The Journal of Neuroscience
October 1986, 6(10): 2941-2949

^{3}H-3-*N*-Methylspiperone Labels D_2 Dopamine Receptors in Basal Ganglia and S_2 Serotonin Receptors in Cerebral Cortex

R. A. Lyon,* M. Titeler,* J. J. Frost,†‡ P. J. Whitehouse,§ D. F. Wong,† H. N. Wagner, Jr.,† R. F. Dannals,† J. M. Links,† and M. J. Kuhar‡**

*Department of Pharmacology and Toxicology, Albany Medical College, Albany, New York 12208; †Division of Nuclear Medicine and Radiation Health Sciences, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205; ‡Department of Neuroscience and §Neuropathology Laboratory, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; and **Neuroscience Branch, Addiction Research Center, National Institute on Drug Abuse, Baltimore, Maryland 21224

Detailed studies of the properties of ^{3}H-3-*N*-methylspiperone (NMSP) binding in rat and human brain homogenates were performed at 37°C. In homogenates of rat striatum and frontal cortex and human caudate and frontal cortex tissues, the specific binding was found to be saturable. Rat caudate contained 33.2 pmol/gm wet-weight tissue and displayed an equilibrium dissociation constant (K_d) of 8.7×10^{-11} M; rat frontal cortex contained 18.5 pmol/gm wet-weight tissue and displayed a K_d of 1.5×10^{-10} M. Human caudate contained 8.96 pmol/gm wet-weight tissue and displayed a K_d of 1.1×10^{-10} M; human frontal cortex possessed 9.8 pmol/gm wet-weight tissue and a K_d of 4.4×10^{-10} M. Kinetic studies revealed a very rapid rate of association in all the tissues studied. The rate of dissociation was relatively slow in all 4 tissue preparations; the dissociation rate was somewhat slower in rat striatum and human caudate relative to rat and human frontal cortex. This was consistent with the somewhat higher affinity, relative to frontal cortex, displayed by ^{3}H-NMSP in rat striatal and human caudate tissue. The pharmacological properties of the specific binding in rat striatal and human caudate tissues were very similar and indicated the presence of brain D_2 dopamine receptors. In rat and human frontal cortex tissue homogenates, the pharmacological characteristics of the specific binding indicated the presence of 5-HT_2 receptors.

Dopamine and serotonin receptors have been studied biochemically in a large number of *in vitro* and *in vivo* receptor binding assays. Many radiolabeled ligands have been used in these studies (Creese et al., 1983; Titeler, 1983). 3-*N*-Methylspiperone (NMSP) containing ^{11}C in the methyl group has been used as a receptor-labeling radioligand in positron emission tomographic (PET) studies in humans (Wagner et al., 1983; Wong et al., 1984). High levels of binding were detected in human caudate and putamen, and moderate levels in the frontal cortex. These results and the fact that NMSP is a derivative of spiperone, a well documented D_2 and 5-HT_2 receptor ligand, has led researchers to conclude that NMSP is labeling predominantly D_2 dopamine receptors in the caudate and 5-HT_2 receptors in cerebral cortical areas in the PET scan studies (Wagner et al., 1983; Wong et al., 1984). Since kinetic constants are needed for modeling and for calculations in PET, the goal of this study was to characterize the interaction of NMSP with D_2 dopamine and S_2 serotonin receptors.

Received Dec. 2, 1985; revised Mar. 24, 1986; accepted Apr. 1, 1986.

This work was supported by BRSG Grant S07RR05394-23, NIH Grants NS 15080, MH 25951, and MH 00053, and by a McKnight Foundation grant. The authors thank Dr. Ian Creese for generously carrying out binding competition studies with the D receptor and Drs. Itoh and Kebabian for generously carrying out the dopamine-stimulated adenylate cyclase assays. We are grateful to Susan Krout and Mary Flutka for manuscript preparation and to Ms. Kendal Marcus for technical assistance.

Correspondence should be addressed to Dr. Michael J. Kuhar, Neuroscience Branch, NIDA Addiction Research Center, P. O. Box 5180, Baltimore, MD 21224.

Materials and Methods

^{3}H-NMSP (76.7 Ci/mmol) was synthesized and purified by New England Nuclear (Boston, MA). Serotonin, dopamine, and noradrenaline were purchased from Sigma. Other drugs were donated by pharmaceutical firms.

Membrane preparation

The rat tissue was obtained by rapid dissection of brain tissue from male Sprague-Dawley rats. Human tissue was obtained at autopsy from 4 male subjects who had no history of neurological or psychiatric disease and were not known to be taking any psychoactive medication. Their average age was 55 years, and the average postmortem delay between death and freezing of the brain was 11.3 hr. Neuropathological examination of the tissues verified the absence of neurological disease.

For the human tissues, 1 hemisphere was chosen at random for receptor studies, sliced coronally in 2 cm sections, frozen on dry ice and stored at −80°C until dissected further. The remaining hemisphere was placed in formalin for neuropathological analysis. The frontal cortex tissue was obtained from the most anterior coronal slice near the frontal pole. The caudate specimens were dissected from the anterior caudate nucleus, present in the third or fourth coronal slice.

Tissue preparation was performed as described by Leysen et al. (1982) for the rat and human frontal cortex and according to List and Seeman (1981) for the rat and human caudate, with minor modifications (Battaglia et al., 1984). The prefrontal and parietofrontal cortices and striata of male Wistar rats, and the frontal cortical and caudate tissue of postmortem human brains were homogenized in 50 mM Tris-HCl buffer, pH 7.4 (1:40 wt/vol) and centrifuged at 35,000 × *g* for 10 min. The pellets were resuspended in buffer and recentrifuged. The final suspension was in 50 mM Tris-HCl, pH 7.4, at 37°C for the cortical tissue and 50 mM Tris-HCl, 140 mM NaCl, pH 7.4, at 37°C for the rat striatal and human caudate tissue. The addition of NaCl to frontal cortex tissue preparations has been found to be deleterious to 5-HT_2 receptor binding of ^{3}H-ketanserin (Leysen et al., 1982); NaCl has been found to be useful in D_2 receptor binding studies, as well as being necessary in order to use sulpiride as the excess displacing nonradioactive ligand (List and Seeman, 1981; Woodruff and Freedman, 1981).

Radioligand binding assay

Assays were performed in triplicate (for saturation and competition studies) or sextuplicate (for kinetic studies) in 2.0 ml volume containing 4 mg wet-weight of tissue (which was added last) for rat and human cortical tissue, and 2.5 mg for rat striatal and human caudate tissue.

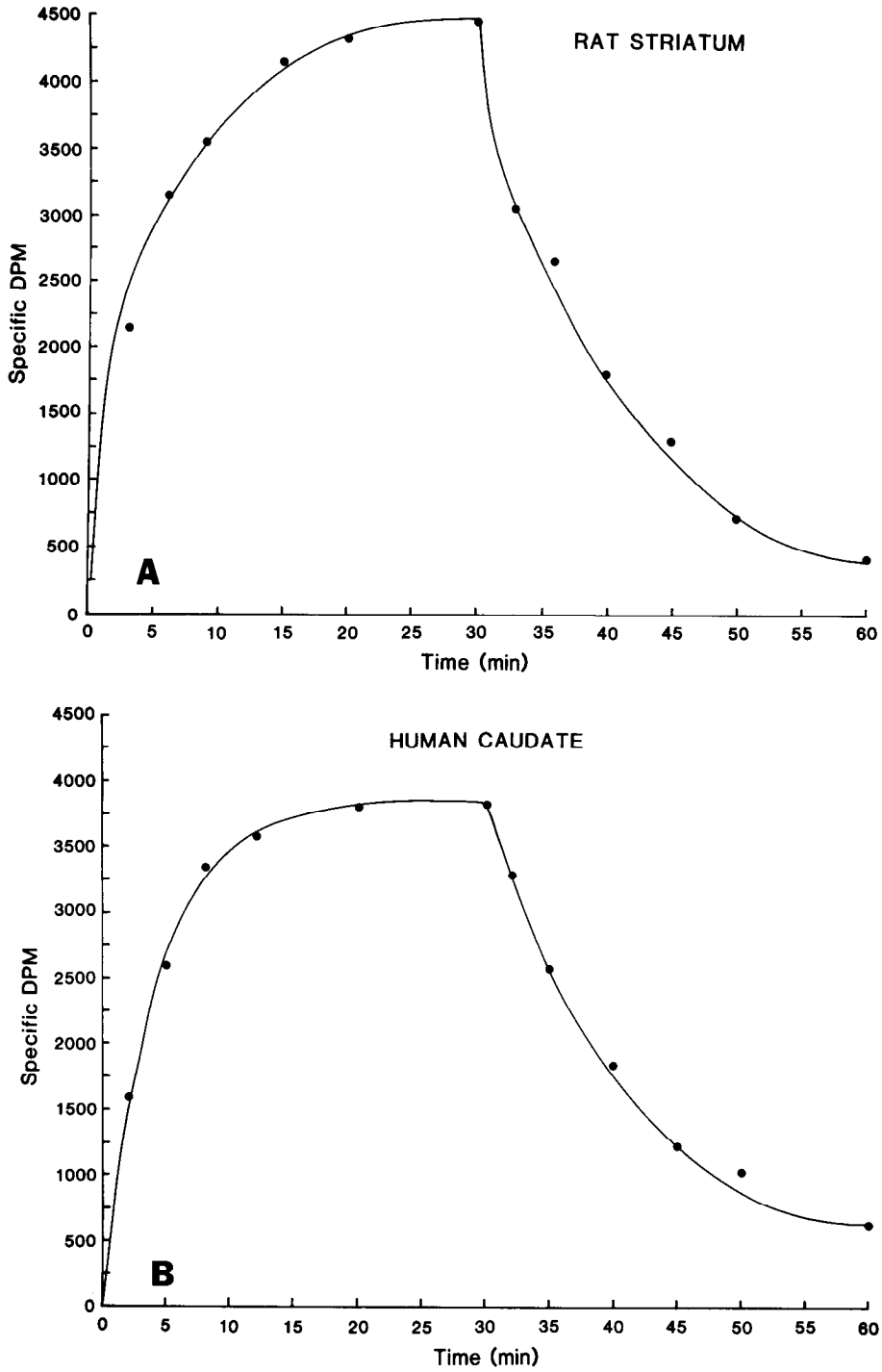


Figure 1. Association and dissociation of ^{3}H-NMSP binding in rat striatum (*A*), human caudate (*B*), rat frontal cortex (*C*), and human frontal cortex (*D*). ^{3}H-NMSP, 10^{-10} M, was coincubated with 2.5 mg of rat striatal or human caudate tissue or 4 mg of rat or human frontal cortex tissue. At 30 min, 10^{-6} M cinanserin (rat and human frontal cortex tissue) or 10^{-5} M sulpiride was added to the incubation mixture to initiate the dissociation phase of the experiments. Each *point* was performed in sextuplicate; all experiments were performed 3× except that on the human cortex, which was performed twice. Data are means (n = 3). SEM of all points was less than 10%.

^{3}H-NMSP saturation analyses were performed at varying concentrations of radioligand using 10^{-6} M cinanserin to define nonspecific binding in cortical tissue and 10^{-5} M (−)-sulpiride to define nonspecific binding in rat striatal and human caudate tissue. Competition and kinetic experiments were performed using 10^{-10} M ^{3}H-NMSP; at this concentration, 74, 65, 89, and 60% of the total binding was specific in the rat striatal, rat cortical, human caudate, and human cortical tissue, respectively. Tubes were incubated for 15 min at 37°C, filtered on Whatman GF/B glass fiber filters, and washed with 10 ml buffer (50 mM Tris-HCl, pH 7.4). The filters were counted by liquid scintillation spectrometry in 5 ml of aqueous counting scintillant (Scintiverse, Fisher) at an efficiency of 50%. In the kinetic studies, 10^{-6} M cinanserin (for 5-HT$_2$ studies) or 10^{-5} M sulpiride (for the D$_2$ studies) was added to the membranes at the 30 min time point to initiate the dissociation phase of the experiments.

Results

Figure 1 presents the kinetic characteristics of ^{3}H-NMSP specific binding in rat and human tissues at 37°C. The rate of association in all 4 tissues is very rapid; measuring this parameter is difficult, since at the critical early time points, high levels of binding have already occurred. The dissociation rates in the rat striatum and human caudate appear to be very similar—7.82 and 6.23 × 10^2 min^{-1}, respectively. These kinetic studies are consistent with the results of the equilibrium studies shown in Figure 2.

Saturation studies in rat striatal and human caudate tissue reveal a similar affinity for specific ^{3}H-NMSP binding, i.e., K_ds of 87 and 93 pM, respectively; however, there is significantly less binding in the human tissue (B_{max} = 8.96 pmol/gm wet-

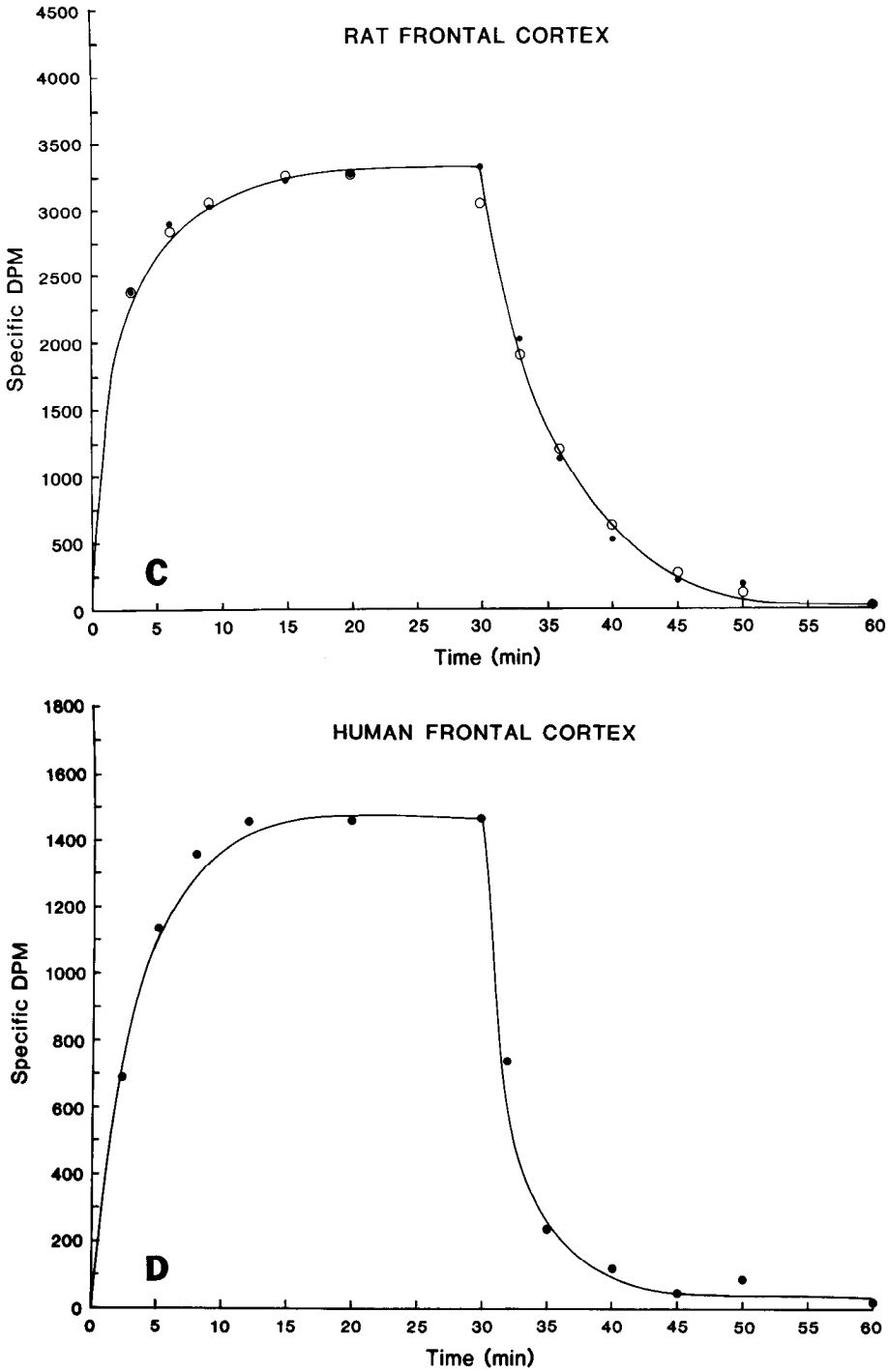


Figure 1. Continued.

weight) than in the rat (B_{max} = 33.2 pmol/gm wet-weight). The saturation studies in rat and human frontal cortex tissue reveal high-affinity binding with a significantly lower affinity than that in the corresponding striatal or human caudate tissue (Fig. 2, *C* and *D*). Again, the level of binding in the human cortical tissue is lower (B_{max} = 9.8 pmol/gm) than in the rat cortical tissue (B_{max} = 18.5 pmol/gm). The lower affinity observed in the frontal cortex tissues is presumably due to the more rapid dissociation rate observed in the kinetic studies (Table 1).

In rat striatal and human caudate tissue, the pattern of affinities of a series of drugs is clearly indicative of a D_2 dopamine receptor (Fig. 3; Table 2). Sulpiride, a specific D_2 dopamine receptor blocker, potently inhibits the ³H-NMSP specific bind-

Table 1. Kinetic constants for ³H-NMSP

Region	k_1 (min^{-1} M^{-1})	k_{-1} (min^{-1})
Rat striatum	1.68×10^9 (±0.40)	7.82×10^{-2} (±0.30)
Human caudate	2.96×10^9 (±0.17)	6.23×10^{-2} (±0.46)
Rat frontal cortex	9.40×10^8 (±0.30)	1.56×10^{-1} (±0.20)
Human frontal cortex	1.60×10^9 (±0.10)	1.30×10^{-1} (±0.10)

Kinetic rate constants for the association and dissociation of ³H-NMSP binding in the rat and human tissues. k_1 is the kinetic association constant, determined according to the formula $k_1 = (k_{obs} - k_{-1})/[^3\text{H-NMSP}]$ (Bennett and Yamamura, 1985), where k_{obs} is the experimentally observed association rate constant and k_{-1} is the dissociation rate constant.

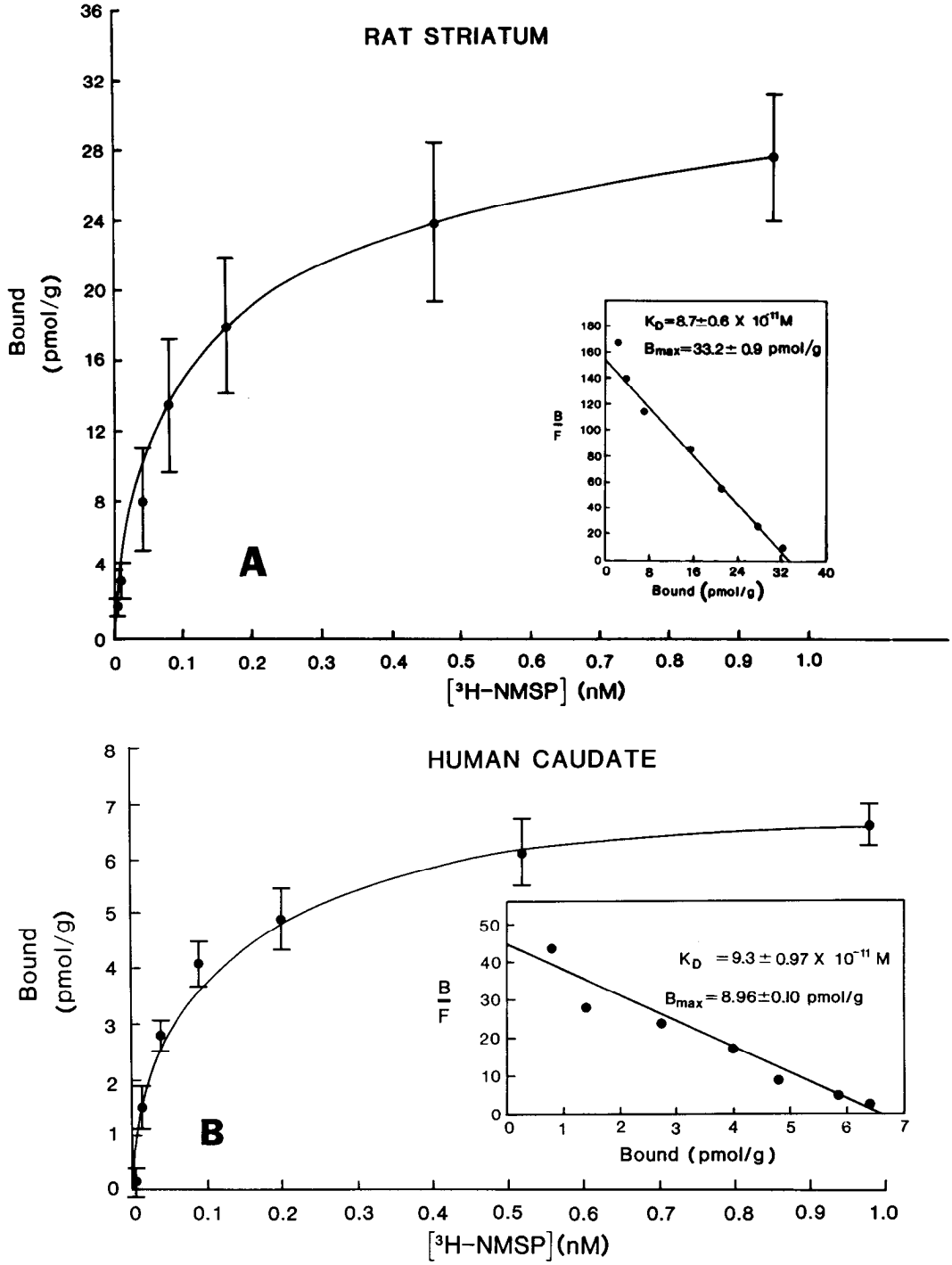


Figure 2. Saturable specific binding of ^{3}H-NMSP to rat striatum (*A*), human caudate (*B*), rat frontal cortex (*C*), and human frontal cortex (*D*). Membranes were incubated with increasing concentrations of radioligand in the absence (total binding) or presence (nonspecific binding) of either 10^{-5} M sulpiride (striatal and caudate tissue) or 10^{-6} M cinanserin (frontal cortex tissue). Results are from 3 independent experiments, each *point* in each experiment being performed in triplicate. *Error bars* are SEM. *Insets* are the Scatchard transformations of the saturation data.

ing in both rat striatal and human caudate tissue (Fig. 3, *A, B*). Potent D_2 dopamine receptor blockers, such as spiperone, haloperidol, and chlorpromazine, inhibit the rat striatal and human caudate tissue binding with potencies clearly indicative of a D_2 dopamine receptor interaction (Seeman, 1980). Consistent with this interpretation are the results from competition studies using dopamine, serotonin, and noradrenaline (Fig. 3, *A, B*; Table 3). In rat striatal and human caudate tissue, dopamine is the only one of these 3 neurotransmitters to compete for specific ^{3}H-NMSP binding.

The competition studies in rat and human frontal cortex tissue revealed significantly different pharmacological properties of ^{3}H-NMSP binding as compared to rat striatal and human caudate tissue (Fig. 3; Table 2). Sulpiride, the potent and specific D_2 dopamine receptor blocker, does not compete for ^{3}H-NMSP binding in the rat or human frontal cortex tissue at 0.1 mM

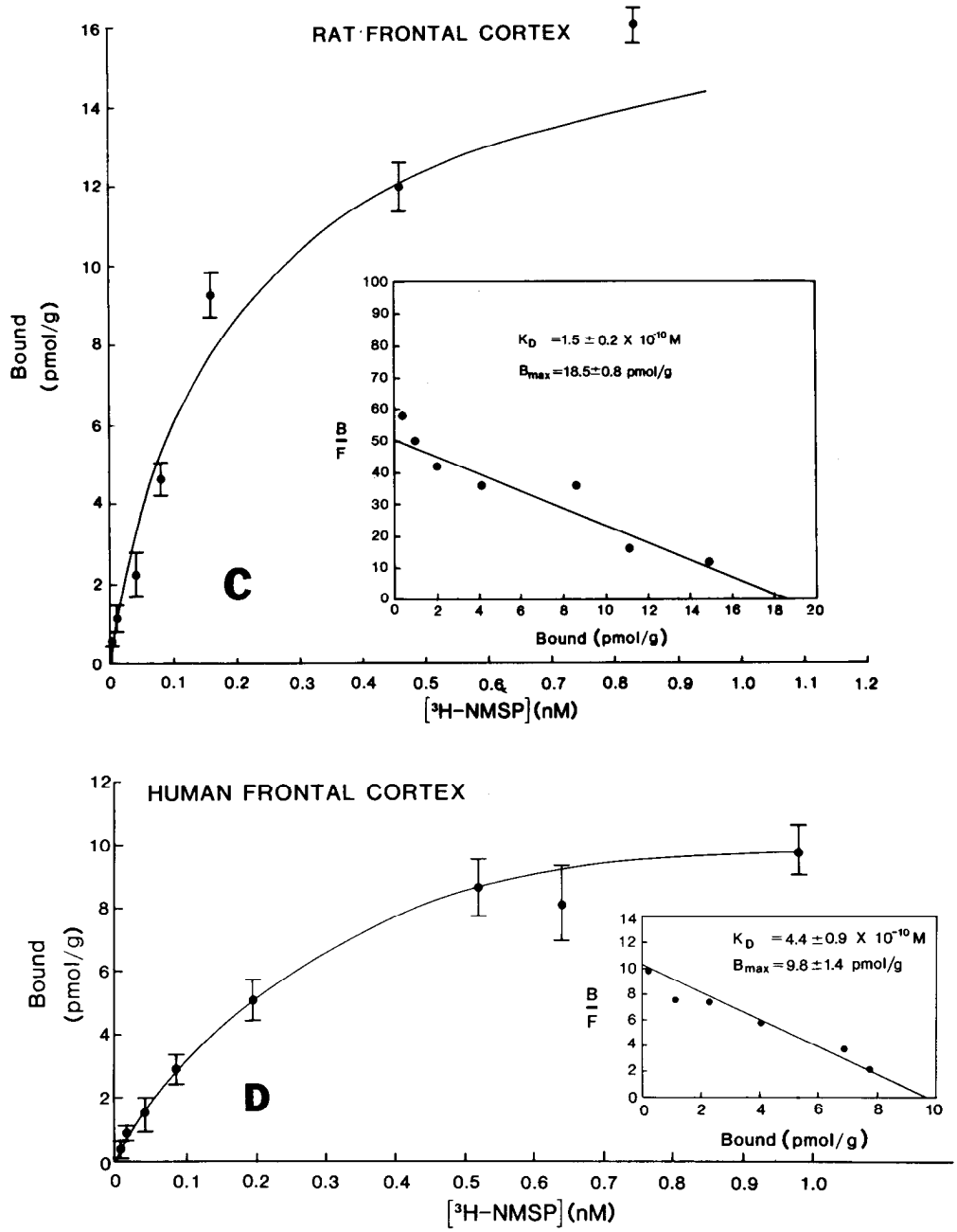


Figure 2. Continued.

concentration. Potent 5-HT_2 blockers, such as ketanserin and cinanserin, which display low affinity for rat striatal and human caudate binding, potently inhibit the binding in rat and human frontal cortex tissue. Serotonin, which does not inhibit the rat striatal or human caudate binding, inhibits the rat and human frontal cortex binding with a potency consistent with a 5-HT_2 receptor interaction. Neither dopamine nor noradrenaline inhibits the rat or human frontal cortex binding.

Table 3 lists the IC_{50} values for unlabeled NMSP in competing for a series of radiolabeled receptors. The only receptor with an affinity at all approaching that of NMSP for D_2 or 5-HT_2 receptors is the alpha receptor. However, the affinity of NMSP for that receptor is approximately 10-fold lower than the affinity of NMSP for the 5-HT_2 receptor and approximately 30-fold lower than the affinity of an NMSP for the D_2 receptor. Table 2 lists the K_i values for a series of drugs, as determined by competition assays in rat striatal, human caudate, rat frontal cortex, and human frontal cortex tissues. There is a very high degree of correlation between the rat striatal and human caudate affinities; there is a similar, high degree of correlation between the rat and human cortical tissue values.

Discussion

The similarity of the structure of ³H-3-*N*-methylspiperone to that of the compound spiperone would lead one to expect a similarity in the receptor binding profiles of the 2 drugs. Considering that NMSP is being used as a PET scan ligand (Wagner et al., 1983; Wong et al., 1984), it is clear that a detailed char-

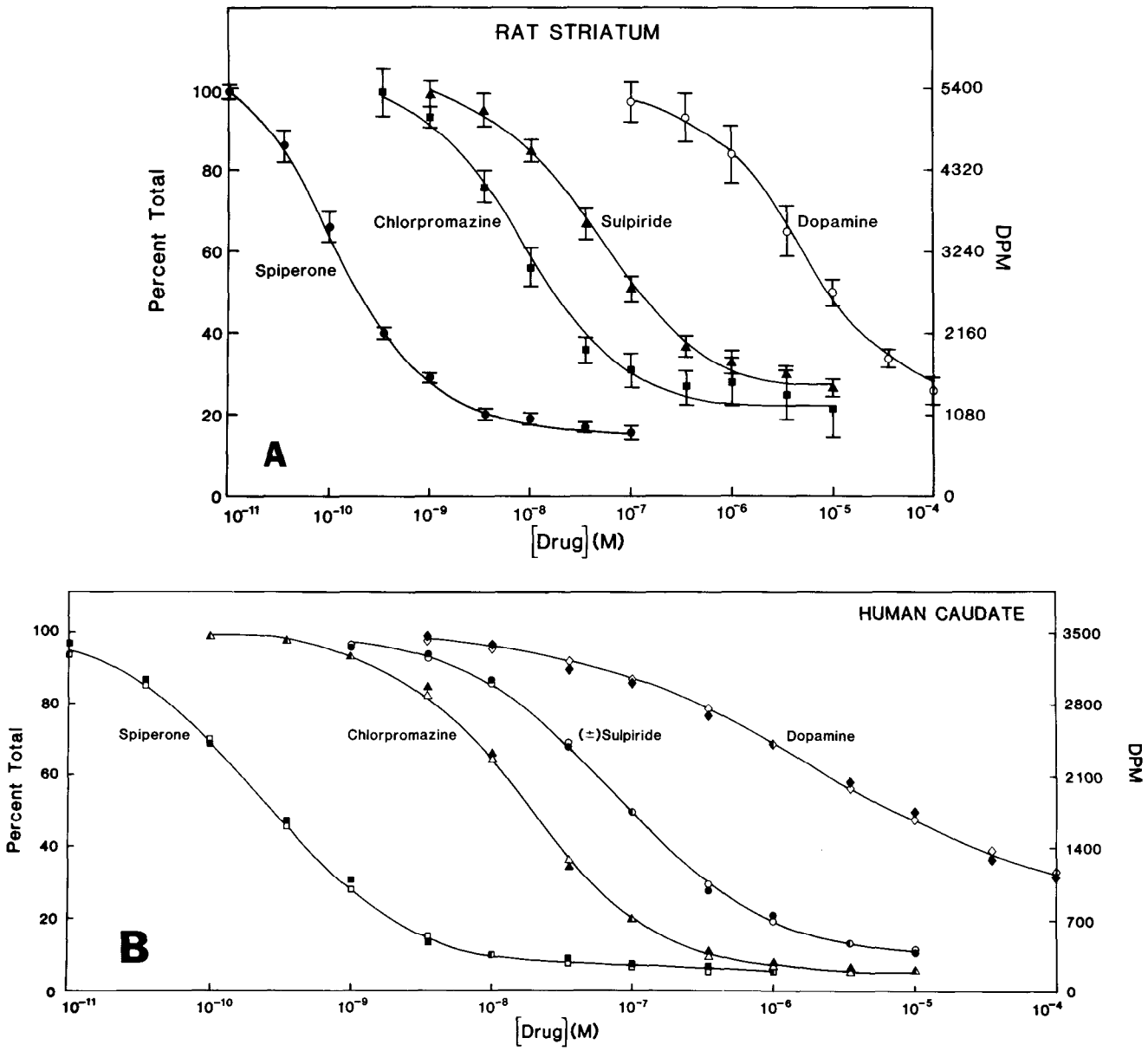


Figure 3. Competition curves of representative drugs competing for ^{3}H-NMSP binding to rat striatal (*A*), human caudate (*B*), rat frontal cortex (*C*), and human frontal cortex (*D*) tissue. Membranes were prepared as described in Materials and Methods and incubated with 10^{-10} M ^{3}H-NMSP

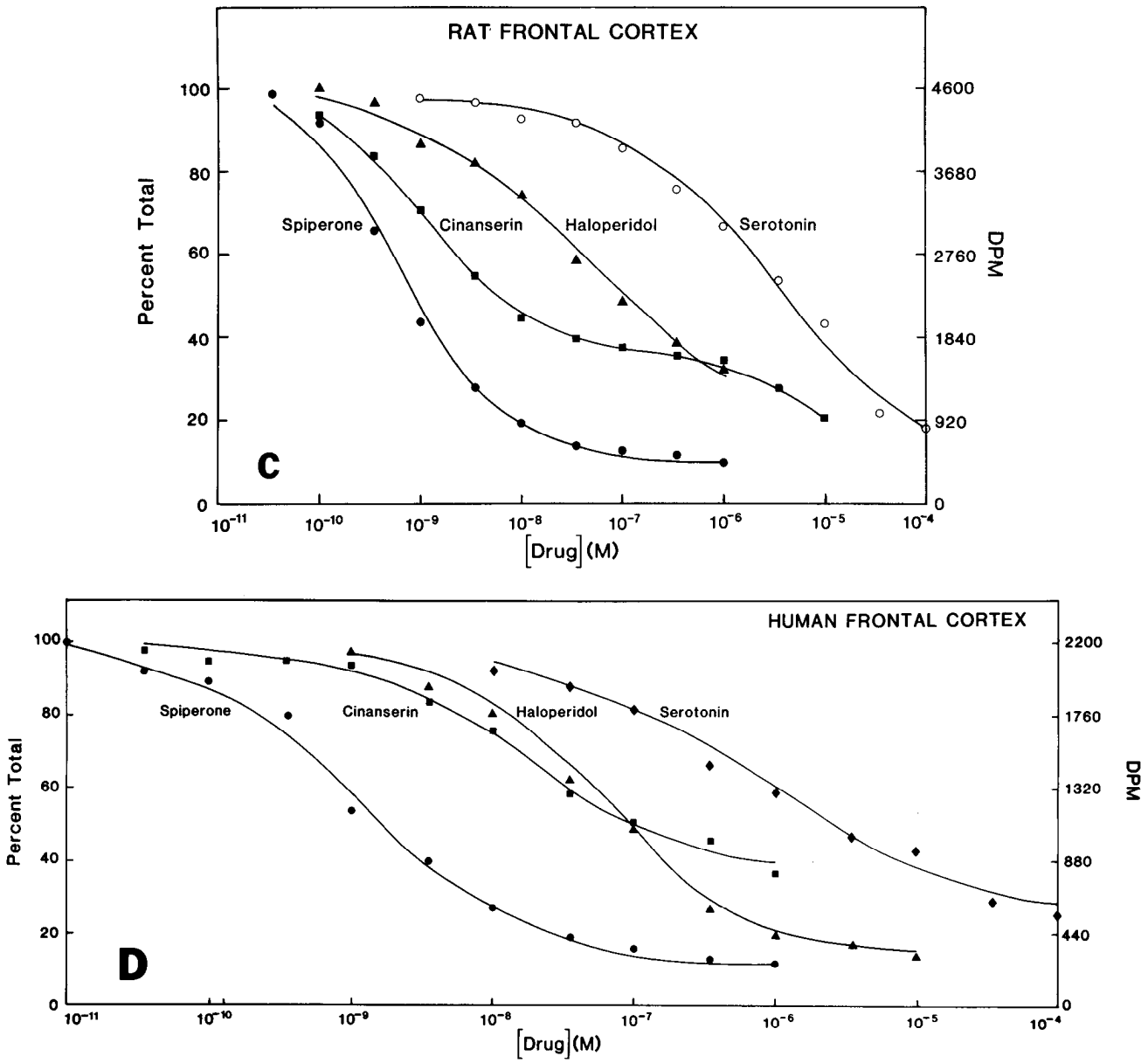
acterization of the receptor binding properties of this compound is needed.

The studies presented herein in rat striatal and human caudate tissue clearly demonstrate a specific interaction with brain D_2 dopamine receptors (Seeman, 1980). The order of potencies of a series of antagonists and agonists is very similar in the rat striatal and human caudate tissue and indicative of an interaction with a D_2 dopamine receptor.

The results of the experiments in rat and human frontal cortex tissue indicate that the binding of ^{3}H-NMSP binding is to 5-HT_2 receptors. The order of potency of a series of drugs is very similar in rat and human cortex tissues. Effective 5-HT_2 receptor blockers such as ketanserin and cinnanserin potently inhibit the binding, while sulpiride, a specific D_2 dopamine receptor blocker, does not. Serotonin potently inhibited the binding, while dopamine and noradrenaline did not.

In general, the results of these studies with ^{3}H-NMSP are similar to those reported using ^{3}H-spiperone as a radioligand for rat and human D_2 and 5-HT_2 receptors. For instance, Creese et al. (1979) compared the receptor-labeling properties of ^{3}H-spiperone in rat and human striatal tissues (calf tissue was also examined); the studies revealed that rat and human D_2 receptors appear to have very similar pharmacological properties, although some subtle differences in specific drug interactions could not be ruled out. Kinetic studies of rat D_2 dopamine receptors, using ^{3}H-spiperone as the radioligand (Leysen et al., 1978), revealed an association rate constant of 2.086×10^9 min^{-1} M^{-1}. This is similar to the k_1 of 1.68×10^9 min^{-1} M^{-1} reported herein for ^{3}H-NMSP. The dissociation rate constant for ^{3}H-spiperone was measured as 0.292 min^{-1}, as compared to 7.82×10^{-2} for the ^{3}H-NMSP binding to D_2 receptors reported herein. Overall, these values are similar, although the dissociation rate of ^{3}H-NMSP appears to be somewhat slower.

The B_{max} values obtained in the rat brain tissues in this study



and increasing concentrations of the drugs. The *points* represent experimental data determined from the means of 3 experiments, each point determined in triplicate. SEM were less than 5% for all points.

using ³H-NMSP are in reasonable agreement with published values for D_2 receptors using ³H-spiperone as the radioligand (Seeman, 1980), or for 5-HT_2 receptors using ³H-ketanserin as the radioligand (Battaglia et al., 1984). The B_{max} values found here in the human tissues for both D_2 and 5-HT_2 receptors are slightly lower than previously reported for D_2 or 5-HT_2 receptors. For instance, Seeman et al. (1985) recently reported an overall average B_{max} value of 11.3 pmol/gm wet-weight of tissue in control postmortem caudate tissue; we report a B_{max} value of 8.96 pmol/gm wet-weight of tissue. One significant difference in the methodology used in this study was the use of sulpiride to define nonspecific binding. This ligand has been demonstrated to selectively reveal D_2 receptor binding, eliminating 5-HT_2 and spirodecanone binding from the assay (List and Seeman, 1981). List and Seeman (1981) demonstrated that using sulpiride does indeed produce a lower B_{max} value than using another, less specific, ligand such as (+)-butaclamol. The B_{max} values for 5-HT_2 receptors in the human cortical tissue revealed with ³H-NMSP are also slightly lower than a previously reported value (Schotte et al., 1983). Possible variables accounting for the lower B_{max} values could be tissue handling and postmortem delay, although efforts were made to reduce these problems by prompt freezing of tissue (Whitehouse et al., 1984).

We did not attempt to identify 5-HT_2 receptors in the caudate and striatum, or D_2 receptors in the cerebral cortex. List and Seeman (1981) and Schotte et al. (1983) found 5-HT_2 receptors in basal ganglia, but their levels were only a fraction of the D_2 receptor levels. Also, NMSP has a higher affinity for D_2 receptors so that lower levels of 5-HT_2 receptors would not readily be detected under the conditions of our assay. Conversely, D_2 receptor levels in cerebral cortex are very difficult to detect; only very sensitive autoradiographic assays have found very low levels of D_2 receptors in cerebral cortex (Martres et al., 1985).

Recent PET scanning studies have yielded D_2 receptor con-

Table 2. Pharmacological characteristics of ^{3}H-NMSP binding

Drug	Rat striatum	Human caudate	Rat frontal cortex	Human frontal cortex
(+)-Butaclamol	1.4 ± 0.7	1.5 ± 0.1	4.2 ± 1.5	32 ± 3.5
(−)-Butaclamol	458 ± 25	228 ± 8	1312 ± 45	2597 ± 132
Chlorpromazine	6.8 ± 1.7	5.9 ± 2	4.6 ± 1.8	5.9 ± 0.3
Cinanserin	1811 ± 410	683 ± 24	1.1 ± 0.1	14.2 ± 1
Haloperidol	1.4 ± 0.1	2.7 ± 0.1	21 ± 9	45 ± 2
Ketanserin	1354 ± 288	1438 ± 124	0.5 ± 0.1	8.8 ± 0.6
N-Methylspiperone	0.2 ± 0.02	0.18 ± 0.1	0.6 ± 0.1	1.4 ± 0.1
Spiperone	0.2 ± 0.1	0.08 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
(−)-Sulpiride	53 ± 17	31 ± 4	10,000	3886 ± 398
Dopamine	3750 ± 1685	710 ± 124	23,453 ± 4310	45,548 ± 2289
(−)-Noradrenaline	>10,000	3813 ± 319	10,000	6141 ± 1150
Serotonin	>10,000	7370 ± 33	1679 ± 290	560 ± 35

K_i values (nM) of a series of drugs determined in competition for ^{3}H-NMSP binding in rat and human brain homogenates. Drugs were coincubated with 0.1 nM ^{3}H-NMSP and tissue. Cinanserin, 10^{-6} M (rat and human frontal cortex studies) or 10^{-5} M sulpiride (rat striatum and human caudate) were used to define the level of nonspecific binding. Values shown are the means and SEM of 3 experiments, each point determined in triplicate. K_i values were calculated from experimentally determined IC_{50} values according to Cheng and Prusoff (1973). The correlation coefficients, determined by linear regression, for the K_i values for rat striatum and human caudate were $r = 0.92$, and, for the K_i values for rat frontal cortex and human frontal cortex, were also 0.92.

Table 3. NMSP competition in radioreceptor assays

Receptor	Ligand	Tissue	IC_{50} (nM)	Ref.
D_2 dopamine	^{3}H-Spiperone	Rat striatum	0.23	Creese et al., 1979
D_1 dopamine	^{3}H-Flupenthixol	Rat striatum	350	Hyttel, 1981
D_1 dopamine	(DA-stimulated cyclase)	Goldfish retina	1400	
S_1 serotonin	^{3}H-5-HT	Rat hippocampus and striatum	>1000	Nelson et al., 1980
S_2 serotonin	^{3}H-Spiperone	Rat frontal cortex	1.3	Leysen et al., 1978
Alpha-1 adrenergic	^{3}H-Prazosin	Rat cerebral cortex	10.1	Greengrass and Bremner, 1979
Alpha-2 adrenergic	^{3}H-*p*-Aminoclondine	Rat cerebral cortex	>1000	Rouot and Snyder, 1979
Alpha-2 adrenergic	^{3}H-Rauwolcine	Rat cerebral cortex	155	Perry and U'Prichard, 1981
Beta-adrenergic	^{125}I-Cyanopindolol	Rat cerebral cortex	>1000	Engel et al., 1981
Muscarinic cholinergic	^{3}H-*N*-Methylscopolamine	Rat cerebral cortex	>10,000	Hammer et al., 1980
Benzo-diazepine	^{3}H-Diazepam	Rat cerebral cortex	>10,000	Braestrup and Squires, 1977
Benzo-diazepine	^{3}H-Ro 15-1788	Rat cerebral cortex	>10,000	Mohler et al., 1981
Benzo-diazepine	^{3}H-Ro 5-4864	Rat adrenals	>100,000	Mohler et al., 1981
Calcium channel	^{3}H-Nitrendipine	Bovine cerebral cortex	217	Gould et al., 1982
Dopamine uptake	^{3}H-Mazindol	Rat striatum	>1000	Javitch et al., 1983

See references listed for details of assay conditions. IC_{50} values were corrected for radioligand concentration according to Cheng and Prusoff (1973) in order to determine the reported K_i.

centrations in normal, young, healthy volunteers of 9–15 pmol/gm (Wong et al., 1986a, b). These values were calculated from a 3-compartment model and are in agreement with the values found here.

The results presented in this study indicate that, in rat striatal and human caudate tissue, ^{3}H-NMSP predominantly labels D_2 dopamine receptors and that in rat and human frontal cortex, this ligand predominantly labels 5-HT_2 receptors. The availability of these quantitative binding data will aid in the evaluation of models for *in vivo* labeling of receptors in PET scanning.

References

Battaglia, G., M. Shannon, and M. Titeler (1984) Guanyl nucleotide and divalent cation regulation of cortical S_2 serotonin receptors. J. Neurochem. *43:* 1213–1219.

Bennett, J. P., Jr., and H. I. Yamamura (1985) Neurotransmitter, hormone, or drug receptor binding methods. In *Neurotransmitter Receptor Binding,* H. I. Yamamura et al., eds., pp. 61–89, Raven, New York.

Cheng, Y. C., and W. H. Prusoff (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent of an enzymatic reaction. Biochem. Pharmacol. *22:* 3099–3108.

Creese, I., K. Stewart, and S. H. Snyder (1979) Species variations in dopamine receptor binding. Eur. J. Pharmacol. *60:* 55–66.

Creese, I., D. R. Sibley, M. W. Hamblin, and S. E. Leff (1983) The classification of dopamine receptors: Relationship to radioligand binding. Annu. Rev. Neurosci. *6:* 43–71.

Engel, G. D., D. Hoyer, R. Berthold, and H. Wagner (1981) ^{125}Iodocyanopindolol, a new ligand for β-adrenoceptors: Identification and quantitation of subclasses of β-adrenoceptors in guinea pig. Naunyn-Schmiedebergs Arch. Pharmacol. *317:* 277–285.

Gould, R. J., K. M. Murphy, and S. H. Snyder (1982) ^{2}H-Nitrendipine-labeled calcium channels discriminate inorganic calcium agonists and antagonists. Proc. Natl. Acad. Sci. USA *79:* 3656–3660.

Greengrass, P., and R. Bremner (1979) Binding characteristics of ^{3}H-

prazosin to rat brain alpha-adrenergic receptors. Eur. J. Pharmacol. *55:* 323–326.

Hammer, R., C. P. Berrie, N. J. M. Birdsall, A. S. V. Burgen, and E. C. Hulme (1980) Pirenzipine distinguished between subclasses of muscarinic receptors. Nature *283:* 90–92.

Hyttel, J. (1981) Similarities between the binding of ³H-pifluthixol and ³H-flupenthixol to rat striatal dopamine receptors *in vitro.* Life Sci. *28:* 563–569.

Javitch, J. A., R. O. Blaustein, and S. H. Snyder (1983) ³H-Mazindol binding associated with neuronal dopamine and norepinephrine uptake sites. Mol. Pharmacol. *26:* 35–44.

Leysen, J. E., W. Gommeren, and P. M. Laduron (1978) Spiperone: A ligand of choice for neuroleptic receptors. Biochem. Pharmacol. *27:* 307–316.

Leysen, J. E., C. J. E. Niemegeers, J. M. Van Neuten, and P. M. Laduron (1982) ³H-Ketanserin (R41 468), a selective ³H-ligand for serotonin$_2$ receptor binding sites. Mol. Pharmacol. *21:* 301–314.

List, S. J., and P. Seeman (1981) Resolution of dopamine and serotonin receptor components of ³H-spiperone binding to rat brain regions. Proc. Natl. Acad. Sci. USA *78:* 2620–2624.

Martres, M. P., M. L. Bouthenet, N. Sales, P. Sokoloff, and J. C. Schwartz (1985) Widespread distribution of brain dopamine receptors evidenced with ^{125}I-Sulpride, a highly selective ligand. Science *228:* 752–755.

Mohler, H., W. P. Burkard, H. H. Keller, J. G. Richards, and W. Haefely (1981) Benzodiazepine antagonist Ro 15-1788: Binding characteristics and interactions with drug-induced changes in dopamine turnover and cerebellar cGMP levels. J. Neurochem. *37:* 714–722.

Nelson, D. L., A. Herbet, A. Enjalbert, J. Bockaert, and M. Hamon (1980) Serotonin-sensitive adenylate cyclase and ³H-serotonin binding sites in the CNS of the rat. Biochem. Pharmacol. *29:* 2445–2454.

Perry, B. D., and D. C. U'Prichard (1981) ³H-Rauwolscine (alpha-yohimbine): A specific antagonist radioligand for brain alpha$_2$ adrenergic receptors. Eur. J. Pharmacol. *76:* 461.

Rouot, B. R., and S. H. Snyder (1979) ³H-Para-amino-clonidine: A novel ligand which binds with high affinity to alpha-adrenergic receptors. Life Sci. *25:* 769–774.

Schotte, A., J. M. Maloteaux, and P. M. Laduron (1983) Characterization and regional distribution of serotonin S_2-receptor in human brain. Brain Res. *276:* 231–235.

Seeman, P. (1980) Brain dopamine receptors. Pharmacol. Rev. *32:* 229–313.

Seeman, P., C. Ulpian, C. Bergeron, P. Riederer, K. Jellinger, E. Gabriel, G. P. Reynolds, and W. W. Tourtellotte (1985) Bimodal distribution of dopamine receptor densities in brains of schizophrenics. Science *225:* 728–731.

Squires, R. F., and C. Braestrup (1977) Benzodiazepine receptors in rat brains. Nature *266:* 732–734.

Titeler, M. (1983) *Multiple Dopamine Receptors.* Dekker, New York.

Wagner, H. N., H. D. Burns, R. F. Dannals, D. F. Wong, B. Langstrom, T. Duelfer, J. J. Frost, H. T. Ravert, J. M. Links, S. B. Rosenbloom, S. E. Lukas, A. V. Kramer, and M. J. Kuhar (1983) Imaging dopamine receptors in the human brain by positron tomography. Science *221:* 1264–1266.

Whitehouse, P. J., D. Lynch, and M. J. Kuhar (1984) Effects of postmortem delay and temperature on neurotransmitter receptor binding in a rat model of the human autopsy process. J. Neurochem. *43:* 553–559.

Wong, D. F., H. N. Wagner, R. F. Dannals, J. M. Links, J. J. Frost, H. T. Ravert, A. A. Wilson, A. E. Rosenbaum, A. Gjedde, K. H. Douglass, J. D. Petronis, M. F. Folstein, J. K. Thomas Toung, H. D. Burns, and M. J. Kuhar (1984) Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. Science *226:* 1393–1396.

Wong, D. F., A. Gjedde, H. N. Wagner, R. F. Dannals, K. H. Douglass, J. M. Links, and M. J. Kuhar (1986a) Quantification of neuroreceptors in the living human brain. II. Inhibition studies of receptor density and affinity. J. Cerebr. Blood Flow Metab. *6:* 147–153.

Wong, D. F., A. Gjedde, and H. N. Wagner (1986b) Quantification of neuroreceptors in the living human brain. I. Irreversible binding of ligands. J. Cerebr. Blood Flow Metab. *6:* 137–146.

Woodruff, G. N., and S. B. Freedman (1981) Binding of ³H-sulpiride to purified rat striatal synaptic membranes. Neuroscience *6:* 407–410.