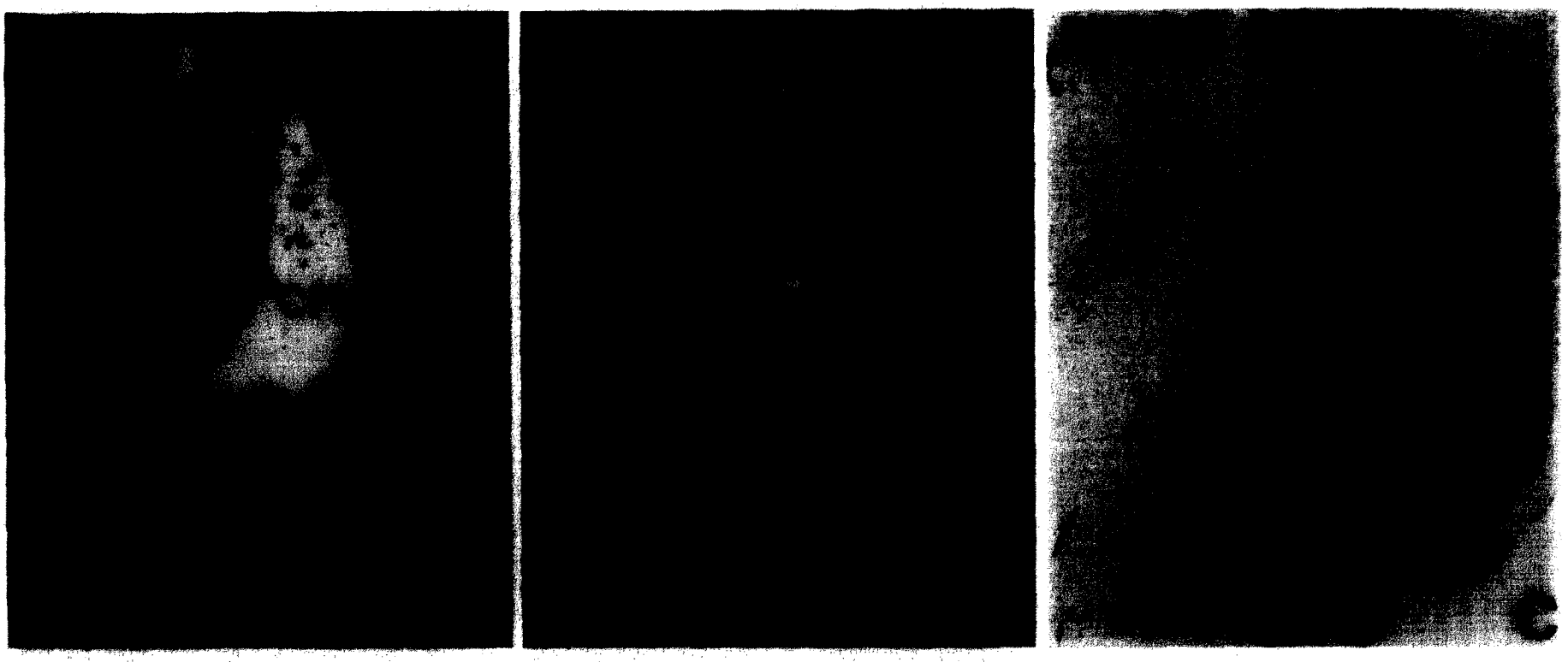

Fig. 3. Dark-field autoradiograms comparing D_1 receptor binding (A) and mRNA (B) in the amygdaloid complex. Moderate to high levels of D_1 binding and mRNA are seen in the cortical (Co) and basomedial nuclei, while moderate levels of D_1 binding and low levels of mRNA are observed in the central (Ce), lateral and basolateral (BL) nuclei. A Nissl-stained section (C) is provided for anatomical reference.

observed. This layer of the cerebellum receives intrinsic projections from the granular cell layer where low to moderate levels of D_1 receptor mRNA can be localized (Fig. 5D).

"Sense"-strand controls

No specific hybridization was observed with sections labeled with the sense-strand probe, while the antisense riboprobe showed specific hybridization (data not shown).

Ibotenic acid lesions

The ibotenic acid lesions were restricted to the caudate–putamen and primarily damaged the head and the rostral portion of the body of the caudate–putamen. Areas of cellular death, as determined by Nissl staining, corresponded precisely to regions of D_1 receptor binding and mRNA loss (Fig. 6A,B). The coordinate and nearly complete loss of both D_1 binding and mRNA suggests that the majority of D_1 receptors are localized in cell bodies in the

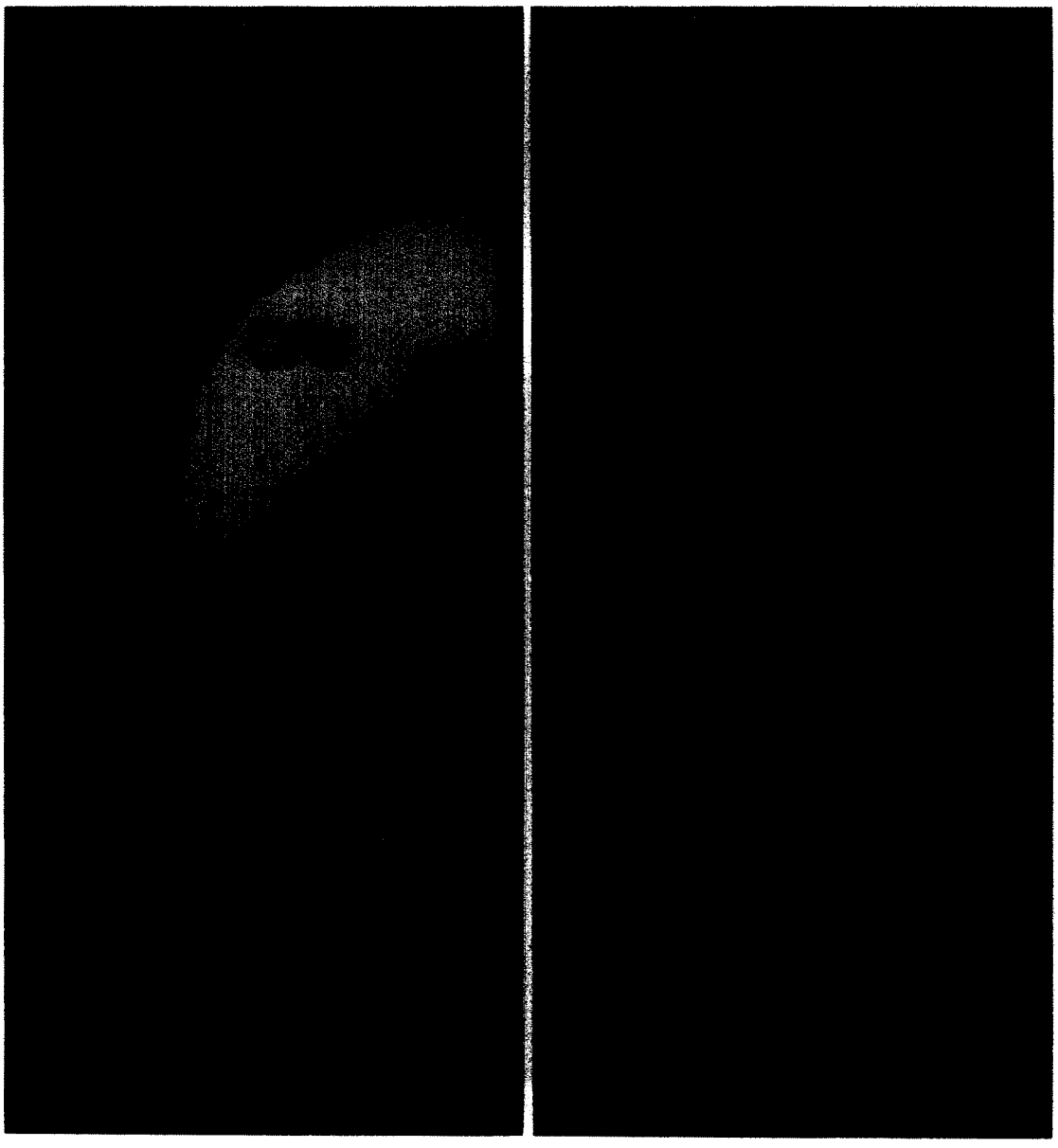

Fig. 4. Horizontal rat brain sections comparing D_1 receptor bidning (A) and mRNA (B). Dark-field images show a good correspondence between D_1 receptor binding and mRNA in the caudate–putamen (CPU) and cortex (CTX). High levels of D_1 receptor binding but no D_1 receptor mRNA are observed in the entopeduncular (EP) and substantia nigra (SN). In addition, D_1 receptors present in fibers of the internal capsule (ic) are probably transported from the striatum to the substantia nigra, pars reticulata. ac, anterior commissure.

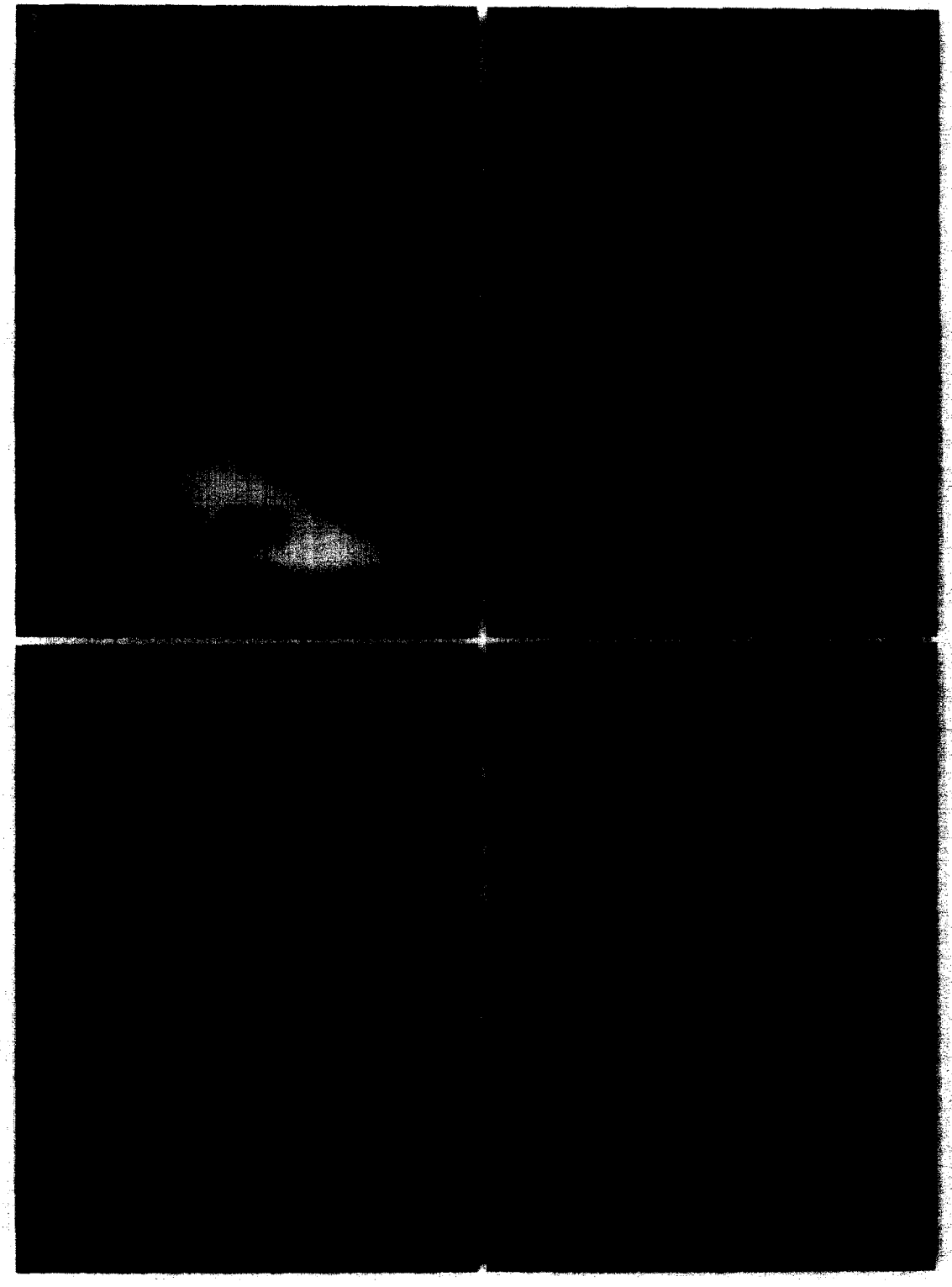

Fig. 5. Dark-field autoradiograms comparing D_1 receptor binding (A, C) and mRNA (B, D) in coronal sections of midbrain and cerebellum. High levels of D_1 binding are observed in the substantia nigra, pars reticulata (SN), while moderate and low levels are seen in the superior colliculus (SC) and periacqueductal gray (PAG), respectively. No D_1 receptor mRNA can be detected in these brain regions. In the cerebellum, D_1 receptor mRNA is localized to the cells of the granular layer (GL), while D_1 receptor binding is predominantly in the molecular layer (ML), suggesting the synthesis and transport of receptors to their terminal fields.

caudate–putamen. Striatal lesions also produced a significant loss of D_1 receptor binding in the medial and central pars reticulata of the substantia nigra (Fig. 6C) suggesting that the D_1 receptors found in this region are localized on fibers originating in the caudate–putamen.

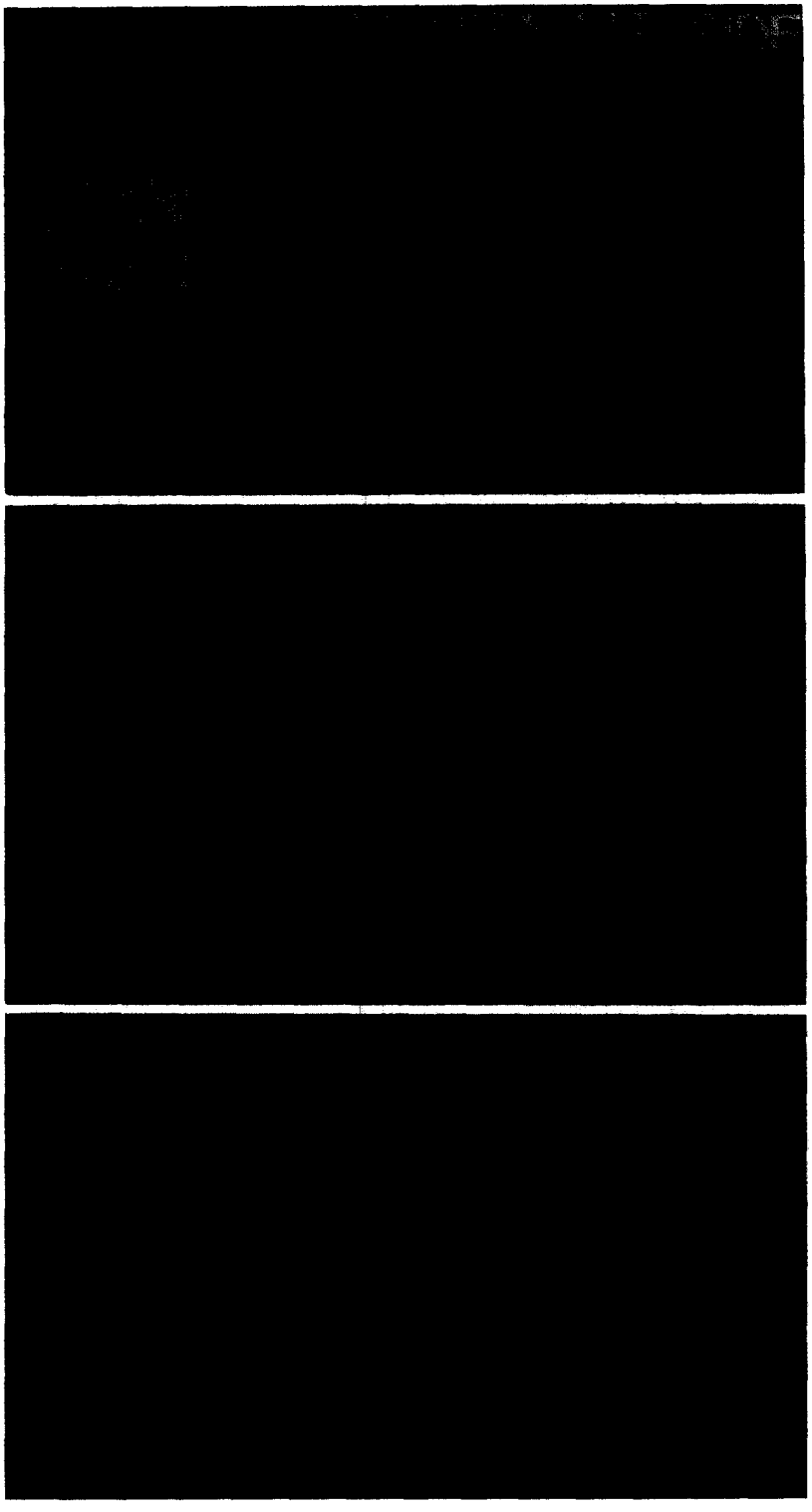

Fig. 6. Dark-field autoradiograms comparing D_1 receptor mRNA (A) and receptor binding (B, C) loss following unilateral striatal ibotenic acid lesions. Note the nearly complete loss of D_1 binding (B) and mRNA (A) in the lesioned (right) caudate–putamen (CPU) and the ipsilateral loss of D_1 receptor binding in the medial and central substantia nigra (SN), pars reticulata (C). ACB, nucleus accumbens.

DISCUSSION

A good correspondence between D_1 receptor binding and mRNA was found in areas such as the paleocortex, caudate–putamen, nucleus accumbens, olfactory tubercle, endopiriform nucleus, suprachiasmatic nucleus and amygdala. In addition, there appears to be a correlation in the relative abundance of both D_1 receptor binding and mRNA, such that regions of high receptor binding generally demonstrate high levels of D_1 receptor mRNA, while areas of low receptor binding demonstrate little or no detectable D_1 receptor mRNA. The amygdaloid complex is a particularly good example of such a correspondence, with specific nuclei displaying varying levels of D_1 receptor binding which correlated precisely to the relative amounts of D_1 receptor mRNA found in these areas.

Similarly, within the dopaminergic terminal fields of the basal ganglia, high levels of both D_1 receptor mRNA and binding are observed. In the nucleus accumbens, dense levels of D_1 receptor binding are seen in the shell and extend to form islands of very dense binding in the core of the nucleus. A gradient of D_1 binding is also seen with binding levels higher in the more rostral extent of the nucleus accumbens. D_1 receptor mRNA is similarly localized in the accumbens with higher levels observed in the rostral extent, and patches of very dense receptor mRNA are observed. In the caudate–putamen, no obvious rostral to caudal gradient in mRNA levels could be detected, while a rostral to caudal gradient in receptor binding was seen in this and in other studies.[3,10,23] The D_1 receptor mRNA distribution within the caudate–putamen is not homogeneous, however, with higher levels observed in the dorsomedial and ventrolateral portions of the nucleus—regions that correspondingly have higher amounts of D_1 receptor binding. The olfactory tubercle, a third major dopaminergic terminal field, has both high levels of D_1 binding and mRNA.

Despite an overall good correspondence in D_1 receptor binding and mRNA in the rostral forebrain, several regions in the CNS fail to show such a relationship. A lack of correspondence can be manifest in one of two ways: either the localization of D_1 receptor binding sites but no D_1 receptor mRNA, or the inverse, a visualization of D_1 receptor mRNA but no D_1 receptor binding. Examples of each type of discordance are observed and may be the result of several factors depending on the brain region involved.

Among the technical considerations that may contribute to this discrepancy in receptor binding and mRNA is ligand and probe selectivity. More specifically, the ligand or riboprobes chosen may label receptors other than D_1 thereby contributing to a lack of correspondence. While a consideration, it is an unlikely factor, as [^{3}H]SCH23390 has been reported to be a highly selective D_1 antagonist[8,22] and any 5-HT_2 receptor effects it may have should have been blocked with the addition of 1 μM ketanserin used in the present study. With regard to the selectivity of the riboprobe, the D_1 receptor is approximately 40% homologous to the D_2 receptor and the other catecholamine receptors that have been cloned. Given such a homology, the conditions used here should produce a selective hybridization to the D_1 receptor and studies from our laboratory have, in fact, demonstrated that the D_1 and D_2 receptor mRNA distributions can be easily differentiated in the CNS using this riboprobe and these hybridization conditions (unpublished observations). However, we cannot rule out the possibility that this riboprobe may hybridize to a yet uncloned receptor or a variant of the D_1 receptor.

A second factor that may contribute to a lack of correspondence is that of sensitivity. This may be an issue in areas with a low density of D_1 receptors, such as most of the nuclei of the hypothalamus and thalamus, inferior colliculus, medial septum, interpeduncular nucleus, periaqueductal gray, raphe and ventral tegmental area. These regions contain low levels of D_1 receptor binding and no detectable mRNA, suggesting a possible lack of sensitivity with present *in situ* hybridization techniques.

A third technical factor that may influence these results is that of differential quenching of the isotopes used to label the binding sites and mRNA. Clearly, [^{3}H]SCH23390 is differentially quenched in the brain, influencing the grain densities observed in various regions. As ^{35}S-labeled riboprobes are stronger beta emitters, differential quenching is less of a problem, but can still contribute to the results. An iodinated ligand could have been used to label the D_1 binding sites; however, differential quenching between ^{35}S and ^{125}I would still need to be considered. Furthermore, no significant anatomical differences have been observed between D_1 binding sites labeled with [^{125}I](5*R*)-8-iodo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol ([^{125}I]SCH23982) and [^{3}H]SCH23390.[15,26]

While such technical considerations can contribute to the results, there are regions in the brain where D_1 receptor mRNA and D_1 receptor binding can be clearly dissociated. Moderate to high densities of D_1 receptor binding are found in the superior colliculus, septum, ventral pallidum, entopeduncular nucleus, subthalamic nucleus, substantia nigra (pars reticulata) and the molecular layers of the denate gyrus, hippocampus and cerebellum, yet no D_1 receptor mRNA can be detected. Such regions of marked discrepancy between D_1 receptor binding and mRNA are likely to be due to a differential localization of mRNA in cell bodies, and binding sites on fibers and strongly suggest the presence of a specific transport of dopaminergic receptors not entirely appreciated using receptor autoradiographic techniques alone.

Based on lesion studies of the nigrostriatal system and receptor autoradiographic results,[2,4,14,19,34,38] sev-

eral investigators have suggested that D_1 receptor-containing cells are localized predominantly in the striatum and project caudally via the internal capsule and terminate in the globus pallidus, entopeduncular nucleus and the pars reticulata of the substantia nigra. Our *in situ* results are consistent with such a scheme and demonstrate D_1 receptor mRNA only in the striatum and not in any of these projection targets. Our ibotenic acid lesion results are also consistent with previous reports[2,4] and demonstrate a coordinate loss of D_1 receptor mRNA and binding in the cells of the striatum and a degeneration of fibers and loss of D_1 receptor binding in the substantia nigra.

Comparison of D_1 receptor binding and mRNA in the nigrostriatal system suggests that striatal neurons can synthesize D_1 receptors which are either transported along their fiber projections, or remain localized within the cell soma itself. This is probably not the case in other brain regions, such as the dentate gyrus and cerebellum. Neurons in these brain areas likely synthesize the D_1 receptor in their cell bodies and transport them mainly to either their dendritic (dentate gyrus) or axonal (cerebellum) fields as evidenced by the differential localization of D_1 receptor mRNA in the granular cell layers and D_1 binding in the molecular layers of both the dentate gyrus and cerebellum.

Within the hippocampal formation, D_1 receptor binding is found predominantly in the molecular layer, but no D_1 mRNA is detected in the pyramidal cell layer, suggesting an extra-hippocampal source, such as cells in the entorhinal cortex that are known to terminate in the molecular layer of the hippocampus.

In addition to long striatonigral projections, D_1 receptors are apparently localized within local circuits. The co-localization of D_1 receptor mRNA and binding in discrete nuclei of the amygdala and the suprachiasmatic nucleus, for example, suggest that the D_1 binding sites in these areas are either found on cell bodies or are on short or intrinsic projections. It is conceivable, however, that despite such a co-distribution, the D_1 receptor mRNA and binding may not be localized in the same cells with, for example, the D_1 binding on terminals of one population of cells and the mRNA on another within the same nuclei. While it is difficult at present to determine the exact nature for such a co-localization, the use of electron microscopy and immunohistochemistry should be able to differentiate these possibilities.

Comparison of D_2 receptor mRNA and binding similarly suggests that the D_2 receptor is also differentially transported within neurons of the CNS.[27] In the zona incerta, for example, high levels of D_2 receptor mRNA can be observed with little or no D_2 binding, suggesting that the D_2 receptor is translated and transported to sites distant from the point of transcription, such as the lateral system or hypothalamus where D_2 binding can be measured. D_2-containing neurons of the substantia nigra, pars compacta, demonstrate yet another example, with the synthesis and transport of the D_2 receptor to both their soma in the substantia nigra and their fibers in the forebrain. It is evident from both the D_1 and D_2 receptor results that different populations of neurons are capable of directing the transport and ultimate insertion of receptors to specific regions of their neuronal membranes affecting neuronal excitability and specificity.

Though by no means complete, this analysis provides a better understanding of the dopamine receptor anatomy in terms of both its circuitry and cellular elements. While the use of receptor autoradiography has been invaluable in understanding the distribution of receptor binding sites, it cannot easily dissociate which binding sites are localized on cell bodies or fibers. Its combination with *in situ* hybridization techniques provides a new means of visualizing potential circuits and the differential cellular transport of receptors. The sensitivity of such an analysis largely depends on the anatomical substrate involved, with regions of discrete receptor binding, such as the suprachiasmatic nucleus, or laminated brain areas, such as the cerebellum, hippocampus and olfactory bulb being more amenable for this analysis. Similarly, well-defined circuits, such as the nigrostriatal system are far easier to examine relative to other brain areas.

What remains unclear, however, is which sites are functional. Receptor sites in long axonal projections may not be coupled to second messenger systems and unable to mediate physiological responses. While nascent receptors may be able to bind a ligand, they may not be functional until they have been inserted into terminals or dendritic membranes. Clearly, the present results need to be combined with microinjection studies to gain a better understanding of the anatomical circuitry and physiology. Future studies combining these techniques with other anatomical methods of visualizing second messengers or phosphinositide turnover[21] will also be important in resolving these biological issues.

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