

## ANATOMICAL AND AFFINITY STATE COMPARISONS BETWEEN DOPAMINE D<sub>1</sub> AND D<sub>2</sub> RECEPTORS IN THE RAT CENTRAL NERVOUS SYSTEM

E. K. RICHFIELD,\*† J. B. PENNEY† and A. B. YOUNG†‡

\*Unit of Functional Neuroanatomy, National Institute of Mental Health, Bethesda, MD 20892, U.S.A.

†Department of Neurology, University of Michigan, Ann Arbor, MI 48104, U.S.A.

**Abstract**—The anatomical distributions and affinity states of dopamine D<sub>1</sub> and D<sub>2</sub> receptors were compared in the rat central nervous system using quantitative autoradiography. [<sup>3</sup>H]SCH23390 and [<sup>3</sup>H]spiperone (in the presence of 100 nM mianserin) were used to label the D<sub>1</sub> and D<sub>2</sub> receptors, respectively. The densities of D<sub>1</sub> and D<sub>2</sub> receptors displayed a positive correlation among 21 brain regions (Pearson correlation coefficient,  $r = 0.80$ ,  $P < 0.001$ ).

The affinity states for the D<sub>1</sub> and D<sub>2</sub> receptors were found to be quite different from each other, and different from the results obtained by others using homogenate preparations. Both the D<sub>1</sub> and D<sub>2</sub> receptors were best modeled using a two-state model. In the absence of exogenous guanine nucleotides and using the nonselective agonist dopamine as the competitor, the D<sub>1</sub> receptor was primarily in a low affinity agonist state ( $R_H = 21 \pm 6\%$ ), whereas the D<sub>2</sub> receptor was primarily in the high affinity agonist state ( $R_H = 77 \pm 3\%$ ). In the presence of 10  $\mu$ M guanylyl-imidodiphosphate or guanosine-5'-O-(2-thiophosphate) both the D<sub>1</sub> and the D<sub>2</sub> receptor were completely in a low affinity agonist state ( $R_L = 100\%$ ). These affinity states were found both in the nucleus accumbens and olfactory tubercle using dopamine as the competitor and in the striatum using selective D<sub>1</sub> or D<sub>2</sub> agonists as competitors.

Receptor occupancy of the D<sub>2</sub> receptor with either an agonist or antagonist did not alter the affinity states of the D<sub>1</sub> receptor, and conversely, receptor occupancy of the D<sub>1</sub> receptor did not alter the affinity states of the D<sub>2</sub> receptor.

The correlation between densities of D<sub>1</sub> and D<sub>2</sub> receptors provides an anatomical framework for evaluating behavioral and electrophysiological evidence of an interaction between the two dopamine receptor subtypes. This interaction does not appear to be due to a sharing or coupling of G-proteins in such a way that binding to one dopamine receptor subtype alters the affinity state of the other receptor subtype. The differences between dopamine receptor distributions described by labeled agonists and antagonists may be due in part to differences in their affinity states. The low proportion of high affinity state D<sub>1</sub> receptors may explain some of the difficulties in assigning specific behavioral roles to the D<sub>1</sub> receptor.

Dopamine (DA) receptors have been classified into two subtypes designated D<sub>1</sub> and D<sub>2</sub>,<sup>31,62</sup> with different biochemical,<sup>31</sup> pharmacological,<sup>9,17,63</sup> anatomical,<sup>17</sup> and behavioral profiles.<sup>3,4,6,23</sup> The continued development and application of selective D<sub>1</sub> and D<sub>2</sub> agonists and antagonists have enabled a better understanding of the roles of each DA receptor subtype in the function of the CNS.<sup>32</sup>

Whereas the parathyroid gland contains only D<sub>1</sub> receptors<sup>5,15</sup> and the pituitary gland contains only D<sub>2</sub> receptors,<sup>60</sup> the majority of the structures in the CNS contain both types of DA receptor subtypes.<sup>10,13,18,22,40,53-56</sup> The existence of a generalized functional interaction between the two types of DA receptor subtypes has not been determined. Areas with both high and low densities of the receptor subtypes have been reported, but detailed quantitative comparisons under similar binding conditions have not been performed. The distributions of both D<sub>1</sub> and D<sub>2</sub> receptors have been studied using labeled selective agonists and antagonists.<sup>2,10,13,16a,18,20a,21,40,45,58,69</sup> Differences between the distributions and densities using an agonist and an antagonist have been described,<sup>16a</sup> but the reason for these differences are incompletely known. Agonist affinity states of the receptors may play a role. Regional differences in affinity states or differences in affinity states between receptor subtypes make it difficult to compare the regional distributions and densities of receptors and receptor subtypes.

Recent behavioral<sup>3,4,6,14,23,37,41,52,57</sup> and electrophysiological<sup>16,70</sup> studies have suggested that the D<sub>1</sub> receptor interacts with the D<sub>2</sub> receptor. These studies have suggested that the D<sub>1</sub> receptor may play a

‡To whom correspondence should be addressed.

**Abbreviations:**  $B_{max}$ , maximal number of binding sites; CV 205-502, *N,N*-diethyl-*N'*-[(3 $\alpha$ ,4 $\alpha$ ,10 $\beta$ )-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-propyl-3-benzo[9]quinolinyl]sulfamide; DA, dopamine; GDP- $\beta$ S, guanosine-5'-O-(2-thiophosphate); GmP-PnP, guanylyl-imidodiphosphate; 5-HT, 5-hydroxytryptamine (serotonin);  $K_D$ , equilibrium dissociation rate constant;  $K_H$ , high affinity rate constant;  $K_I$ , inhibitory rate constant;  $K_L$ , low affinity rate constant; LY 171555, 4,4a,5,6,7,8,8a,9-octahydro-5-*n*-propyl-2H-pyrazolo[3,4-*g*]-quinoline; N-0437, (*N*-propyl-*N*-2-thienylethyl-amino)-5-hydroxytetralin; NMDA, *N*-methyl-D-aspartate;  $R_H$ , high affinity agonist state;  $R_L$ , low affinity agonist state; SCH 23390, (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol; SKF 38393, 2,3,4,5-tetrahydro-7,8-di-hydroxy-1-phenyl-1H-3-benzazepine; TCP, *N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine.

"synergistic", "permissive", or "modulatory" role in the functions of the D<sub>2</sub> receptor in mediating certain behaviors or the tonic single unit activity of cells in the CNS. Since all of these studies relied on the systemic administration of drugs, the sites and mechanisms of this interaction remain unknown. A comparative study between the anatomical distributions and densities of the D<sub>1</sub> and D<sub>2</sub> receptor might help to locate regions where interactions might occur and suggest an underlying mechanism for such interactions.

Analogous to  $\beta$ -adrenergic receptors, DA receptors, when binding an agonist, have been shown to recognize two affinity states designated  $R_H$  (high affinity) and  $R_L$  (low affinity).<sup>22,24,26,28,74</sup> The role of affinity states in the behavioral and biochemical functions of DA receptors remains unresolved. The possibility of an allosteric interaction between D<sub>1</sub> and D<sub>2</sub> receptors has not been demonstrated. A comparison between the affinity states of the D<sub>1</sub> and D<sub>2</sub> receptors might help to determine their contributions to different behaviors mediated by selective and nonselective drugs. We report the results of detailed quantitative comparisons of the CNS distributions and agonist affinity states of the D<sub>1</sub> and D<sub>2</sub> receptors.

## EXPERIMENTAL PROCEDURES

### Tissue preparation

Brains were obtained from male Sprague-Dawley rats, weighing 175–225 g, after rapid decapitation. The brains were frozen on tissue pedestals with Lipshaw embedding matrix using crushed dry ice. The brains were warmed to  $-20^\circ\text{C}$  and coronal sections 14 or 20  $\mu\text{m}$  thick were cut on a Lipshaw cryostat microtome. The 20- $\mu\text{m}$ -thick sections were used for distribution studies, while the 14- $\mu\text{m}$ -thick sections were used for competition studies. The sections were thaw-mounted onto gelatin coated slides and were then dehydrated on a warming plate at  $30^\circ\text{C}$  and stored at  $-20^\circ\text{C}$  until used in assays.

### Receptor assays

Both the D<sub>1</sub> and D<sub>2</sub> receptor assays were performed in the same buffer, consisting of 25 mM Tris-HCl (pH 7.5) containing 100 mM NaCl, 1 mM MgCl<sub>2</sub>, 1  $\mu\text{M}$  pargyline, and 0.001% ascorbate, as previously described.<sup>53,55</sup> Briefly, slides were warmed to room temperature for 1 h then incubated with tritiated ligand at room temperature for either 120 (D<sub>2</sub> assay) or 150 (D<sub>1</sub> assay) min. After the incubation, the slides were washed for 10 min in cold ( $4^\circ\text{C}$ ) buffer, dipped in distilled water for 3 sec, and dried using a stream of cool air.

The D<sub>1</sub> receptor was assayed using [<sup>3</sup>H]SCH 23390. The details of this autoradiographic assay have been described elsewhere.<sup>18,55</sup> All incubations were done at a concentration of [<sup>3</sup>H]SCH 23390 equal to the equilibrium dissociation rate constant ( $K_D$ ) (0.57 nM) in rat striatum. Specific binding was determined by subtracting the amount bound in the presence of 1  $\mu\text{M}$  *cis*-flupentixol (nonspecific binding) from the total amount bound. Specific binding represented 98–99% of the total binding in the striatum. Under these conditions, [<sup>3</sup>H]SCH 23390 binds only to the D<sub>1</sub> receptor.<sup>55</sup>

The D<sub>2</sub> receptor was assayed using [<sup>3</sup>H]spiperone (spiro-peridol). The properties of this autoradiographic assay have been described elsewhere.<sup>2,13,54–56</sup> All incubations were done at a concentration of [<sup>3</sup>H]spiperone equal to the  $K_D$  (0.25 nM) in rat striatum and included 100 nM mianserin to block the binding of [<sup>3</sup>H]spiperone to the serotonin (5-HT) receptor.<sup>2,53,55</sup> Specific binding was determined by sub-

tracting the amount bound in the presence of 25  $\mu\text{M}$  DA (nonspecific binding) from the total amount bound. Specific binding represented 85% of total binding in the striatum. Under these conditions, dopamine does not displace the binding of [<sup>3</sup>H]spiperone to other sites to which it binds.<sup>27,56</sup>

Competition studies were carried out using sections containing the rostral striatum, rostral nucleus accumbens and olfactory tubercle. A competition curve was derived from 24 consecutive 14- $\mu\text{m}$ -thick sections, of which two sections in the middle of the series were incubated only with tritiated ligand to yield total binding, two other sections in the middle of the series were incubated with tritiated ligand plus the blank to yield nonspecific binding, and the remaining 20 sections were incubated with tritiated ligand and decreasing concentrations of competitor. Three competition curves were performed in each animal and two animals were used in each experiment. Some competition curves were derived from sections incubated in the presence of 10  $\mu\text{M}$  of a nonmetabolizable guanine nucleotide. Additional competition curves were derived from sections incubated in fixed concentrations of a selective D<sub>1</sub> (SKF 38393) or D<sub>2</sub> (LY 171555, quinpirole) agonist or a selective D<sub>1</sub> (SCH 23390) or D<sub>2</sub> (sulpiride) antagonist as listed in the results. Quantitative autoradiography was used to determine the amount of binding in various brain regions for all competitions.

### Data analysis

Best-fit least squares iterative curve fitting was performed using the Lunden II software package (Lunden, Inc., Cleveland, OH) to analyse all competition curves. Each curve was first analysed as a one-site fit, and subsequently analysed as a two-site fit. A two-site fit was selected only if the *F*-test comparing the sum of squares for errors for the two-site fit to that for the one-site fit indicated that the sum of squares for errors was significantly reduced using the more complex (two-site) model ( $P < 0.05$ ).<sup>36</sup> All curves reported (having one or two sites) had an insignificant runs test ( $P > 0.05$ ) supporting the goodness of the fit.<sup>8,36</sup> All curves were constrained by having the dissociation constant for the tritiated ligand remain fixed and equal to its  $K_D$  for both one- and two-site fits. The  $K_{DS}$  for the [<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone binding to the D<sub>1</sub> and D<sub>2</sub> receptors, respectively, were determined from saturation experiments done independently and indicated binding to single sites.<sup>55</sup>

### Autoradiography

Dried slides were placed in an x-ray cassette with <sup>14</sup>C plastic standards previously calibrated with <sup>3</sup>H brain paste sections.<sup>48,50</sup> The sections and standards were apposed to LKB Ultrafilm-<sup>3</sup>H at  $4^\circ\text{C}$  for either 7–10 (D<sub>1</sub> receptor) or 14–21 (D<sub>2</sub> receptor) days. The LKB Ultrafilm-<sup>3</sup>H was developed in Kodak D19 for 3 min at room temperature and fixed in Kodak rapid fix for 3.5 min.

All binding data, in units of fmol/mg protein, were determined directly from film densities in regions of interest using a video based densitometer<sup>56</sup> and fourth degree polynomial curve fitting to the standards. Regions of interest were outlined bilaterally with a mouse controlled cursor, sampled, and the template was stored for use on subsequent consecutive sections. Sections through the striatum were analysed for medial to lateral gradients in binding by generating receptor density versus distance histograms.

Four rats were used to study the distribution of D<sub>1</sub> and D<sub>2</sub> receptors in different regions of the CNS. Each rat had adjacent sections throughout the forebrain assayed for the density of D<sub>1</sub> and D<sub>2</sub> receptors. The same 21 regions (Table 1), at identical levels,<sup>49</sup> were measured in each animal for each receptor subtype. These 21 regions were selected to represent portions of the basal ganglia, cerebral cortex and subcortical areas. The areas were selected *a priori* without regard to their receptor densities. The densities of the D<sub>1</sub> and D<sub>2</sub> receptors for the four animals were averaged for each region. Because binding was determined at  $K_D$  ligand con-

Table 1. Summary of D<sub>1</sub> and D<sub>2</sub> receptor densities in the rat CNS

| Region†                       | Receptor subtype<br>(amount bound, fmol/mg protein*) |                |
|-------------------------------|--|----------------|
|                               | D <sub>1</sub>                                       | D <sub>2</sub> |
| 1. Olfactory tubercle         | 1472 ± 36  | 530 ± 21       |
| 2. Med. sub. nigra reticulata | 1457 ± 34  | 95 ± 26        |
| 3. Rostral n. accumbens       | 1420 ± 18  | 348 ± 27       |
| 4. Med. sub. nigra compacta   | 1354 ± 33  | 153 ± 33       |
| 5. Rostral striatum           | 1342 ± 44  | 562 ± 18       |
| 6. Ventral pallidum           | 689 ± 26   | 70 ± 14        |
| 7. Entopeduncular n.          | 600 ± 48   | 19 ± 14        |
| 8. Basolateral amygdaloid n.  | 358 ± 27   | 61 ± 20        |
| 9. Perirhinal cortex          | 288 ± 43   | 91 ± 53        |
| 10. Entorhinal cortex         | 206 ± 43   | 69 ± 12        |
| 11. Occipital cortex          | 200 ± 50   | 17 ± 6         |
| 12. Olfactory bulb            | 200 ± 50   | 38 ± 5         |
| 13. Globus pallidus           | 192 ± 33   | 28 ± 8         |
| 14. Sup. gray superior coll.  | 170 ± 27   | 55 ± 9         |
| 15. Lat. prefrontal cortex    | 92 ± 6   | 48 ± 4         |
| 16. Lat. sep. n. dorsal       | 79 ± 34  | 59 ± 11        |
| 17. Temporal cortex           | 78 ± 59  | 23 ± 8         |
| 18. Parietal cortex           | 49 ± 10  | 13 ± 6         |
| 19. Ventral hypothalamic n.   | 49 ± 24  | 13 ± 10        |
| 20. Central gray              | 46 ± 25  | 31 ± 10        |
| 21. Frontal cortex            | 41 ± 6   | 26 ± 15        |

\*The values in the table are the number of receptors (fmol/mg protein) from four animals (mean ± S.E.M.). The values represent binding at a concentration equal to the  $K_D$  for each ligand as determined in the rat striatum.

†The atlas of Paxinos and Watson<sup>49</sup> was used to assist in determining anatomical regions. In regions of the cerebral cortex where binding is heterogeneous in different laminae, the values represent the average number of receptors in a rectangular box extending from the surface of the cortex to the underlying white matter. In regions where binding was nonlaminar, but heterogeneous (nucleus accumbens, olfactory bulb and tubercle), the entire region was averaged. Averaged values for each of the cerebral cortical regions used were used to eliminate the possibility that multiple layers would have skewed the analysis by giving excess weight to those areas.

centrations, values represent 50% of the maximal number of binding sites ( $B_{max}$ ) in any region. A Pearson correlation was performed between the D<sub>1</sub> and D<sub>2</sub> receptor densities.

Rostral to caudal gradients have been reported for the D<sub>1</sub> and D<sub>2</sub> receptors in regions of the striatum, nucleus accumbens, and olfactory tubercle.<sup>2,13,55</sup> However, rostral to caudal gradients were not detected in these areas in the short distances examined in this study (data not shown).

#### Materials

[<sup>3</sup>H]SCH 23390 (specific gravity 74–85 Ci/mmol) and [<sup>3</sup>H]spiperone (76–95 Ci/mmol) were obtained from Amersham Corporation (Arlington Heights, IL). Mianserin was obtained from Organon (Oss, The Netherlands), dopamine from Sigma (St Louis, MO), *cis*-flupentixol from Dr John Hyttel of H. Lundbeck and Co. (Copenhagen, Denmark), SKF 38393 from Smith, Kline and French Laboratories (Philadelphia, PA), LY 171555 from Lilly Research Laboratories (Indianapolis, IN), sulpiride from Ph. Delagrangue (Paris, France), SCH 23390 from Schering (Bloomfield, NJ), and guanylyl-imidodiphosphate (GmP-PnP) and guanosine-5'-O-(2-thiophosphate) (GDP-βS) were obtained from Boehringer-Mannheim (Indianapolis, IN).

#### RESULTS

##### Dopamine receptor distributions in the rat CNS

DA D<sub>1</sub> and D<sub>2</sub> receptors have heterogeneous distributions in the rat CNS (Table 1 and as reported by

others<sup>2,10,13,18,40</sup>). The amount of binding varied 36-fold for the D<sub>1</sub> receptor and 43-fold for the D<sub>2</sub> receptor among the structures sampled (Table 1). Medial to lateral gradients for the D<sub>2</sub> receptor have been reported in the striatum,<sup>29</sup> but were not seen in these animals for either the D<sub>1</sub> or the D<sub>2</sub> receptors (Fig. 1).

A plot of the log transformed densities of the two receptor subtypes revealed a linear increase of one subtype with the other (Fig. 2). Pearson correlation between the two receptor subtypes was 0.80 ( $P < 0.001$ ) for the 21 regions of Table 1.

##### Affinity states of dopamine receptors in striatum

The two DA receptor subtypes were found to have different affinity states in the absence of exogenous guanine nucleotides (Fig. 3; Tables 2 and 3). The D<sub>1</sub> receptor was primarily in the low affinity state for both the nonselective agonist DA ( $R_H = 21 \pm 6\%$ ,  $K_H = 49 \pm 24$  nM and  $K_L = 2030 \pm 690$  nM) and the selective agonist SKF 38393 ( $R_H = 20 \pm 14\%$ ,  $K_H = 2 \pm 1$  nM and  $K_L = 41 \pm 5$  nM). The D<sub>2</sub> receptor was primarily in the high affinity state for both the nonselective agonist DA ( $R_H = 77 \pm 3\%$ ,  $K_H = 43 \pm 4$  nM and  $K_L = 4550 \pm 530$  nM) and the

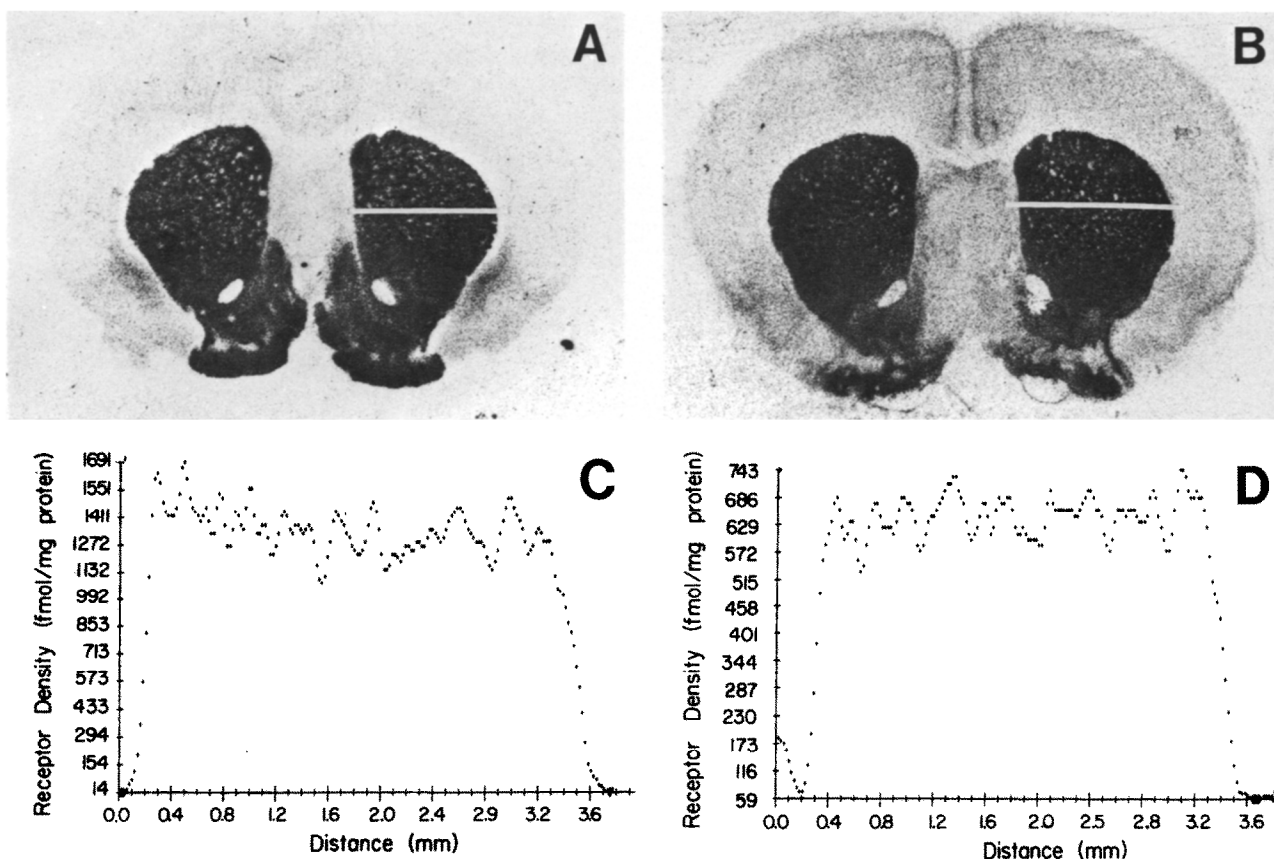


Fig. 1. Striatal dopamine receptor histograms.  $D_1$  (A) and  $D_2$  (B) receptor autoradiograms were used to generate receptor density versus distance histograms through the right striata for both the  $D_1$  (C) and  $D_2$  (D) receptors. Minor increases and decreases in receptor density can be attributed to white matter bundles coursing through the striatum and to minor random fluctuations in receptor density or ligand binding. No significant medial to lateral gradient was observed at any of the rostral levels used in this study. The LKB Ultrafilm- $^3H$  was apposed to the tissue sections for different periods of time for the  $D_1$  and  $D_2$  receptors, so direct comparison of the amount of binding from the photographs cannot be made. Valid comparisons can be made using quantitative autoradiography and those results are presented in Table 1.

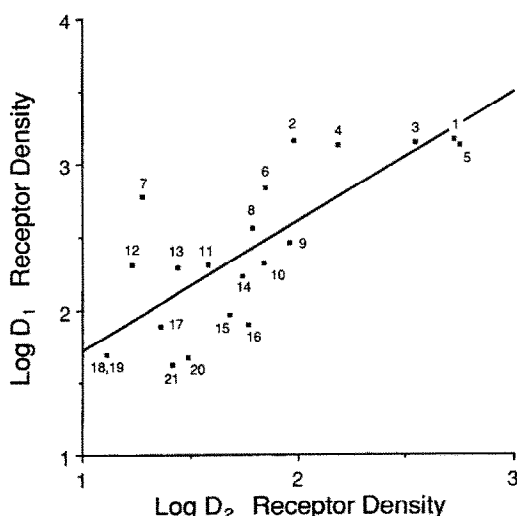


Fig. 2. Scatter plot of log  $D_1$  and  $D_2$  receptor densities. The  $D_1$  and  $D_2$  receptor densities for 21 regions of the rat CNS (Table 1) were log transformed and plotted. A Pearson correlation for these 21 regions was performed ( $r = 0.80$ ,  $P < 0.001$ ).

selective agonist LY 171555 ( $R_H = 90 \pm 1\%$ ,  $K_H = 63 \pm 18$  nM and  $K_L = 3330 \pm 750$  nM).

In the presence of  $10 \mu\text{M}$  GmP-PnP, both  $D_1$  and  $D_2$  receptors were entirely converted to a low affinity state ( $R_L = 100\%$ ) for both nonselective and selective agonists (Table 2). The dissociation constant ( $K_L$ ) determined in the presence of exogenous guanine nucleotide was higher than the dissociation constant ( $K_L$ ) determined in the absence of guanine nucleotides from the two-site model for both receptor subtypes using both selective and nonselective agonists (Table 2). The guanine nucleotide GDP- $\beta\text{S}$  ( $10 \mu\text{M}$ ) produced an affinity state shift similar to that of  $10 \mu\text{M}$  GmP-PnP for both the  $D_1$  and  $D_2$  receptors. In the presence of  $10 \mu\text{M}$  GDP- $\beta\text{S}$ , using DA as the competitor, the  $K_L$  was 8580 nM and  $R_L$  was 100% for the  $D_1$  receptor, and the  $K_L$  was 17100 nM and the  $R_L$  was 100% for the  $D_2$  receptor. Neither  $10 \mu\text{M}$  GDP- $\beta\text{S}$  nor  $10 \mu\text{M}$  GmP-PnP had an effect on the amount of tritiated antagonist bound to the  $D_1$  or  $D_2$  receptor.

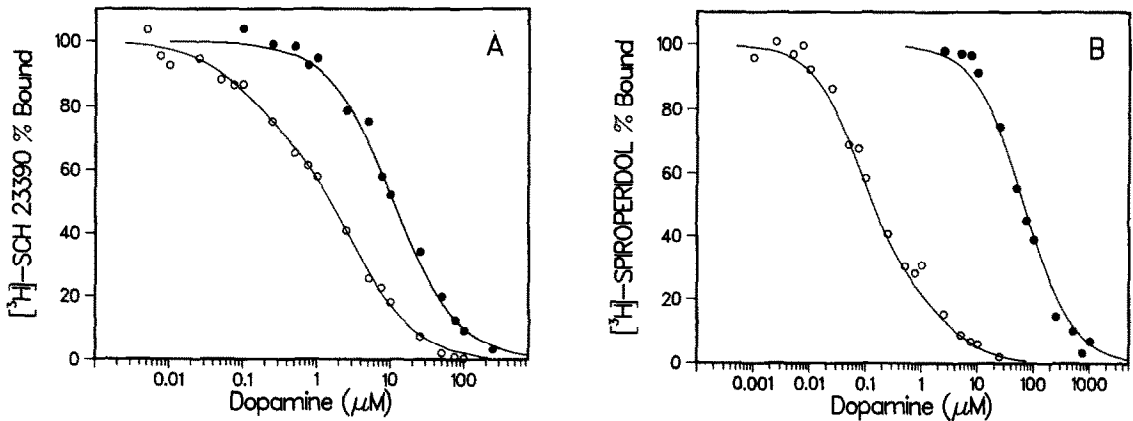


Fig. 3. Dopamine competition curves for the D<sub>1</sub> and D<sub>2</sub> receptors in striatum. Competition curves for the D<sub>1</sub> (A) and D<sub>2</sub> (B) receptors were performed in the striatum using DA as the competitor. Competitions were performed in the absence (open circles) or presence of 10  $\mu$ M GmP-PnP (filled circles). The data for the two curves for each receptor subtype are from the same experiment. Values plotted are actual values, while the line plotted is from the best fit computer generated estimates for that set of data. Values for the D<sub>1</sub> receptor (A) in the absence of exogenous guanine nucleotide (open circles) were  $K_H = 59$  nM,  $K_L = 1570$  nM,  $R_H = 27.7\%$ ,  $R_L = 72.3\%$ ; values for the D<sub>1</sub> receptor in the presence of 10  $\mu$ M GmP-PnP (filled circles) were  $K_L = 5760$  nM,  $R_L = 100\%$ . Values for the D<sub>2</sub> receptor (B) in the absence of exogenous guanine nucleotide (open circles) were  $K_H = 40$  nM,  $K_L = 1350$  nM,  $R_H = 79\%$ ,  $R_L = 21\%$ ; values for the D<sub>2</sub> receptor in the presence of 10  $\mu$ M GmP-PnP (filled circles) were  $K_L = 25,500$  nM,  $R_L = 100\%$ .

#### Affinity states of dopamine receptors in other regions

The affinity states of the D<sub>1</sub> and D<sub>2</sub> receptors were also analysed in the nucleus accumbens and olfactory tubercle. Only competition curves in which the regions were present in all sections were used and only competition curves for DA were analysed. The affinity states for the D<sub>1</sub> receptor in both the nucleus accumbens and the olfactory tubercle were similar to those seen in the striatum (Table 3). Affinity states for the D<sub>2</sub> receptor in nucleus accumbens were also similar to those seen in the striatum (Table 3). The olfactory tubercle demonstrated binding that was too heterogeneous for the D<sub>2</sub> receptor to produce satis-

factory competition curves for autoradiographic analysis.

#### Affinity state interactions

To determine if there was an allosteric interaction between the DA receptor subtypes that would alter binding characteristics, the affinity state of one DA receptor subtype was examined while the other DA receptor subtype was occupied by an agonist or antagonist. DA competitions for the D<sub>1</sub> receptor were performed in the presence of 1  $\mu$ M sulpiride (to block the binding of DA to the D<sub>2</sub> receptor). This concentration of sulpiride did not alter the binding of

Table 2. Affinity states of dopamine D<sub>1</sub> and D<sub>2</sub> receptors in rat striatum\*

| Drug                    | No nucleotide |                |             |             | 10 $\mu$ M GmP-PnP |           |
|-------------------------|---------------|----------------|-------------|-------------|--------------------|-----------|
|                         | $K_H$ (nM)†   | $K_L$ (nM)     | $R_H$ (%)‡  | $R_L$ (%)   | $K_L$ (nM)         | $R_L$ (%) |
| D <sub>1</sub> Receptor |               |                |             |             |                    |           |
| Dopamine                | 49 $\pm$ 20   | 2030 $\pm$ 690 | 21 $\pm$ 6  | 79 $\pm$ 6  | 9550 $\pm$ 5360    | 100       |
| SKF 38393               | 2 $\pm$ 1     | 41 $\pm$ 5     | 20 $\pm$ 14 | 80 $\pm$ 14 | 89 $\pm$ 49        | 100       |
| D <sub>2</sub> Receptor |               |                |             |             |                    |           |
| Dopamine                | 43 $\pm$ 4    | 4550 $\pm$ 930 | 77 $\pm$ 3  | 23 $\pm$ 3  | 28,500 $\pm$ 4270  | 100       |
| LY 171555               | 63 $\pm$ 18   | 3330 $\pm$ 750 | 90 $\pm$ 1  | 10 $\pm$ 1  | 31,900 $\pm$ 310   | 100       |

\*DA receptor autoradiography was performed as described in the text, using both tritiated antagonists ([<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone) at a concentration equal to their respective  $K_D$ s. Competition curves were done in either the absence or presence of 10  $\mu$ M GmP-PnP. Both antagonists displayed Hill slopes equal to one from saturation studies done previously (Richfield *et al.*<sup>35,55</sup>). Computer modeled parameters were constrained with the dissociation rate constants of the receptor for the tritiated antagonist fixed to the  $K_D$  of each ligand. For two-site fits, both  $K_H$  and  $K_L$  for the tritiated ligand were constrained to the same value. All curves were fit using nonlinear, least-squares computer assisted modeling as described in the text.

†Values are in nM  $\pm$  S.D. for 2–3 independent experiments.

‡Values are the percentage of the total number of receptors  $\pm$  S.D. for 2–3 independent experiments. In the absence of exogenous guanine nucleotide, all curves were best modeled as a two-site fit, whereas in the presence of 10  $\mu$ M GmP-PnP all curves were best modeled as a one-site fit.

Table 3. Affinity states of dopamine D<sub>1</sub> and D<sub>2</sub> receptors in rat nucleus accumbens and olfactory tubercle\*

| Region†                 | $K_H$ (nM)‡ | $K_L$ (nM)  | $R_H$ (%)§ | $R_L$ (%) |
|-------------------------|-------------|-------------|------------|-----------|
| D <sub>1</sub> receptor |             |             |            |           |
| Nucleus accumbens       | 22 ± 18     | 1570 ± 320  | 17 ± 2     | 73 ± 2    |
| Olfactory tubercle      | 9 ± 4       | 2040 ± 700  | 23 ± 1     | 77 ± 1    |
| D <sub>2</sub> receptor |             |             |            |           |
| Nucleus accumbens       | 52 ± 31     | 1610 ± 1480 | 66 ± 19    | 34 ± 19   |

\*Dopamine receptor autoradiography was performed as described in the text, using both tritiated antagonists ([<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone) at a concentration equal to their  $K_D$ . Competition curves were done in the absence of exogenous guanine nucleotide. Computer modeled parameters were constrained with  $K_H$  and  $K_L$  equal to the  $K_D$  for the tritiated ligand. All curves were fit using nonlinear least-squares computer assisted modeling as described in the text. All curves in the absence of exogenous guanine nucleotide were best modeled using a two-site model.

†The regions of nucleus accumbens and olfactory tubercle were selected from sections previously analysed in the striatum. Both the nucleus accumbens and olfactory tubercle had heterogeneous binding for both D<sub>1</sub> and D<sub>2</sub> receptors. The heterogeneous D<sub>1</sub> receptor binding in nucleus accumbens and olfactory tubercle, as well as the heterogeneous D<sub>2</sub> binding in the nucleus accumbens was slight, and the entire region was averaged as a single value. The heterogeneous binding produced curves with more scatter to the data, producing greater variability in the parameters estimated. The D<sub>2</sub> binding in olfactory tubercle was too heterogeneous to produce satisfactory curves for analysis.

‡Values are in nM ± S.D. for 2–3 independent experiments.

§Values are the percentage of the total number of receptors ± S.D. for 2–3 independent experiments.

[<sup>3</sup>H]SCH 23390 to the D<sub>1</sub> receptor (inhibitory rate constant,  $K_i > 1000 \mu\text{M}$ ) and blocked most of the binding of DA to the D<sub>2</sub> receptor. No change in the D<sub>1</sub> receptor affinity state was seen. The selective D<sub>1</sub> agonist SKF 38393 was used as a competitor at the D<sub>1</sub> receptor in the presence of the selective D<sub>2</sub> agonist LY 171555 (10  $\mu\text{M}$ ). This concentration of LY 171555 did not alter binding of [<sup>3</sup>H]SCH 23390 to the D<sub>1</sub> receptor ( $K_i > 100 \mu\text{M}$ ) and was adequate to block most of the D<sub>2</sub> receptors ( $K_H = 63 \text{ nM}$  and  $K_L = 3330 \text{ nM}$  for the D<sub>2</sub> receptor). Under these conditions, no change in the affinity state of the D<sub>1</sub> receptor was seen. A similar set of experiments was performed for the D<sub>2</sub> receptor in which DA competitions were done in the presence of 100 nM SCH 23390 ( $K_i = 0.57 \text{ nM}$  for the D<sub>1</sub> receptor, with no change in the amount of D<sub>2</sub> receptor binding assessed using [<sup>3</sup>H]spiperone) and LY 171555 competitions were done in the presence of 10  $\mu\text{M}$  SKF 38393 ( $K_H = 2 \text{ nM}$  and  $K_L = 41 \text{ nM}$  for the D<sub>1</sub> receptor and no change in the amount of D<sub>2</sub> receptor binding assessed using [<sup>3</sup>H]spiperone). Similar to the results obtained for the D<sub>1</sub> receptor, no change in the affinity state of the D<sub>2</sub> receptor was seen in either case.

## DISCUSSION

### Anatomical distribution

Although the distribution of DA receptors in the rat has been reported for the D<sub>1</sub>,<sup>18,58</sup> D<sub>2</sub>,<sup>10,30,40,45,66</sup> or both D<sub>1</sub> and D<sub>2</sub> receptors,<sup>13,22</sup> no studies have

specifically examined the quantitative relationship between the distributions of the two receptors. In the present study, assay conditions were similar for both receptor subtypes. The identical incubation buffer was used, both ligands were used at a concentration equal to their respective  $K_D$ , in order to label the same proportion of receptors (50% of the total number of receptors), and tritiated antagonists were used to avoid differences in affinity states recognized by agonists. Under these conditions a positive correlation was seen between the densities of the D<sub>1</sub> and D<sub>2</sub> receptors.

Attempts were made to avoid bias in the correlation performed. The same animals and adjacent tissue sections were used. Regions were selected *a priori* to include a variety of areas with previously known dopaminergic innervation, as well as areas with unverified dopaminergic innervation. The areas included had densities that spanned the range from highest densities of receptors seen in portions of the basal ganglia to areas with barely detectable levels of receptors in some regions of the cerebral cortex. Nevertheless, there are sources of potential bias that might affect the correlation seen. Some areas with heterogeneous binding were averaged to yield a single value. Although averaged values of D<sub>1</sub> and D<sub>2</sub> receptors might correlate well, smaller areas within a region might not have, thus introducing a bias that artifactually increased the correlation. In addition, some regions might not have been included that would have reduced the correlation seen. However,

no regions were eliminated from the analysis, or ignored from selection, based on density values.

The Pearson correlation seen in this study (0.80) can be compared to the correlations seen between other binding sites in the rat CNS. For example, the correlation between *N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine (TCP) and *N*-methyl-D-aspartate (NMDA) binding is 0.94.<sup>38</sup> The correlation between benzodiazepine and GABA-stimulated benzodiazepine binding is 0.88.<sup>68a</sup> While the correlation between the NMDA and quisqualate subtypes of glutamate receptors is similar to that seen in this study (0.76), the correlations of other glutamate subtypes are low; NMDA and kainate, 0.07, quisqualate and kainate, 0.11.<sup>75</sup> Furthermore, the correlation of the GABA receptor subtypes GABA<sub>A</sub> and GABA<sub>B</sub> is only 0.39 (Pearson correlation calculated from the data of Bowery *et al.*<sup>11</sup>). Correlations between unrelated GABA and glutamate receptor subtypes were also low (0.2, data calculated from unpublished observations of D.C.M. Chu and W. F. Maragos). The intermediate correlation seen between DA D<sub>1</sub> and D<sub>2</sub> receptors in this study suggests that a relationship may exist between the receptor subtypes, but the stoichiometry and nature of the relationship are unclear and will require other types of investigations for clarification.

Despite the regional correlation seen between D<sub>1</sub> and D<sub>2</sub> receptors, there are some regions where there is a marked discrepancy in the proportion of the two receptor subtypes, including the substantia nigra pars reticulata, endopeduncular nucleus, and the globus pallidus. The neuronal location of DA receptors in these regions differs.<sup>43,59,71</sup> The substantia nigra pars reticulata and entopeduncular nucleus are also unique in their metabolic responses to D<sub>1</sub> and D<sub>2</sub> agonists following 6-hydroxydopamine lesions to the substantia nigra.<sup>67,68</sup> These two regions had the largest deviations from the linear relationship seen between the log transformed D<sub>1</sub> and D<sub>2</sub> receptors' densities in this study. If the Pearson correlation is repeated without including these two regions, the correlation between D<sub>1</sub> and D<sub>2</sub> receptors improves to 0.90. This supports the notion that these two regions have dopaminergic characteristics that may differ from those seen in other regions of the CNS.

Autoradiographic studies using a radiolabeled agonist<sup>16a,20a,22,58,69</sup> are likely to underestimate the total number of DA D<sub>1</sub> and D<sub>2</sub> receptors because they bind primarily to high affinity sites at the concentration used. Guanine nucleotide insensitive D<sub>2</sub> agonists (CV 205-502) exist and appear to label a single homogeneous site.<sup>16a</sup> These noncatechol D<sub>2</sub> agonists label a different proportion of D<sub>2</sub> sites compared to other D<sub>2</sub> agonists (N-0437).<sup>69</sup> Surprisingly, the guanine nucleotide-insensitive agonist (CV-205-502) labeled only about one-quarter the number of sites that the guanine nucleotide-sensitive agonist (N-0437) labeled.<sup>69</sup> If the regional percentage of high and low affinity sites is similar throughout the brain, as it was

for the three regions examined in this report, then the distributions described by an agonist will be approximately similar to those described with an antagonist. However, if the affinity state of a receptor changes from region to region, or under experimental or pathological conditions, then distributions described by an agonist will reflect this. This suggests that both the total number of receptors and their affinity states need to be considered in determining the regional distributions and densities of dopamine receptors.

Determination of receptor densities using agonists will also give an incorrect comparison between the densities of D<sub>1</sub> and D<sub>2</sub> receptors because the proportions of high affinity sites are quite different between them. For example, the density of D<sub>2</sub> receptors is reported to be greater than that of the D<sub>1</sub> receptors in rat striatum, when tritiated agonists are used.<sup>22</sup> However, when antagonists are used D<sub>1</sub> receptors are in considerable excess of D<sub>2</sub> receptors in the rat striatum.<sup>13,55</sup>

One question regarding the cellular location of D<sub>1</sub> and D<sub>2</sub> receptors is whether they are both located on the same neuron in a given region. Striatal neurons were hypothesized to contain both D<sub>1</sub> and D<sub>2</sub> receptors based on adenylate cyclase studies.<sup>62</sup> Electrophysiological evidence has also suggested that some neurons of the cat caudate nucleus may possess both functional D<sub>1</sub> and D<sub>2</sub> receptors.<sup>46</sup> In view of the regional correlation between DA receptor subtypes, the location of D<sub>1</sub> and D<sub>2</sub> receptors on the same neurons in one region might suggest a common mechanism of interaction in other selective regions. The reason for two receptors binding the same endogenous neurotransmitter (with nearly the same dissociation constants), but having opposing actions on adenylate cyclase remains unclear however. Recent studies continue to suggest that more than one direct biochemical effect might be ascribed to the D<sub>1</sub> or D<sub>2</sub> receptor.<sup>12,34,42,51,61</sup>

The recent description of behavioral<sup>6,23,39,41,57,68</sup> and electrophysiological<sup>16,70,72</sup> studies demonstrating a synergistic effect of D<sub>1</sub> receptor activation on D<sub>2</sub> receptor actions suggests that the DA receptor subtypes may have a common mode of interaction. A variety of different behaviors (including stereotypy, turning behavior, catalepsy, and reserpine induced akinesia) have been found to be potentiated when DA D<sub>1</sub> and D<sub>2</sub> receptors were simultaneously stimulated.<sup>6,23,39,41,57,70</sup> Whether the regions mediating different behaviors contain both D<sub>1</sub> and D<sub>2</sub> receptors or receive projections from areas containing different proportions of D<sub>1</sub> or D<sub>2</sub> receptors is not known. In view of the widespread distributions of both D<sub>1</sub> and D<sub>2</sub> receptors, determining the region(s) responsible for a particular behavior may be difficult.

#### *Affinity state comparisons*

In this study, D<sub>1</sub> and D<sub>2</sub> receptors differed in the proportions of high and low affinity receptors. The D<sub>2</sub> receptor was primarily in the high affinity state

( $R_H$  between 77 and 90%), whereas the  $D_1$  receptor was primarily in the low affinity state ( $R_H$  between 17 and 23%). This difference was seen using both selective and nonselective agonists as competitors, and in different regions of the CNS including striatum and nucleus accumbens. These regions share characteristics that may not be representative of other parts of the CNS, including their high levels of DA,  $D_1$  and  $D_2$  receptors. However, the innervation of these regions is from both the substantia nigra pars compacta (nigrostriatal pathway) and the ventral tegmental area (mesolimbocortical pathway), which together supply most of the innervation to the remaining telencephalon. Whether these affinity state proportions are true in the other regions of the CNS will require further study. In view of the regional correlation between  $D_1$  and  $D_2$  receptors, it would be noteworthy if this relationship was maintained throughout the CNS. It would also be interesting to determine if the characteristics of the presynaptic  $D_1$  heteroreceptors in the entopeduncular nucleus and substantia nigra pars reticulata were similar.

The  $D_1$  and  $D_2$  receptors also shared a number of affinity state characteristics in this study. Agonist binding to both  $D_1$  and  $D_2$  receptors was clearly biphasic in the absence of guanine nucleotide, and monophasic in the presence of either GDP- $\beta$ S or GmP-PnP. In the absence of exogenous guanine nucleotide, both receptors had similar high affinities for DA ( $K_H$ ) that were near 40 nM (range 9–74 nM) and low affinities for DA ( $K_L$ ) that were between 2 and 4  $\mu$ M. The similarities in dissociation rate constants for DA at the two DA receptor subtypes suggest that neither receptor will predominate in its binding of endogenous DA. If both receptors are found in the same dendritic area of a neuron, both receptors are likely to bind released DA.

Although the affinities of the two DA receptor subtypes were found to be similar for DA the magnitude of the cellular response depends on the number of receptors, as well as their affinity states. The  $D_1$  receptor accounts for an average of 78% of the total number of DA receptors in most regions (Table 1), but only 20% of the receptors may be in the high affinity state. Conversely, the  $D_2$  receptor accounts for the remaining 22% of the total number of  $D_2$  receptors, but 80–90% of the receptors may be in the high affinity state. There is evidence to suggest that a portion of the  $D_1$  receptors in the rat striatum and substantia nigra may be spare receptors.<sup>1</sup> How spare DA receptors contribute to the proportion of high and low affinity states in these regions is not known. However, if both  $D_1$  and  $D_2$  receptors are located on the same neurons in any region, and if these affinity state proportions exist *in vivo*, these proportions will have a major influence on the cellular effects of DA.

The affinity states for the  $D_1$  and  $D_2$  receptors obtained from this autoradiographic study differ from those typically seen using homogenate prepara-

tions.<sup>20,24–26,34,44,60</sup> Many of the characteristics relating to affinity states seem to be influenced by the method used to measure them, including the dissociation constants ( $K_H$  and  $K_L$ ), the proportion of high and low affinity sites ( $R_H$  and  $R_L$ ), and the ability of guanine nucleotides to produce a complete shift in the affinity state.

For the  $D_1$  receptor studied using tissue homogenates, the dissociation constants ( $K_H$  and  $K_L$ ) for DA (1–197 and 740–3971) and SKF 38393 (3–8 and 114–600) were quite variable between different studies.<sup>26,44</sup> Likewise for the  $D_2$  receptor, the dissociation constants determined for DA ( $K_H$  varied from 16 to 460 nM, and  $K_L$  varied from 2 to 67  $\mu$ M) by different investigators were quite different.<sup>20,25,34,60</sup>

Of greater interest is the difference in the proportion of high ( $R_H$ ) and low ( $R_L$ ) affinity states determined using tissue sections compared to those determined using tissue homogenates. The proportion of high affinity state  $D_2$  receptors in striatum was found to be much higher in tissue sections ( $R_H = 77$ –90%) than in homogenate preparations, where the values of  $R_H$  ranged from 28 to 56%.<sup>20,24,71</sup> The high percentage of  $R_H$  seen for the  $D_2$  receptor in tissue sections was confirmed by obtaining similar values using both selective and nonselective DA agonists and in two different regions of the brain the striatum and the nucleus accumbens. This finding was originally ascribed to improved preservation of membrane properties or membrane proteins.<sup>53</sup> It was, therefore, unexpected that the  $D_1$  receptor would have a proportion of high affinity sites ( $R_H = 20\%$ ) much lower than that seen for the  $D_2$  receptor. This percentage was also confirmed by the use of both selective and nonselective agonists and by examining three different regions in the rat brain. This low proportion of high affinity state receptors for the  $D_1$  receptor in tissue sections was also lower than that seen in homogenate studies. Values of  $R_H$  for the  $D_1$  receptor in striatum using tissue homogenates have been reported to average 40%.<sup>26,44</sup> The reason for observing a low proportion of  $R_H$  for the  $D_2$  receptor when using tissue homogenates might be due to the processes used in preparing tissues for homogenate studies. However, the reason for the observed lower proportion of  $R_H$  for the  $D_1$  receptor in tissue sections is not clear, since identical methods and buffer were used for both the  $D_1$  and  $D_2$  receptors in this study. Perhaps homogenization is capable of uncoupling G-proteins from their intended receptor and allows them to interact with another receptor type, thus increasing the proportion of  $D_1$  receptors in the high affinity agonist state determined using homogenate preparations. The role of the relative differences in affinity state proportions in terms of biochemical and behavioral functions remains a complex function.<sup>73</sup>

Despite the differences in  $R_H$  for the  $D_1$  and  $D_2$  receptors, both could be shifted entirely to a one site low affinity state with the use of exogenous guanine nucleotide. This property also differs from that seen



in homogenate studies, where a complete shift to a single low affinity state was frequently not possible for the D<sub>1</sub><sup>26,44</sup> nor for the D<sub>2</sub><sup>19,24,28,74</sup> receptors. This may indicate that some uncoupling or alteration of G-proteins occurs during homogenization.

Affinity states have been examined using homogenate preparations in different regions of the bovine brain for D<sub>2</sub> receptors.<sup>19,35</sup> Biphasic curves were seen in caudate nucleus, putamen, olfactory tubercle, and globus pallidus in the absence of exogenous guanine nucleotide. Complete shifts to a single site were seen in the caudate nucleus in one report,<sup>19</sup> but not another,<sup>35</sup> and in the other regions examined. A maximal shift was seen only with 100  $\mu$ M guanine nucleotide,<sup>35</sup> which is 10 times higher than that required with tissue sections in this study.

Taken together, this information suggests that the agonist binding properties of receptors in tissue sections are quite different from those in homogenate preparations. The variability in tissue preparation techniques may account for the differences between the various homogenate studies. The differences between tissue sections and homogenates may reflect differences in the preservation or coupling of integral membrane components including G-proteins. Whether the properties seen using tissue sections are a better reflection of the properties of DA receptors *in vivo* has not yet been demonstrated.

#### *Relationship of affinity states to adenylate cyclase activity*

The relationship of the affinity states for the D<sub>1</sub> and D<sub>2</sub> receptors in tissue sections is difficult to relate to the findings from studies on adenylate cyclase activity due to DA receptor binding. The D<sub>1</sub> receptor has been shown to produce very strong stimulation of adenylate cyclase in striatum.<sup>62</sup> For a long time the D<sub>2</sub> receptor was thought not to be linked to adenylate cyclase due to the difficulty in demonstrating DA inhibition in the striatum.<sup>17</sup> Subsequently, reports have demonstrated inhibition of adenylate cyclase

in rat striatum due to D<sub>2</sub> receptor activation.<sup>7,47,64</sup> The magnitude of the changes in adenylate cyclase due to D<sub>2</sub> activation<sup>62</sup> and in the nucleus accumbens a lack of inhibition of adenylate cyclase has been reported,<sup>7,33,64</sup> despite the presence of substantial numbers of D<sub>2</sub> receptors, their high proportion of high affinity states, and their regulation by guanine nucleotides. Perhaps the central nervous system D<sub>2</sub> receptor is also linked to G<sub>O</sub> (guanine nucleotide-binding protein not linked to adenylate cyclase), which might have an intracellular effect different from inhibition of adenylate cyclase via G<sub>i</sub> (inhibitory guanine nucleotide-binding protein).<sup>22,34,42,51,61,65</sup>

While the D<sub>1</sub> receptor has been recently shown to have synergistic behavioral and electrophysiological actions on the D<sub>2</sub> receptor,<sup>6,23,39,41,57,68</sup> behaviors mediated solely by the D<sub>1</sub> receptor have been difficult to find.<sup>52</sup> The reason for this difficulty is not clear, but perhaps the low proportion of high affinity sites ( $R_H$ ) seen in tissue sections contributes to this. The *in vivo* activity mediated by D<sub>1</sub> receptors may be small in comparison to that mediated by the D<sub>2</sub> receptor, making the separation of behaviors mediated by the D<sub>1</sub> receptor subtype difficult.

#### CONCLUSION

The present study has demonstrated a quantitative relationship between the regional distributions of DA D<sub>1</sub> and D<sub>2</sub> receptors. The significance of this correlation will depend on the cellular location and mechanism of interaction between D<sub>1</sub> and D<sub>2</sub> receptors. The proportion of high affinity sites was quite different between the two receptor subtypes, although other affinity state characteristics were similar. These results may have implications for understanding the role of the DA system in mediating different cerebral functions in normal and pathological conditions.

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#### REFERENCES

1. Ackerman M. S., Artman L. D., Mackenzie R. G. and Keabian J. W. (1987) Evidence for dopamine D-1 receptor reserve in rat striatum and substantia nigra. *Proc. Soc. Neurosci. Abstr.* **13**, 490 (Abstract).
2. Altar C. A., O'Neil S., Walter R. J. and Marshall J. F. (1985) Dopamine and serotonin receptor sites revealed by digital subtraction autoradiography. *Science* **228**, 597–600.
3. Amalric M., Koob G. F., Creese I. and Swerdlow N. R. (1986) "Selective" D-1 and D-2 receptor antagonists fail to differentially alter supersensitive locomotor behavior in the rat. *Life Sci.* **39**, 1985–1993.
4. Arnt J. (1985) Behavioral stimulation is induced by separate dopamine D-1 and D-2 receptor sites in reserpine-pretreated but not in normal rats. *Eur. J. Pharmac.* **113**, 79–88.
5. Attie M. F., Brown E. M., Gardner D. G., Spiegel A. M. and Aurbach G. D. (1980) Characterization of dopamine-responsive adenylate cyclase of bovine parathyroid cells and its relationship to parathyroid hormone secretion. *Endocrinology* **107**, 1776–1781.
6. Barone P., Davis T. A., Braun A. R. and Chase T. N. (1986) Dopaminergic mechanisms and motor function: characterization of D-1 and D-2 dopamine receptor interactions. *Eur. J. Pharmac.* **123**, 109–114.
7. Battaglia G., Norman A. B., Hess E. J. and Creese I. (1985) D<sub>2</sub> dopamine receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in rat striatum. *Neurosci. Lett.* **59**, 177–182.
8. Bennett C. A. and Franklin N. L. (1954) *Statistical Analysis in Chemistry and the Chemical Industry*. John Wiley, New York.
9. Billard W., Ruperto V., Crosby G., Iorio L. C. and Barnett A. (1984) Characterization of the binding of [<sup>3</sup>H]-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. *Life Sci.* **35**, 885–893.

10. Bouthenet M. L., Matres M.-P., Sales N. and Schwartz J.-C. (1987) A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [<sup>125</sup>I]iodosulpiride. *Neuroscience* **20**, 115–155.
11. Bowery N. G., Hudson A. L. and Price G. W. (1987) GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience* **20**, 365–383.
12. Boyar W. C., Kim H. S. and Altar C. A. (1987) Opposing role for D-1 and D-2 dopamine receptors in the neostriatal content of cyclic GMP. *Proc. Soc. Neurosci. Abstr.* **13**, 1346.
13. Boyson S. J., McGonigle P. and Molinoff P. B. (1986) Quantitative autoradiographic localization of the D<sub>1</sub> and D<sub>2</sub> subtypes of dopamine receptors in rat brain. *J. Neurosci.* **6**, 3177–3188.
14. Braun A. R. and Chase T. N. (1986) Obligatory D-1/D-2 receptor interaction in the generation of dopamine agonist related behaviors. *Eur. J. Pharmac.* **131**, 301–306.
15. Brown E. M., Attie M. F., Reen S., Gardner D. G., Kebebian J. and Aurbach G. D. (1980) Characterization of dopaminergic receptors in dispersed bovine parathyroid cells. *Molec. Pharmac.* **18**, 335–340.
16. Carlson J. H., Bergstrom D. A. and Walters J. R. (1987) Stimulation of both D<sub>1</sub> and D<sub>2</sub> dopamine receptors appears necessary for full expression of postsynaptic effects of dopamine agonists: a neurophysiological study. *Brain Res.* **400**, 205–218.
- 16a. Charuchinda C., Supavilai P., Karobath M. and Palacios J. M. (1987) Dopamine D<sub>2</sub> receptors in the rat brain: autoradiographic visualization using a high-affinity selective agonist ligand. *J. Neurosci.* **7**, 1352–1360.
17. Creese I., Sibley D. R., Hamblin M. W. and Leff S. E. (1983) The classification of dopamine receptors: relationship to radioligand binding. *A. Rev. Neurosci.* **6**, 43–71.
18. Dawson T. M., Gehlert D. R., McCabe R. T., Barnett A. and Wamsley J. K. (1986) D-1 dopamine receptors in the rat brain: a quantitative autoradiographic analysis. *J. Neurosci.* **6**, 2352–2365.
19. De Keyser J., De Backer J.-P., Convents A., Ebinger G. and Vaquelin G. (1985) D<sub>2</sub> dopamine receptors in calf globus pallidus: agonist high- and low-affinity sites not regulated by guanine nucleotides. *J. Neurochem.* **45**, 977–979.
20. De Lean A., Kilpatrick B. F. and Caron M. B. (1982) Dopamine receptors of the porcine anterior pituitary gland; evidence for two affinity states discriminated by both agonists and antagonists. *Molec. Pharmac.* **22**, 290–297.
- 20a. Dubois A. and Scatton B. (1985) Heterogeneous distribution of dopamine D<sub>2</sub> receptors within the rat striatum as revealed by autoradiography of [<sup>3</sup>H]N-n-propylorapomorphine binding sites. *Neurosci. Lett.* **57**, 7–12.
21. Dubois A., Savasta M., Curet A. and Scatton B. (1986) Autoradiographic distribution of the D<sub>1</sub> agonist [<sup>3</sup>H]SKF 38393, in the rat brain and spinal cord. Comparison with the distribution of D<sub>2</sub> dopamine receptors. *Neuroscience* **19**, 125–137.
22. Enjalbert A., Sladeczek F., Guillon G., Bertrand P., Shu C., Epelbaum J., Garcia-Saint A., Jard S., Lombard C., Kordon C. and Bockaert J. (1986) Angiotensin II and dopamine modulate both cAMP and inositol phosphate production in anterior pituitary cells. *J. biol. Chem.* **261**, 4071–4075.
23. Gershanki O., Heikkila R. E. and Duvoisin R. C. (1983) Behavioral correlations of dopamine receptor activation. *Neurology* **33**, 1489–92.
24. Hall H. and Sällermärk M. (1987) Effects of chronic neuroleptic treatment on agonist affinity states of the dopamine-D<sub>2</sub> receptor in the rat brain. *Pharmac. Toxicol.* **60**, 359–363.
25. Hancock A. A. and Marsh C. L. (1984) Distinctions between ligand-binding sites for [<sup>3</sup>H]dopamine and D<sub>2</sub> dopaminergic receptors characterized with [<sup>3</sup>H]-spiroperidol. *Molec. Pharmac.* **26**, 439–451.
26. Hess E. J., Battaglia G., Norman A. B., Torio L. C. and Creese I. (1986) Guanine nucleotide regulation of agonist interactions at [<sup>3</sup>H]-SCH 23390-labeled D<sub>1</sub> dopamine receptors in rat striatum. *Eur. J. Pharmac.* **121**, 31–38.
27. Howlett D. R., Morris H. and Nahorski S. R. (1979) Anomalous properties of [<sup>3</sup>H]-spiperone binding sites in various areas of the rat limbic system. *Molec. Pharmac.* **15**, 560–564.
28. Huff R. M. and Molinoff P. B. (1982) Quantitative determination of dopamine receptor subtypes not linked to activation of adenylate cyclase in rat striatum. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7561–7565.
29. Joyce J. N., Loesch S. K. and Marshall J. F. (1985) Dopamine D-2 receptors in rat caudate-putamen: the lateral to medial gradient does not correspond to dopaminergic innervation. *Brain Res.* **338**, 209–218.
30. Joyce J. N. and Marshall J. F. (1986) Quantitative autoradiography of dopamine D<sub>2</sub> sites in rat caudate-putamen: localization to intrinsic neurons and not to neocortical afferents. *Neuroscience* **20**, 773–795.
31. Kebebian J. W. and Calne D. B. (1979) Multiple receptors for dopamine. *Nature* **277**, 93–96.
32. Kebebian J. W., Agui T., van Oene J. C., Shigematsu K. and Saavedra J. M. (1986) The D<sub>1</sub> dopamine receptor: new perspectives. *Trends Pharmac. Sci.* **5**, 96–99.
33. Kelly E. and Nahorski S. R. (1987) Dopamine D-2 receptors inhibit D-1 stimulated cyclic AMP accumulation in striatum but not limbic forebrain. *Naunyn-Schmiedeberg's Arch. Pharmac.* **335**, 508–512.
34. Lacey M. G., Mercuri N. B. and North R. A. (1987) Dopamine acts on D<sub>2</sub> receptors to increase potassium conductance in neurons of the rat substantia nigra zona compacta. *J. Physiol.* **392**, 397–416.
35. Leonard M. N., Macey C. A. and Strange P. G. (1987) Heterogeneity of D<sub>2</sub> dopamine receptors in different brain regions. *Biochem. J.* **248**, 595–602.
36. Lundeen J. E. and Gordon J. H. (1986) Computer analysis of binding data. In *Receptor Binding in Drug Research* (ed. O'Brien R. A.), pp. 31–49. Marcel Dekker, New York.
37. Mailman R. B., Schulz D. W., Lewis M. H., Staples L., Rollema H. and Dehaven D. L. (1984) SCH-22390: a selective D<sub>1</sub> dopamine antagonist with potent D<sub>2</sub> behavioral actions. *Eur. J. Pharmac.* **101**, 159–160.
38. Maragos W. F., Penney J. B. and Young A. B. (1988) Anatomic correlation of NMDA and <sup>3</sup>H-TCP-labeled receptors in rat brain. *J. Neurosci.* **8**, 493–501.
39. Mashurano M. and Waddington J. L. (1986) Stereotyped behaviour in response to the selective D-2 dopamine receptor agonist RU 24213 is enhanced by pretreatment with the selective D-1 agonist SK&F 38393. *Neuropharmacology* **25**, 947–949.
40. Matres M.-P., Bouthenet M.-L., Sales N., Sokoloff P. and Schwartz J.-C. (1985) Widespread distribution of brain dopamine receptors evidenced with [<sup>125</sup>I]iodosulpiride, a highly selective ligand. *Science* **228**, 752–755.
41. Meller E., Kuga S., Friedhoff A. J. and Goldstein M. (1985) Selective D<sub>2</sub> dopamine receptor agonists prevent catalepsy induced by SCH 22390, a selective D<sub>1</sub> antagonist. *Life Sci.* **36**, 1857–1864.
42. Memo M., Castelletti C., Missale C. and Spano P. (1987) Stimulation of dopamine D-2 receptors increases potassium permeability in mammothrophs. *Eur. J. Pharmac.* **139**, 361–362.

43. Nagy J. I., Lee T., Seeman P. and Fibiger H. C. (1973) Direct evidence for presynaptic and postsynaptic dopamine receptors in brain. *Nature* **274**, 278–281.
44. Niznik H. B., Otsuka N. Y., Dumbrielle-Ross A., Grigoriadis D., Tirpak A. and Seeman P. (1986) Dopamine D<sub>1</sub> receptors characterized with [<sup>3</sup>H]-SCH 23390: solubilization of a guanine nucleotide-sensitive form of the receptor. *J. biol. Chem.* **261**, 8397–8406.
45. Nock B., Sedvall G. and McEwen B. S. (1986) Quantitative autoradiography of [<sup>3</sup>H]piquindone binding sites (dopamine D-2 receptors) in rat brain. *Eur. J. Pharmac.* **121**, 387–393.
46. Ohno Y., Sasa M. and Takaori S. (1987) Coexistence of inhibitory dopamine D-1 and excitatory D-2 receptors on the same caudate nucleus neurons. *Life Sci.* **40**, 1937–1945.
47. Onali P., Olanas M. C. and Gessa G. L. (1985) Characterization of dopamine receptors mediating inhibition of adenylate cyclase activity in rat striatum. *Molec. Pharmac.* **28**, 138–145.
48. Pan H. S., Frey K. A., Young A. B. and Penney J. B. (1983) Changes in [<sup>3</sup>H]muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography. *J. Neurosci.* **3**, 1189–1198.
49. Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York.
50. Penney J. B., Pan H. S., Young A. B., Frey K. A. and Dauth G. W. (1981) Quantitative autoradiography of [<sup>3</sup>H]-muscimol binding in rat brain. *Science* **214**, 1036–1038.
51. Pizzi M., D'Agostini F., Da Prada M., Spano P. F. and Haefely W. E. (1987) Dopamine D<sub>2</sub> receptor stimulation decreases the inositol triphosphate level of rat striatal slices. *Eur. J. Pharmac.* **136**, 263–264.
52. Pugh M. T., O'Boyle K. M., Molloy A. G. and Waddington J. L. (1985) Effects of the putative D-1 antagonist SCH 23390 on stereotyped behavior induced by the D-2 agonist RU24213. *Psychopharmacologica* **87**, 308–312.
53. Richfield E. K., Young A. B. and Penney J. B. (1986) Properties of D-2 dopamine receptor autoradiography: high percentage of high affinity agonist sites and increased nucleotide sensitivity in tissue sections. *Brain Res.* **383**, 121–128.
54. Richfield E. K., DeBowel D., Penney J. B. and Young A. B. (1987) Basal ganglia and cerebral cortical distribution of dopamine D-1 and D-2 receptors in neonatal and adult cat brain. *Neurosci. Lett.* **73**, 203–208.
55. Richfield E. K., Young A. B. and Penney J. B. (1987) Comparative distribution of dopamine D-1 and D-2 receptors in the basal ganglia of turtle, pigeon, rat, cat, and monkey. *J. comp. Neurol.* **262**, 446–463.
56. Richfield E. K., Young A. B. and Penney J. B. (1989) Comparative distribution of dopamine D-1 and D-2 receptors in the cerebral cortex of rats, cats, and monkeys (submitted).
57. Robertson G. S. and Robertson H. A. (1986) Synergistic effects of D<sub>1</sub> and D<sub>2</sub> dopamine agonist on turning behavior in rats. *Brain Res.* **384**, 387–390.
58. Scatton B. and Dubois A. (1985) Autoradiographic localization of D<sub>1</sub> dopamine receptors in the rat brain with [<sup>3</sup>H]SKF 38393. *Eur. J. Pharmac.* **111**, 145–146.
59. Skirboll L. R., Grace A. A. and Bunney B. S. (1979) Dopamine auto- and postsynaptic receptors: electrophysiological evidence for differential sensitivity to dopamine agonists. *Science* **206**, 80–82.
60. Sibley D. R., DeLean A. and Creese I. (1982) Anterior pituitary dopamine receptors: demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *J. biol. Chem.* **275**, 6351–6361.
61. Simmonds S. H. and Strange P. G. (1985) Inhibition of inositol phospholipid breakdown by D<sub>2</sub> dopamine receptors in dissociated bovine anterior pituitary cells. *Neurosci. Lett.* **60**, 267–272.
62. Stoof J. C. and Kebabian J. W. (1981) Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature* **294**, 366–368.
63. Stoof J. C. and Kebabian J. W. (1984) Two dopamine receptors: biochemistry, physiology, and pharmacology. *Life Sci.* **35**, 2281–2296.
64. Stoof J. C. and Verheijden P. F. H. M. (1986) D-2 receptor stimulation inhibits cyclic AMP formation brought about by D-1 receptor stimulation in rat neostriatum, but not nucleus accumbens. *Eur. J. Pharmac.* **129**, 205–206.
65. Stoof J. C., Verheijden P. F. H. M. and Leysen J. F. (1987) Stimulation of D<sub>2</sub>-receptors in rat nucleus accumbens slices inhibits dopamine and acetylcholine release but not cyclic AMP formation. *Brain Res.* **423**, 364–368.
66. Trugman J. M., Geary W. A. II and Wooten G. F. (1986) Localization of D-2 dopamine receptors to intrinsic striatal neurons by quantitative autoradiography. *Nature* **322**, 267–269.
67. Trugman J. M. and Wooten G. F. (1986) The effect of L-dopa on regional cerebral glucose utilization in rats with unilateral lesions of the substantia nigra. *Brain Res.* **379**, 264–274.
68. Trugman J. M. and Wooten G. F. (1987) Selective D<sub>1</sub> and D<sub>2</sub> dopamine agonist differentially alter basal ganglia glucose utilization in rats with unilateral 6-hydroxydopamine substantia nigra lesions. *J. Neurosci.* **7**, 2927–2935.
- 68a. Unnerstall J. R., Kuhar M. J., Niehoff D. L. and Palacios J. M. (1981) Benzodiazepine receptors are coupled to a subpopulation of g-aminobutyric acid (GABA) receptors: evidence from a quantitative autoradiographic study. *J. Pharmac. exp. ther.* **218**, 797–804.
69. Van der Weide J., Camps M., Horn A. S. and Palacios J. M. (1987) Autoradiographic localization of dopamine D<sub>2</sub> receptors in the rat brain using the new agonist [<sup>3</sup>H]N-0437. *Neurosci. Lett.* **83**, 259–263.
70. Walters J. R., Bergstrom D. A., Carlson J. H., Chase T. N. and Braun A. R. (1987) D<sub>1</sub> dopamine receptor activation required for postsynaptic expression of D<sub>2</sub> agonist effects. *Science* **236**, 719–722.
71. Wamsley J. K., Filloux F. M., Gehlert D. R. and Dawson T. M. (1986) Lesions in the rat brain alter the density and distribution of dopamine D-1 and D-2 receptors as ascertained by quantitative autoradiography. *Proc. Soc. Neurosci. Abstr.* **12**, 192.
72. Weick B. G. and Walters J. R. (1987) Do D-1/D-2 receptor interactions occur directly in the substantia nigra pars reticulata? *Proc. Soc. Neurosci. Abstr.* **13**, 489.
73. Wreggett K. A. and De Léan A. (1984) The ternary complex model. Its properties and application to ligand interactions with the D<sub>2</sub>-dopamine receptor of the anterior pituitary gland. *Molec. Pharmac.* **26**, 214–227.
74. Wreggett K. A. and Seeman P. (1984) Agonist high- and low-affinity states of the D<sub>2</sub>-dopamine receptor in calf brain: Partial conversion by guanine nucleotide. *Molec. Pharmac.* **25**, 10–17.
75. Young A. B., Cha J.-H. J., Greenamyre J. T., Maragos W. F. and Penney J. B. (1989) Distribution of PCP and glutamate receptors in normal and pathologic mammalian brain. In *The Allosteric Modulation of Amino Acid Receptors and its Therapeutic Implications* (eds Costa E. and Bernard E.) pp. 357–375. Raven Press, New York.