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Localization of D_1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission

(papez circuit/entorhinal cortex/prefrontal cortex/amygdala/hippocampal formation)

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ABSTRACT **Expression of a D_1 dopamine receptor was examined in the rat brain by using a combination of *in situ* hybridization and *in vitro* receptor autoradiography. Cells expressing D_1 receptor mRNA were localized to many, but not all, brain regions receiving dopaminergic innervation. The highest levels of hybridization were detected in the caudate-putamen, nucleus accumbens, and olfactory tubercle. Cells expressing D_1 receptor mRNA were also detected throughout the cerebral cortex, limbic system, hypothalamus, and thalamus. D_1 receptor mRNA was differentially expressed in distinct regions of the hippocampal formation. Dentate granule cells were labeled in dorsal but not ventral regions, whereas the subicular complex was prominently labeled in ventral but not dorsal regions. Intermediate to high levels of D_1 binding sites, but no hybridizing D_1 receptor mRNA, were detected in the substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus, and subthalamic nucleus. In these brain regions, which are involved in the efferent flow of information from the basal ganglia, D_1 receptors may be localized on afferent nerve terminals originating in other brain regions. These results indicate that in addition to a role in control of motor function, the D_1 receptor may also participate in the cognitive, affective, and neuroendocrine effects of dopaminergic neurotransmission.**

The diverse physiological effects of dopamine in the mammalian brain are mediated by receptors, which have been classically subdivided on the basis of pharmacological, biochemical, and functional differences into two subtypes referred to as D_1 and D_2 (1). Activation of D_1 receptors, which are the most abundant dopamine receptor subtype in brain, stimulates adenylyl cyclase activity (1). Activation of D_2 receptors inhibits adenylyl cyclase activity and increases K^+ channel conductance (2).

Traditionally, the behavioral actions of dopaminergic agents have been attributed to interaction with D_2 receptors, whereas the role of D_1 receptors in the central nervous system has been less clear. Thus, the therapeutic efficacy of an antipsychotic drug correlates with its antagonist affinity for the D_2 receptor (3). Similarly, the motor, endocrine, and antiparkinsonian effects of ergot derivatives, such as bromocryptine, are believed to result from stimulation of D_2 receptors (4). Recent studies, however, suggest that D_1 receptors in the brain may have important functions. D_1 receptor activation has been reported to inhibit neurite outgrowth and neuronal growth cone motility (5), regulate cerebral glucose utilization (6), inhibit serotonin release in the substantia nigra (7), stimulate γ-aminobutyric acid release in the substantia nigra (8) and striatum (9), and modify the activity of D_2 receptors (reviewed in ref. 10). Furthermore, D_1 receptor stimulation mediates the activation of immediate-early genes by psychomotor stimulants (11).

Previous receptor autoradiography studies have revealed a widespread distribution of D_1 receptor binding sites in brain (12–16). These studies are limited, however, to regional topographic localization of the D_1 receptor protein. In addition, the radioligands used to label D_1 receptors also bind to other neurotransmitter receptors, in particular, type 1c and 2 serotonin receptors (16). Therefore, localization of the D_1 receptor at the cellular level has been difficult to determine with certainty by this technique.

Recently, we (17), and others (18–20), have isolated cDNA and genomic clones encoding a D_1 dopamine receptor subtype that is coupled to the stimulation of adenylyl cyclase. The deduced amino acid sequence reveals that the D_1 receptor shares sequence and structural similarity with the large family of guanine nucleotide binding protein-coupled receptors thought to traverse the plasma membrane seven times. To gain insight into the possible functional role of the cloned D_1 receptor, we have examined the sites of synthesis of D_1 receptor mRNA in rat brain by *in situ* hybridization histochemistry. *In situ* hybridization provides a very sensitive and specific means for localizing cell bodies expressing D_1 receptor mRNA and thus complements studies of the regional distribution of D_1 receptor protein.

METHODS

Animals and Tissue Preparation. Male Sprague–Dawley rats (300–375 g; Charles River Breeding Laboratories) were killed by decapitation. Brains were removed and frozen on an aluminum block cooled with liquid nitrogen. Frozen sections (10 μm) were prepared in a cryostat, thaw-mounted onto polylysine-coated slides at room temperature, and stored in the cryostat (−28 to −30°C) for 4–7 hr. The slide-mounted sections were subsequently stored at −70°C to −85°C until processed for *in situ* hybridization or radioligand binding.

***In Situ* Hybridization.** A 0.45-kilobase (kb) *Eco*RI–*Cla* I restriction fragment and a 2.1-kb *Eco*RI restriction fragment from the rat striatal D_1 receptor cDNA (17) were subcloned into pBluescript (Stratagene). Uridine 5′-[α-[^{35}S]thio]triphosphate-labeled antisense or sense-strand RNA probes were prepared by *in vitro* transcription of linearized templates with the appropriate RNA polymerases, to a specific activity >10^9

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Abbreviation: TM, transmembrane domain.

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cpm/μg. The 2.1-kb *Eco*RI restriction fragment codes for a portion of the rat D_1 receptor including transmembrane domain (TM) 7, the carboxyl-terminal tail, and the 3′ untranslated region. A 0.45-kb *Eco*RI–*Cla* I restriction fragment, coding for a portion of the rat D_1 receptor including TM 4, extracellular loop 2, TM 5, the third intracellular loop, TM 6, and a portion of extracellular loop 3, was also used in these studies. These regions of the D_1 receptor share <50% nucleotide sequence identity with other cloned guanine nucleotide binding protein-coupled receptors.

In situ hybridization was conducted essentially as described (21) with the following modifications. Tissue sections were thawed and fixed for 10 min in 4% paraformaldehyde in phosphate-buffered saline at 4°C. After hybridization with 2.5–5.0 × 10^6 cpm of heat-denatured (15 min at 65°C), ^{35}S-labeled RNA probes per ml, the sections were treated with RNase A at 50 μg/ml (21). The final high-stringency wash was 4 liters of 0.1× standard saline citrate, 14 mM 2-mercaptoethanol, and 0.05% sodium pyrophosphate for 3 hr at 55–58°C, after which the slides were gradually cooled to room temperature in the same solution overnight. The slides were dipped in Kodak NTB3 or NTB2 nuclear track emulsion (Eastman Kodak), exposed for 2–4 weeks at −15°C, and analyzed with a Wild microscope (Leitz) equipped for low-power dark-field photomicroscopy.

Receptor Autoradiography. To label D_1 receptors, sections were incubated for 1 hr at 23°C with 0.5 nM ^{125}I-labeled SCH 23982 (a D_1 receptor antagonist) in binding buffer (50 mM Tris·HCl, pH 7.5/120 mM NaCl) containing 40 nM ketanserin to mask type 2 serotonin receptors. Sections were then washed in two changes of ice-cold Tris buffer (50 mM Tris·HCl, pH 7.5) for 1 min each, followed by a 15-sec rinse in distilled water. Sections were dried with a stream of cool air and apposed to Kodak SR-5 film for 1–3 weeks.

RESULTS AND DISCUSSION

Specificity of Hybridization. To examine the specificity of the *in situ* hybridization autoradiographic signal, adjacent sections were hybridized with two complementary RNA probes derived from different regions of the mRNA encoding the D_1 receptor (see *Methods*). Hybridization with uridine 5′-[α-[^{35}S]thio]triphosphate-labeled antisense RNA probes derived from the 2.1-kb *Eco*RI fragment (Fig. 1*A*) or the 0.45-kb *Eco*RI–*Cla* I fragment (data not shown) gave identical hybridization patterns. In contrast, hybridization of adjacent sections with a sense-strand control probe resulted in background labeling (Fig. 1*B*). Furthermore, background labeling was observed over dopaminergic neurons of the substantia nigra, ventral tegmental area, hypothalamus, and olfactory bulb (Figs. 2 and 3; R.T.F., unpublished observations). Therefore, under these stringent hybridization conditions, the D_1 receptor probe does not cross-hybridize with the mRNAs coding for other previously characterized guanine nucleotide binding-protein coupled receptors including the D_2 and D_3 dopamine receptors (22–25).

Distribution of D_1 Receptor mRNA in Rat Brain. *Basal ganglia.* The most intense hybridization signal in the central nervous system was observed over the caudate-putamen, nucleus accumbens, and olfactory tubercle (Fig. 1). Within the basal ganglia, labeled cells were also observed in the islands of Calleja, substantia innominata, and ventral pallidum.

In contrast, no specific hybridization signals were observed in the globus pallidus, entopeduncular or subthalamic nuclei, or substantia nigra pars compacta or pars reticulata (Fig. 2 *A* and *C*). These brain regions, which are involved in the efferent flow of information from the basal ganglia (26), do, however, exhibit intermediate to high levels of ^{125}I-labeled SCH 23982 binding (Fig. 2 *B* and *D*). D_1 receptors in these brain regions may be present on afferent nerve terminals originating in other brain regions. Consistent with previous studies indicating that dopamine selectively stimulates the release of γ-aminobutyric acid in substantia nigra, D_1 receptors in the substantia nigra pars reticulata may be located on nerve terminals of striatonigral afferents containing γ-aminobutyric acid (8). We cannot rule out the possibility that a distinct gene may code for a related D_1 receptor subtype in these brain regions that we cannot detect with our stringent hybridization conditions.

Cerebral cortex. Cells expressing D_1 receptor mRNA were observed throughout the cerebral cortex (Fig. 3). Allocortical regions that exhibited prominent labeling include the cingulate, entorhinal, infralimbic, insular, perirhinal, piriform, and retrosplenial cortices. In the entorhinal cortex, labeled neurons were observed in layers II and V–VI. In ventral regions of the lateral entorhinal cortex, labeled neurons often occurred as clusters. This pattern of labeling corresponds well with the previously described patches of dopaminergic terminals that innervate ventral aspects of the lateral entorhinal cortex (27).

In the isocortex, prominent labeling was observed in the anteromedial and suprarhinal regions of the prefrontal cortex (Fig. 3*A*). Furthermore, labeling of neurons in layer 6 of the isocortex was observed, especially the pericallosal region, at all levels examined (Fig. 3). Cells containing D_1 receptor mRNA were also sparsely distributed throughout the cortical mantle. These results are consistent with previous reports of the topographical distribution of cortical dopaminergic innervation (27–29) and D_1 binding sites (12–16). The colocalization of D_1 receptor mRNA and binding sites in the cerebral cortex reinforces previous lesion studies indicating that a significant proportion of cortical D_1 receptors are postsynaptic (30).

Hippocampal formation. Recent studies support a transmitter role for dopamine in the rat hippocampal formation

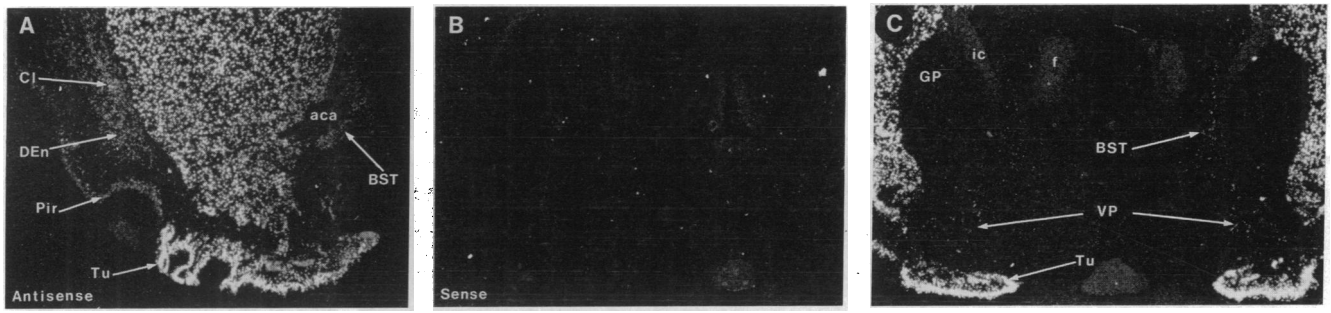

FIG. 1. Localization of D_1 receptor mRNA in rat brain by *in situ* hybridization. Coronal sections were hybridized with ^{35}S-labeled single-stranded RNA probes in the antisense (*A* and *C*) or sense (*B*) orientation derived from the 2.1-kb *Eco*RI fragment of the rat D_1 receptor cDNA. In these dark-field photomicrographs, the silver grains representing hybridized probe appear white. Tu, olfactory tubercle; Pir, piriform cortex; DEn, dorsal endopiriform nucleus; Cl, claustrum; BST, bed nucleus of the stria terminalis; aca, anterior commissure; VP, ventral pallidum; GP, globus pallidus; ic, internal capsule; f, fornix.

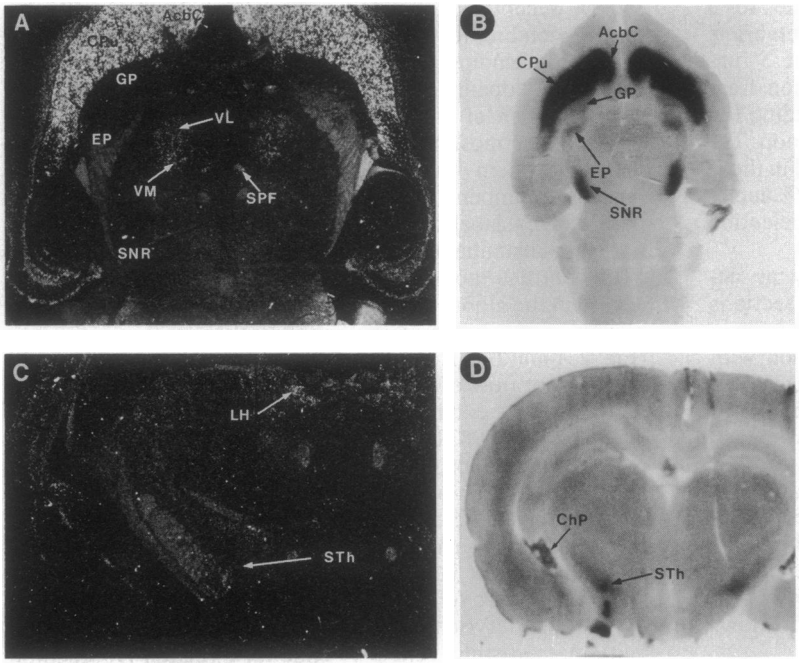

FIG. 2. Comparison of the distribution of D_1 receptor mRNA and ^{125}I-labeled SCH 23982 binding in adjacent rat brain sections. (*A*) Horizontal section through the mesencephalon demonstrating high levels of D_1 receptor mRNA in the caudate-putamen (CPu) and nucleus accumbens (AcbC) as well as the ventral lateral (vL), ventral medial (VM), and subparafascicular (SPF) nuclei of the dorsal thalamus. In contrast, background labeling was observed over the substantia nigra pars compacta or pars reticulata (SNR), globus pallidus (GP), and entopeduncular nucleus (EP). (*C*) Similarly, background labeling was observed over the subthalamic nucleus (STh) following *in situ* hybridization. Labeled cells were observed in this section, however, in the lateral habenula (LH). (*B* and *D*) Intermediate to high levels of ^{125}I-labeled SCH 23982 binding (in the presence of 40 nM ketanserin to mask type 2 serotonin receptors) were observed in the caudate-putamen, nucleus accumbens, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus, and subthalamic nucleus. ^{125}I-labeled SCH 23982 binding in the choroid plexus (ChP) most likely represents binding to type 1c serotonin receptors.

(31). The hippocampal formation receives a sparse dopaminergic innervation (32), dopamine exerts electrophysiological actions within the hippocampus (33), and ^{125}I-labeled SCH 23982 binding sites exist in the molecular layer of the dentate gyrus (13). However, the cellular localization of hippocampal D_1 receptors is not known. A differential localization of cells expressing D_1 receptor mRNA was observed in different regions of the hippocampal formation (Fig. 4). In the ventral hippocampus (Fig. 4*C*), prominent labeling of the subicular complex was observed, including the subiculum proper, the pre- and parasubiculum. Also, a small number of labeled pyramidal cells were observed dispersed throughout

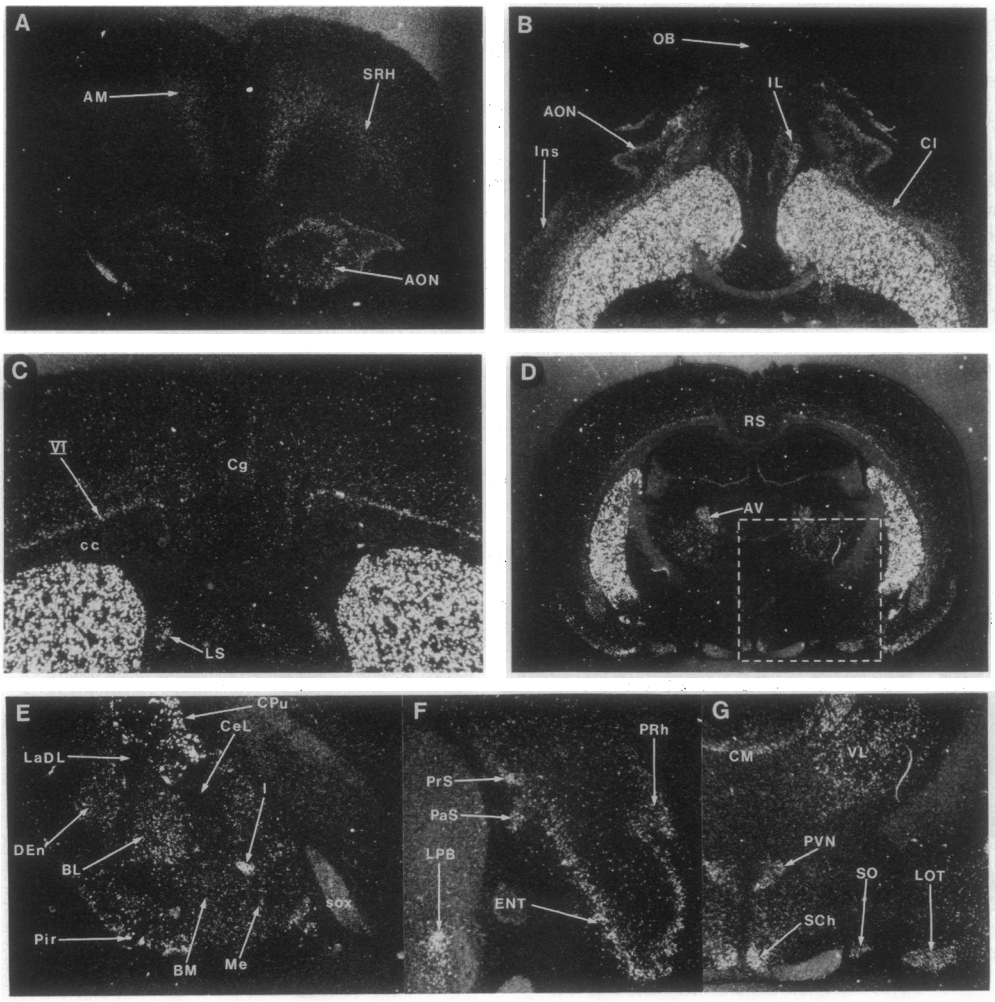

FIG. 3. Localization of D_1 receptor mRNA in coronal (*A*, *C–F*) and horizontal (*B*) sections of adult rat brain. (*G*) Inset from *D* showing neurons expressing D_1 receptor mRNA in the hypothalamic paraventricular (PVN), supraoptic (SO), and suprachiasmatic (SCh) nuclei, as well as in the central median (CM) and ventral lateral (VL) dorsal thalamic nuclei, and in the nucleus of the lateral olfactory tract (LOT) of the amygdala. AM, anteromedial prefrontal cortex; SRH, suprarhinal prefrontal cortex; AON, anterior olfactory nuclei; Ins, insular cortex; IL, infralimbic cortex; Cl, claustrum; OB, olfactory bulb; Cg, cingulate cortex; VI, layer VI of the isocortex; cc, corpus callosum; LS, lateral septum; RS, retrosplenial cortex; AV, anteroventral nucleus of the dorsal thalamus; BL, basolateral nucleus; BM, basomedial nucleus; CeL, central lateral nucleus; CPu, caudate-putamen; DEn, dorsal endopiriform nucleus; I, intercalated nucleus; LaDL, dorsal lateral region of the lateral nucleus; Me, medial nucleus; Pir, piriform cortex; sox, supraoptic decussation; LPB, lateral parabrachial nucleus; PRh, perirhinal cortex; ENT, entorhinal cortex; PrS, presubiculum; PaS, parasubiculum.

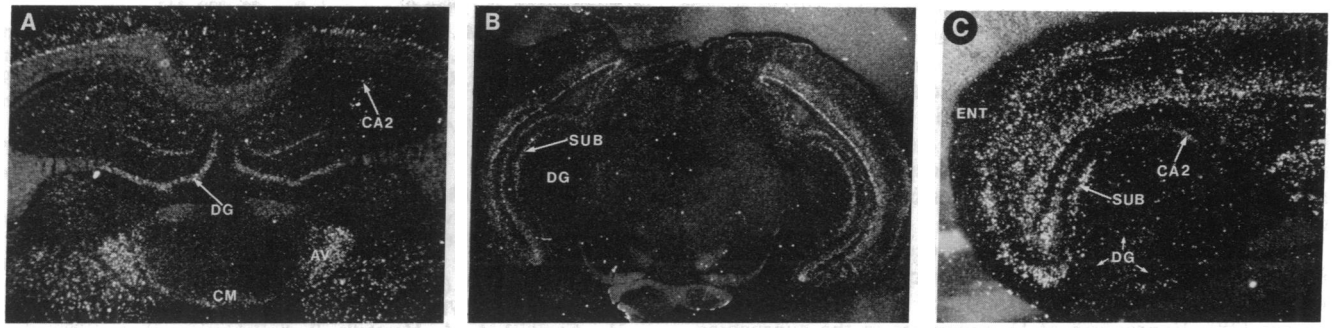

FIG. 4. Differential localization of D_1 receptor mRNA in distinct regions of the hippocampus. (*A*) In anterior regions of the dorsal hippocampus, granule cells of the dentate gyrus (DG) were prominently labeled. A small number of pyramidal neurons in CA2 were also labeled. (*B*) In the posterior region of the dorsal hippocampus, neurons in the subiculum (SUB) were labeled; the dentate granule cells were unlabeled. (*C*) In the ventral hippocampus shown in this horizontal section, the subiculum was prominently labeled. Also, labeled pyramidal neurons were observed in the CA2 field. Labeled cells were rarely observed in the dentate granule layer in the ventral hippocampus. ENT, entorhinal cortex; CM, central median.

the CA1, CA2, and CA3 fields; the frequency of labeling was higher in CA2 pyramidal neurons. Labeled cells were also observed in the stratum oriens and stratum radiatum of fields CA1–CA3. In the ventral dentate gyrus, labeled cells were observed in the molecular layer and the dentate hilar region. In contrast, specific labeling of dentate granule neurons was rarely observed in the ventral hippocampus (one to three cells per section).

In the anterior region of the dorsal hippocampus (Fig. 4*A*), dentate granule cells were prominently labeled. In contrast, few cells were labeled in the anterior subicular complex or CA1 and CA3 pyramidal layers. Pyramidal neurons in CA2 were labeled in the anterior region of the dorsal hippocampus, but the frequency and intensity of labeling were less than in the ventral hippocampus. Labeled neurons were rarely observed in the anterior stratum oriens or stratum radiatum of fields CA1–CA3. In the posterior region of the dorsal hippocampus, cells expressing D_1 receptor mRNA were detected in the subiculum, but the dentate granule cells were unlabeled.

Information from multimodal association areas of the cortex, including the temporal, prefrontal, cingulate, and insular regions, converges on the entorhinal cortex and is transmitted to the hippocampal formation via the perforant pathway (34). Hippocampal efferent output is organized such that information from various subregions of the hippocampus is transmitted via intrahippocampal association pathways to the subicular complex before leaving the hippocampus (34). Thus, neurons expressing D_1 receptor mRNA in the entorhinal cortex and discrete subregions of the hippocampus, including the ventral subiculum, could influence information entering and leaving the hippocampus.

Amygdaloid complex. Cells expressing D_1 receptor mRNA were heterogeneously distributed throughout the component nuclei of the amygdaloid complex (Fig. 3*E*). The most intense hybridization was detected in the basolateral and intercalated cell groups. Labeled cells were also observed in the medial division of the central nucleus, the medial, lateral, basomedial, and cortical nuclei, and the nucleus of the lateral olfactory tract. Little or no specific labeling was observed over the central lateral nucleus or the dorsolateral region of the lateral nucleus (Fig. 3*E*).

The amygdaloid complex appears to play a significant role in cognitive functions involving sensory-affective integration and associative learning. Through prominent and often reciprocal connections with the olfactory system, hippocampal formation, thalamus and neocortex, striatum, basal forebrain, hypothalamus, and brain stem, the amygdaloid complex appears to regulate ongoing endocrine, autonomic, and behavioral responses appropriate to past experience (35). The results described in this study indicate that the D_1 receptor may have a significant role in mediating the functional role of dopaminergic neurotransmission in the amygdaloid complex.

Other subcortical limbic regions that exhibited cells expressing D_1 receptor mRNA include the lateral septal nucleus and the bed nucleus of the stria terminalis (Figs. 1*A* and 3*C*). Cells expressing D_1 receptor mRNA were also detected in the anterior olfactory nucleus, dorsal endopiriform nucleus, and the claustrum (Figs. 1 and 2). In contrast, no specific hybridization signal was detected in the olfactory bulb (Fig. 3*B*).

Thalamus. Cells expressing D_1 receptor mRNA were detected in a number of thalamic nuclei thought to play a role in the integration of sensory and motor activities (36). Prominent labeling was observed in the ventral lateral, ventral medial, central medial, and subparafascicular thalamic nuclei (Figs. 2 and 3 *D* and *G*). Labeled cells were also observed in the central lateral, dorsal lateral geniculate, paracentral, and posterior thalamic nuclei (data not shown). A small number of labeled cells were observed in the lateral habenula (Fig. 2*C*). In contrast, background labeling was observed over the ventroposterior complex of somatosensory relay nuclei.

Labeled cells were also observed in limbic regions of the thalamus including the anteroventral (Fig. 3*D*), anterodorsal, and lateral dorsal thalamic nuclei. These nuclei are reciprocally connected with limbic areas of the cortex including the cingulate and retrosplenial areas and the subicular complex (36). The anteroventral and anterodorsal nuclei also receive a prominent afferent input from the mammillary bodies of the hypothalamus. The pathway from the mammillary body to the cingulate cortex via the anterior thalamic nucleus is part of a circuit proposed to represent a structural basis of emotion (37). Thus D_1 receptors may regulate the flow of information through the Papez' circuit.

In contrast to the high levels of D_1 receptor mRNA observed in certain thalamic nuclei, only low levels of ^{125}I-labeled SCH 23982 binding were observed in the dorsal thalamus (Fig. 2), in agreement with previous studies (12–16). These results suggest that D_1 receptors, translated locally in cell bodies in the dorsal thalamus, are transported to nerve terminals located in distant sites. In this regard, the major efferent target of the dorsal thalamus is the cerebral cortex (36). However, the dorsal thalamus also projects to the striatum, olfactory tubercle, and amygdala (36). An alternative explanation for the lack of correspondence between D_1 receptor mRNA and D_1 receptor binding in the dorsal thalamus could be due to technical limitations (see ref. 24). Thus, the visualization of D_1 receptor mRNA following hybridization with ^{35}S-labeled RNA probes and emulsion autoradiography may be more sensitive than the visualization of the corresponding protein by receptor autoradiography.

Hypothalamus. Cells expressing D_1 receptor mRNA were detected in a number of hypothalamic nuclei. Prominent hybridization signals were detected in the paraventricular, supraoptic, and suprachiasmatic nuclei (Fig. 3 *D* and *G*). Labeled cells were also observed in the preoptic, dorsal hypothalamic, ventromedial, dorsal medial, and lateral hypothalamic areas. However, the diencephalic dopamine neurons did not express detectable levels of D_1 receptor mRNA (data not shown).

These results indicate that the D_1 receptor may participate in a wide variety of hypothalamic functions involved in the integration of endocrine, autonomic, and behavioral processes (38). Expression of D_1 receptors in the suprachiasmatic nucleus, site of an endogenous circadian clock (39), suggests that D_1 receptors may participate in the regulation of circadian rhythms.

Comparison of the Distribution of Cells Synthesizing the D_1 Receptor and DARPP-32. Previously, a dopamine- and cyclic AMP-regulated phosphoprotein of molecular weight 32,000 (DARPP-32), similar in structure to phosphatase inhibitor 1, was described to be enriched in dopaminoceptive neurons that possess D_1 receptors (40). In general, a good correspondence exists in many brain regions between the distribution of cells synthesizing the D_1 receptor and DARPP-32 (41–43). However, significant differences exist in certain brain regions. For example, the D_1 receptor is synthesized by neurons in the lateral septum and dorsal thalamus (Figs. 2 and 3), which do not appear to express DARPP-32 (41–43). Therefore, DARPP-32 may be expressed by a subset of neurons that express the D_1 receptor. Depending upon whether DARPP-32 subserves a role in D_1 receptor-mediated events, these results raise the interesting possibility that differential properties of signaling events could occur depending upon whether a cell also expresses DARPP-32.

DARPP-32 mRNA and immunostaining have been reported in cerebellar Purkinje neurons and in certain glial cells including tanycytes and ependymal cells of the third ventricle and the choroid plexus (41–43). However, we were unable to detect D_1 receptor mRNA in these cell types. Thus DARPP-32 may have functions in these cell types that do not involve D_1 receptors.

Conclusions. We have used *in situ* hybridization and receptor autoradiography to examine the expression of the D_1 dopamine receptor in rat brain. Consistent with a role for the D_1 receptor in modulating motor functions (10), high levels of both D_1 receptor mRNA and binding were observed in striatum and nucleus accumbens, supporting a postsynaptic localization. Interestingly, in brain regions involved in the efferent flow of information from the basal ganglia, D_1 receptors appear to be localized on afferent nerve terminals, suggesting a presynaptic regulatory function for these receptors. The widespread expression of D_1 receptor mRNA throughout cortical, limbic, hypothalamic, and thalamic brain regions contrasts with the previously reported distribution of D_2 receptor mRNA in rat brain (refs. 22–24; R.T.F. and G.E.D., unpublished observations). These results further underscore the important role for the D_1 receptor in cognitive (44), affective, and neuroendocrine aspects of dopaminergic neurotransmission.

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